Praca oryginalna *Original paper*

Comparison of cardiopulmonary, hematological, biochemical, and sedation effects of intramuscular, intranasal, and intraosseous methods of administering midazolam and butorphanol combination to pigeons

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Received 08.04.2025 *Accepted 02.07.2025*

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Summary

This study compared the effects of midazolam-butorphanol (MID-BUT) administration via different routes in pigeons (*Columba livia*). Twenty-four healthy adult pigeons (1-2 years old, 494.2 ± 22.3 g) were divided into three groups, each receiving MID (4 mg/kg) + BUT (2 mg/kg) by intramuscular (IM), intraosseous (IO), or intranasal (IN) routes. Cardiopulmonary, hematological, and biochemical parameters, along with sedation depth and anesthesia duration, were evaluated. While intergroup differences were not significant, temporal changes occurred within groups. Cloacal temperature was significantly lower 30 and 40 min after sedation administration in all groups. SpO_2 was significantly decreased 5 min after sedation adminstration in the IM and IO groups, while $EtCO_2$ was significantly increased 10, 15, and 20 min after sedation administration in the IO group. Hematological changes in the IM group included lower WBC, RBC, HCT, HGB, and CREA, which were significantly reduced 15 and 30 min after sedation administration. Sedation onset was fastest in the IO group (4.22 ± 0.7 min), followed by the IN group (4.37 ± 0.89 min) and the IM group (4.86 ± 0.9 min). Sedation duration was longest in the IO group (37.23 ± 3.21 min), significantly longer than in the IM (34.53 ± 3.4 min) and IN (30 ± 1.21 min) groups. In conclusion, the IN method is recommended as a preferable option for short-term procedures, whereas the IO method can be considered more suitable for long-term procedures, but presents potential risks in pigeons with respiratory complications. The IM method should be used with greater caution because of its observed effects on hematological parameters.

Keywords: intramuscular, intranasal, intraosseous, midazolam, butorphanol

The use of anesthesia and analgesia in avian species has gained increasing prominence in veterinary medicine, particularly for physical examinations, diagnostic sampling, radiography, surgical interventions, and fracture repair (6, 8, 24, 32, 36). These procedures, often stressful for birds, trigger rapid activation of the sympathetic nervous system, resulting in marked alterations in heart rate, respiratory rate, body temperature, and hematological parameters (8, 16, 33). The choice of anesthetic agents and administration methods is critical for ensuring successful outcomes and accurate physiological assessments (14, 19, 24).

In 2018, the European Society of Anesthesiology defined procedural sedation and analgesia (PSA) as the use of sedative and analgesic drugs to facilitate various diagnostic and therapeutic procedures while minimizing cardiopulmonary depression, anxiety, and stress (10). PSA is applied in both noninvasive and invasive procedures, including radiography, biopsies, punctures, fracture repair, blood collection, catheterization, suturing, arterial stenting, dental procedures, and endoscopic diagnostics (13, 18). Agents such as midazolam (MID), commonly used for sedation and anxiolysis, propofol for rapid induction and short-duration anesthesia, and opioids for potent analgesia, have proven effective in a variety of veterinary contexts, including companion animals, birds, and exotic species (3, 4).

PSA is increasingly used in avian medicine because of its efficacy in reducing complications and improving clinical outcomes. Specifically, PSA offers advantages over inhalation anesthetics, such as isoflurane, which, though effective, may cause hypotension and other adverse effects (8, 33). By using parenteral PSA methods, veterinarians can achieve appropriate sedation and analgesia while minimizing risks, ultimately enhancing safety and reducing both morbidity and mortality in avian patients.

Injectable anesthetics in avian species are typically administered via intravenous (IV), intramuscular (IM), intranasal (IN), or intraosseous (IO) routes (5, 6, 11, 16). However, IV administration is often less preferred due to the fragility of avian vasculature and the technical difficulty of catheter placement (14, 16, 37). IO administration, which involves injection into the ulna or other long bones, provides rapid and effective vascular access, but carries risks, such as infection and pneumatic bone penetration, and therefore requires specialized skills (5, 34). IM injection, most commonly into the pectoral muscle, is widely used due to its practicality, though it carries risks, such as inadvertent penetration of the thoracic cavity, especially in smaller species (6, 33). IN administration offers a less invasive option with fewer biochemical changes and reduced stress compared to IM injections, but its use is largely limited to hydrophilic drugs (6, 17, 33).

The combination of midazolam (MID) and butorphanol (BUT) has been widely used for procedural sedation in avian species. MID, a water-soluble benzodiazepine, is known for its sedative, anxiolytic, anticonvulsant, and amnesic effects in birds (14, 26). BUT, an opioid with agonist-antagonist properties, exerts its analgesic effects through kappa receptor activation and is commonly used in avian clinical settings (3, 8, 20, 31). A previous study demonstrated that the MID-BUT combination is effective for PSA in cockatiels, highlighting its potential application in avian anesthesia (8). However, data on the effects of different administration routes for this combination in avian species remain limited.

This study aims to compare the anesthetic and analgesic effects of the MID-BUT combination administered via intramuscular, intraosseous, and intranasal routes in pigeons (*Columba livia*). The evaluation focuses on cardiopulmonary, hematological, and biochemical parameters, as well as reflex responses, to determine the safety and efficacy of each administration method for procedural sedation and analgesia in avian species.

Material and methods

The birds. The study was conducted at the Harran University Animal Experiments Research Center (HDAM), Farm Animals and Avian Unit. In this study, 24 healthy adult pigeons (*Columba livia*) aged 1-2 years and weighing 494.2 ± 22.3 g (mean ± SD) were used. The pigeons were housed in the avian unit of HDAM, in spacious and airy cages located in a clean, quiet, and stress-free environment, at room temperature (25°C), with *ad libitum* access to feed water. All clinical and parasitological (fecal) examinations were performed there.

The study protocol was approved by the Local Ethics Committee on Animal Experiments, Harran University (session and permit number: 2024/002/02).

Study groups. Regarding the anesthesia application methods in the study, the subjects were randomly divided into 3 groups of 8 pigeons each. The anesthesia protocol combination comprised MID (Zolamid® 15 mg/3 ml, Vem İlaç, Ankara, Turkiye) and BUT (Butomidor® 10 mg/ml, richterpharma ag, Wels/Austria) at doses of 4 mg/kg MID + 2 mg/kg BUT (23). Group 1 (n = 8) was the IM group, where intramuscular anesthesia was administered through the pectoral muscle using a 28-gauge, 1.27 cm long needle, and a 1 ml insulin syringe (22, 23). Group 2 (n = 8) was the IO group. For this purpose, the anesthesia protocol (MID 4 mg/kg and BUT 2 mg/kg) was diluted 1 : 1 with isotonic saline prior to administration. This dilution was performed to reduce the viscosity of the solution, minimize injection resistance, and ensure homogeneous distribution within the medullary cavity. It has been reported in the literature that dilution can facilitate drug delivery by reducing intramedullary pressure and preventing backflow or compartmental pressure-related complications during IO infusion in birds and small animals (11, 22, 34). In this study, IO administration was performed via the ulnar bone using an 18G (green) cannula. After feather plucking and aseptic preparation of the site, the ulna was stabilized manually and the cannula was introduced at a 45-70° angle into the bone's long axis. Insertion was achieved through gentle rotational motion until the loss of resistance indicated entry into the medullary cavity. Correct placement was confirmed radiographically (mediolateral and craniocaudal position). Group 3 (n = 8) constituted the IN group. The procedure was performed using a 1 ml insulin syringe with a 28-gauge, 1.27 cm long needle, which was removed before intranasal administration to avoid injury. For intranasal administration, the pigeons were restrained with a towel, and the drug to be administered was applied equally to both nostrils for approximately 10 seconds and absorbed from the nasal mucosa (23). The pigeon was then placed on a flat surface and allowed to enter anesthesia (17, 37).

Measurements. Initial cardiopulmonary, hematological, and biochemical measurements and evaluations of the pigeons were performed 5 min before anesthesia (T0) in the dorsoventral position without any pressure on the head and neck of each pigeon.

Cardiopulmonary evaluation was performed 5 (T5), 10 (T10), 15 (T15), 20 (T20), 30 (T30) and 40 (T40) minutes after sedation administration. Mindray UMEC12VET (Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China) was used for this evaluation. To assess the heart rate, the electrodes were attached directly to the skin on the anterior surface of both wings and on the legs to the skin fold on the inner surface of both thighs (femurs).

The cloacal temperature (CT) was determined by inserting a thermometer into the cloaca. Blood pressure was measured indirectly and a medium-sized winged blood pressure cuff was placed on the dorsal metatarsal artery of the right leg. The cuff width was maintained at approximately 40% of the leg circumference. For SpO_2 monitoring, a pulse oximeter probe was placed on the muscle mass of the right radial bone. $EtCO_2$ was monitored by placing the $EtCO_2$ tube over the nose and covering the entire beaker for $EtCO_2$ measurements. Multiparametric monitoring of the heart rate, respiratory rate, SPO_2, cloacal temperature (CT), end-tidal CO_2, and mean arterial pressure (MAP) was recorded (16, 27).

Hematologic evaluations (to assess the efficacy of anesthesia and its impact on blood health) and biochemical evaluations (to ensure the safety of the anesthetic agents and detect any potential liver or kidney dysfunction) were performed before anesthesia administration (T0) as well as 15 min (T15) and 30 min (T30) thereafter. Hematological and biochemical evaluations were performed in line with previous studies conducted on avian species (2, 9, 11). Blood (2 ml) for biochemical and hematologic analyses was collected from the vena cava ulnaris (28). For hemogram analysis, white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), and hematocrit (HCT) values were obtained from 1 ml venous blood samples with K3EDTA using an MS4 autoanalyzer (CFE 279, Hematology Analyzer, France). One milliliter of blood was collected for biochemical analysis and placed in gel tubes without anticoagulants. When clotting occurred, serum was separated by centrifugation at 3000 rpm for 10 min. For biochemical analysis, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea (BUN), and creatinine (CREA) levels were measured using a SPOTCHEM EZ SP-4430 dry system biochemistry analyzer (Arkray Inc., Kyoto, Japan).

The depth of sedation, reflexes, and level of anesthetic immobilization were determined by modified reflex assessment methods described in previous studies (23, 29, 30). Accordingly, the evaluation included the eyelid gap (G) (degree of eyelid opening and closing when the eyelid margin was touched at the medial canthus with a dry cotton swab), pedal reflex (P) (degree of pulling of the leg when the fingers were pulled), wing withdrawal reflex (K) (degree of gathering of the wings when the wings were pulled outward), response to needle prick (R) (sensation control with needle pricking of one leg), as well as head and neck posture (BB) (degree of head-neck movement). Clinically tested reflex grades were scored as 0: no response to stimuli, 1: very weak and partial response to stimuli, and 2: evident and strong response to stimuli. The time of sedation onset (loss of reflexes in the dorsal position) and duration of sedation (from T0 to spontaneous recovery of reflexes from the dorsal position) were monitored by the same person.

Statistical analysis. Data were evaluated using the SPSS 22.1 statistical software (SPSS for Windows®). The Shapiro-Wilk normality test was applied to determine whether all data were parametric. Because the data did not meet the parametric test assumptions, the differences between the experimental groups (IN, IO, and IM) in all quantitative characteristics were determined using the Kruskal-Wallis and Mann-Whitney U tests. Differences between measurements repeated in the same pigeon at different times were determined separately using the Freidman test for each experimental group. For variables exhibiting significant differences in the Friedman test, pairwise comparisons were performed using the Wilcoxon test only for differences between the baseline and other measurements. The results of the study are presented as the mean ± SD and median values. Statistical significance was set at $p < 0.05$.

Results and discussion

Administration of anesthetic agents IM, IO, and IN was performed safely in all study groups. No complications related to the application method were observed during or after the study. The pigeons were healthy at the end of the study. In the follow-up of the pigeons at the end of the study, no lesions were observed in the muscle or bone tissue at the IM and IO application sites. No cases of sneezing or respiratory distress were observed in the IN group.

No statistically significant differences were observed between the groups in the comparative statistical analysis of the experimental groups ($p > 0.05$). However, statistically significant results were obtained for the in-group time intervals of the application method ($p < 0.05$).

According to cardiopulmonary evaluation findings, only cloacal temperature was statistically significant at T30 and T40 ($p < 0.05$). The heart rate and MAP values were not statistically significant in any groups at the intra-group time intervals. However, SpO_2 at T5 in the IM and IO groups was statistically significantly lower than it was at T0 ($p < 0.05$). On the other hand, a statistically significant increase was observed in $EtCO_2$ at T10, T15, and T20 in the IO group compared to T0, whereas an increase was observed only at T10 in the IN group ($p < 0.05$). Statistically significant changes in intra-group time intervals were observed in respiratory rate and cloacal temperature ($p < 0.05$). Respiratory rate showed a significant decrease at T5, T10, T15, T30, and T40 in the IM group, at all time intervals except T0 in the IO group, and at T10, T15, T20, and T30 in the IN group ($p < 0.05$). There was a statistically significant decrease in cloacal temperature at T10, T15, T20, T30, and T40 in the IM and IO groups and at T20, T30, and T40 in the IN group ($p < 0.05$). The values for cardiopulmonary evaluation are presented in Table 1.

In hematologic and biochemical evaluation, there was a significant difference between the IM, IO, and IN groups in HCT, HGB, AST, ALP, BUN, and CREA values at T15 and T30, and in ALT values only at T30 ($p < 0.05$). There was a statistically significant decrease in ALP values at T15 and T30 compared to the T0 value at the in-group time intervals. Furthermore, a statistically significant decrease was observed in

Tab. 1. Mean ± SD and median cardiopulmonary values over time for IM, IO, and IN midazolam-butorphanol (4 mg/kg and 2 mg/kg) in pigeons

Pm	Gr	T0	T5	T10	T15	T20	T30	T40
HR (beats per minute)	IM	207.87 ± 66.32 185	201.75 ± 63.34 228	198.25 ± 80.37 187.5	210.38 ± 85.05 224.5	212.5 ± 60.23 221.5	221.25 ± 71.77 225	212.63 ± 61.99 209.5
	IO	224.12 ± 23.18 220.5	210.50 ± 45.44 234	212.38 ± 53.33 232	204.38 ± 43.52 227.5	203.00 ± 46.42 214	190.88 ± 43.94 176	207.38 ± 24.04 193
	IN	260.50 ± 0.92 269	229.75 ± 52.31 214	207.63 ± 59.47 189	220.00 ± 56.29 212.5	200.38 ± 41.39 214.5	204.88 ± 47.80 224	211.00 ± 57.23 199
SpO_2 (%)	IM	89.88 ± 4.15 91	80.00 ± 11.91* 82	86.38 ± 10.28 88	88.38 ± 7.50 90.5	91.13 ± 12.36 93	90.38 ± 13.34 93.5	89.38 ± 7.48 90.5
	IO	92.25 ± 2.43 93	86.63 ± 4.34* 86	90.00 ± 4.27 90	88.13 ± 4.32 86	90.88 ± 3.39 90	87.75 ± 3.88 87	93.13 ± 2.8 93
	IN	93.25 ± 3.28 93.5	90.38 ± 3.88 90	89.13 ± 4.54 90.5	90.13 ± 3.27 89.5	91.50 ± 3.11 91	92.00 ± 5.70 93.5	92.13 ± 4.32 93
$EtCO_2$ (%)	IM	35.50 ± 3.02 36	36.25 ± 12.57 39.5	40.75 ± 6.45 40.5	35.50 ± 4.95 37	41.38 ± 7.24 41	42.38 ± 9.59 41	37.00 ± 6.50 37.5
	IO	33.25 ± 1.58 33	36.75 ± 6.38 38.5	46.13 ± 5.61* 46	43.88 ± 3.22* 44.5	37.75 ± 3.49* 36.5	39.00 ± 6.52 38	35.13 ± 5.46 34
	IN	37.13 ± 3.22 37.5	42.50 ± 7.61 41.5	49.13 ± 9.73* 50	45.75 ± 12.12 41.5	40.75 ± 7.44 41.5	41.50 ± 9.42 41.5	39.63 ± 4.37 38
Respiration (breaths per minute)	IM	40.75 ± 7.42 40.5	29.50 ± 6.82* 29.5	28.50 ± 8.43* 29.5	28.00 ± 7.17* 30	32.00 ± 8.97 29	29.00 ± 7.91* 29	32.75 ± 9.73* 32.5
	IO	42.50 ± 3.25 43.5	32.88 ± 4.94* 34	33.75 ± 7.53* 32.5	32.50 ± 6.61* 31	29.88 ± 2.29* 30.5	30.13 ± 3.60* 32	34.00 ± 6.71* 35.5
	IN	51.25 ± 9.19 49.5	31.88 ± 4.51* 30.5	29.63 ± 9.81* 29.5	26.00 ± 8.03* 26.5	29.75 ± 7.45* 31	28.63 ± 9.41* 25	33.88 ± 17.12 27
MAP (mm/hg)	IM	106.50 ± 23.77 111.5	106.13 ± 29.54 108.5	85.88 ± 26.69 87	97.00 ± 18.69 97.5	96.50 ± 28.35 93	102.63 ± 34.37 88	108.00 ± 51.48 81
	IO	114.75 ± 12.92 114.5	122.63 ± 15.62 130	112.50 ± 25.31 110	118.88 ± 28.13 114	128.38 ± 41.56 138	108.38 ± 29.89 116.5	113.50 ± 33.43 113
	IN	135.63 ± 32.00 136	99.75 ± 41.02 99	112.75 ± 33.26 110.5	129.13 ± 39.62 133	125.25 ± 40.95 108.5	132.25 ± 45.22 122	134.00 ± 47.46 144
CT (°C)	IM	41.22 ± 1.18 41.55	40.95 ± 1.24 41.3	40.53 ± 1.05* 40.65	40.33 ± 1.05* 40.6	40.06 ± 1.06* 40.35	39.97 ± 1.13^{a}* 40.2	39.86 ± 0.92* 40.05
	IO	41.52 ± 0.63 41.3	41.00 ± 0.78 41	40.27 ± 0.81* 40.3	40.10 ± 0.81* 40.2	39.72 ± 0.76* 39.7	39.51 ± 0.75^{b}* 39.55	39.30 ± 0.69* 39.5
	IN	41.63 ± 0.50 41.5	41.21 ± 0.57 41.3	41.02 ± 0.65 40.95	40.75 ± 0.97 40.3	40.43 ± 0.70* 40.3	40.43 ± 0.68^{c}* 40.25	40.21 ± 0.47* 40.3

Explanations: Pm – parameter; Gr – group; IM – intramuscular; IO – intraosseous; IN – intranasal; T0 – pre-injection, baseline; T5, T10, T15, T20, T30, T40 – 5, 10, 15, 20, 30, 40 min, respectively; after T0; HR – heart rate; MAP – mean arterial pressure; RR – respiratory rate; SpO_2 – oxyhemoglobin saturation; $EtCO_2$ – end-tidal CO_2; BT – body temperature. * – Differences within the same line are statistically significant ($p < 0.05$). a-c – Differences between means of time carrying different letters in the same line are statistically significant ($p < 0.05$).

WBC, RBC, HCT, and HGB values at T15 and T30 and in CREA values at T15 compared to T0 in the IM group. The hematological and biochemical parameters are presented in Table 2.

In the majority of pigeons across all groups, the depth of sedation observed at T5 was insufficient compared to T0, as evidenced by persistent reflex activity and inadequate anesthesia-induced immobilization. Pigeons treated by the IO method underwent anesthesia faster than those treated by the other methods. They were placed in the dorsal position without manual intervention. From T10 onwards, reflexes and sensitivity to the environment decreased, and deep sedation was observed in most pigeons. In particular, in the IO and IN groups, more pigeons showed very weak reflexes and deep sedation at T15, T20, and T30. In the IM and IN groups, sedation started to wear off and reflexes began to return in pigeons at T40, whereas in the IO group, weak reflexes and deep sedation persisted in more pigeons. Pigeon scores at the time intervals for each group are presented in Table 3. The onset of sedation was earlier in the IO group (4.22 ± 0.7 min) than in the IN group (4.37 ± 0.89 min) and the IM group (4.86 ± 0.9 min) ($p = 0.006$). The sedation duration was significantly longer in the IO group (37.23 ± 3.21 min) than in the IN group (30 ± 1.21 min) and the IM group (34.53 ± 3.4 min) ($p = 0.01$). The onset and duration of sedation for each group are shown in Table 4.

This study examined the cardiopulmonary, hematological, and biochemical parameters, as well as reflexes and sedation efficacy for the MID-BUT combination, which can be categorized within the scope of PSA, by comparing IM, IO, and IN administration routes in pigeons. The three procedures were performed without complications, and the clinically significant aspects were particularly noteworthy. The IM route provided effective sedation, but hematologic effects should also be considered. Although the IO route induced rapid

Tab. 2. Hematological values (mean ± SD and median) over time for IM, IO, and IN midazolam-butorphanol (4 mg/kg and 2 mg/kg) in pigeons

Pm	Gr	T0	T15	T30
WBC (10^3/ul)	IM	181.38 ± 65.82 174.01	225.07 ± 61.39* 236.7	168.86 ± 98.96* 147.16
	IO	187.01 ± 93.15 186.51	202.96 ± 72.11 282.70	204.72 ± 96.32 185.21
	IN	195.86 ± 140.16 199.67	208.25 ± 128.70 223.03	200.64 ± 110.27 209.67
RBC (10^6/ul)	IM	3.25 ± 0.27 3.35	2.90 ± 0.31* 2.81	3.05 ± 0.18* 3.12
	IO	3.23 ± 0.73 3.49	3.34 ± 0.36 3.30	3.14 ± 0.24 3.09
	IN	3.16 ± 0.34 3.26	3.10 ± 0.33 3.26	3.02 ± 1.05 3.23
HCT (%)	IM	51.26 ± 3.42 52.9	46.02 ± 3.28^{a}* 45.2	48.96 ± 2.54^{a}* 50.7
	IO	51.12 ± 12.53 52.05	51.40 ± 4.69^{b} 52	49.80 ± 7.32^{b} 48.05
	IN	48.10 ± 5.69 50.35	47.12 ± 5.35^{c} 49	47.78 ± 4.38^{c} 50.2
HGB (g/dL)	IM	12.10 ± 1.38 13	9.88 ± 0.81^{a}* 9.80	11.32 ± 0.67^{a}* 11.8
	IO	13.68 ± 2.31 13.15	14.11 ± 1.95^{b} 14.25	13.73 ± 3.20^{b} 13.3
	IN	10.12 ± 0.91 10.5	9.53 ± 1.03^{c} 9.4	9.77 ± 1.65^{c} 10.15
ALT (U/L)	IM	17.05 ± 5.88 18.1	16.77 ± 0.53 16.05	12.45 ± 2.43^{a} 10.95
	IO	13.27 ± 1.18 13.45	12.92 ± 1.08 13.15	13.08 ± 3.35^{b} 11.85
	IN	15.03 ± 6.76 12.75	14.51 ± 5.53 12.8	13.72 ± 6.10^{c} 11.1
AST (U/L)	IM	102.31 ± 27.97 175.55	102.75 ± 12.77^{a} 87.15	106.96 ± 7.47^{a} 113.15
	IO	92.37 ± 20.46 94.35	85.17 ± 18.40^{b} 84.55	93.28 ± 16.21^{b} 97.6
	IN	99.00 ± 7.62 89.25	103.73 ± 12.80^{c} 97.4	113.82 ± 5.57^{c} 104.85
ALP (U/L)	IM	117.63 ± 51.19 109	103.63 ± 47.80^{a}* 100.5	100.63 ± 45.63^{a}* 95.5
	IO	96.38 ± 66.03 64	86.75 ± 58.74^{b}* 58	80.88 ± 56.38^{b}* 54
	IN	116.63 ± 97.01 117	94.00 ± 59.16^{c}* 99.5	88.25 ± 53.94^{c}* 81.5
BUN (mg/dL)	IM	12.25 ± 11.68 6.9	12.70 ± 12.73^{a} 6.4	12.65 ± 11.86^{a} 7.05
	IO	20.13 ± 8.69 19.9	19.23 ± 9.21^{b} 18.2	19.48 ± 9.02^{b} 19.4
	IN	5.42 ± 2.36 4.55	5.38 ± 2.35^{c} 4.65	5.43 ± 2.12^{c} 4.4
CREA (mg/dL)	IM	0.18 ± 0.08 0.22	0.09 ± 0.17^{a}* 0.08	0.18 ± 0.12^{a} 0.16
	IO	0.15 ± 0.05 0.17	0.17 ± 0.09^{b} 0.17	0.18 ± 0.09^{b} 0.20
	IN	0.26 ± 0.17 0.20	0.29 ± 0.07^{c} 0.22	0.21 ± 0.15^{c} 0.16

Explanations: Pm – parameter; Gr – group; IM – intramuscular; IO – intraosseous; IN – intranasal; T0 – pre-injection, baseline; T5, T15, T30 – 5, 15, 30 min, respectively, after T0; WBC – white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; BUN – urea; CREA – creatinine. * – Differences shown in the same line are statistically significant ($p < 0.05$). a-c – Differences between means of time carrying different letters in the same line are statistically significant ($p < 0.05$).

and profound sedation, caution is necessary due to the risk of respiratory depression. Conversely, the IN route yielded safer outcomes, but the duration of sedation was shorter. These findings offer valuable insights into the potential clinical applications of these administration methods.

Although anesthesia and sedation methods in pigeons remain current topics, studies have evaluated IM, IO, and IN methods with a focus on cardiopulmonary effects. One such study on the use of dexmedetomidine and midazolam separately and in combination with IN administration reported that midazolam alone did not affect cardiopulmonary values, but significant decreases in heart rate, respiratory rate, and cloacal temperature were observed for the midazolam/dexmedetomidine combination (14). Another study involving IN application reported no change in cardiopulmonary values with the acepromazine/diazepam combination (33). A study comparing the IO and IM methods of ketamine administration in pigeons reported that the IM method resulted in a greater increase in the respiratory rate and a greater decrease in the heart rate (16). A study evaluating the IO and IV routes of thiopental-Na administration in pigeons observed no significant differences in cardiopulmonary values (5). Furthermore, a study using the IO method with low- and high-dose propofol anesthesia emphasized that high-dose propofol induced severe respiratory depression and hypotension (11). In studies conducted on other avian species, no significant differences were observed in cardiopulmonary values in budgerigars with IM MID-BUT combination and in chickens with IO ketamine and thiopental-Na administration (21, 34). In the present study, the significant effects of the midazolam/butorphanol combination with IO on SpO_2, $EtCO_2$, and respiratory rate, particularly shortly after administration, may be attributed to the rapid vascular access of this method and the potential respiratory suppression caused by opioids (35). It is recommended that, in patients with respiratory problems, the IO method of administering this combination be used with caution and that antagonists (flumazenil) be introduced if necessary. Another important consideration is the cloacal temperature. A slight decrease in cloacal temperature was observed across all methods, which was interpreted as potentially resulting from metabolic deceleration (14). It is suggested that the ambient temperature be increased, especially when using IO and IM methods. In this study, the absence of significant changes in the heart rate with the MID-BUT combination administered by the IN method may be explained by the greater cardiovascular safety profile of dexmedetomidine as an α_2 adrenergic receptor agonist compared to its potential to induce cardiac arrhythmias (1).

In studies on avian species, it has been reported that age is a significant factor in hematological and

Tab. 3. Sedation depth, reflexes, and immobilization at time intervals in pigeons administered IM, IO, and IN midazolam-butorphanol (4 mg/kg and 2 mg/kg)

Pm	Gr	T0	T5	T10	T15	T20	T30	T40
G	IM	$2^{(8)}$	1 $0^{(3)}$ - $1^{(4)}$ - $2^{(1)}$	0 $0^{(6)}$ -$1^{(2)}$	0 $0^{(5)}$ - $1^{(3)}$	1 $0^{(3)}$ - $1^{(5)}$	1 $0^{(3)}$ - $1^{(3)}$ - $2^{(2)}$	1 $0^{(1)}$ - $1^{(6)}$ - $2^{(1)}$
	IO	$2^{(8)}$	1 $1^{(7)}$ - $2^{(1)}$	0 $0^{(4)}$ - $1^{(4)}$	0 $0^{(6)}$ - $1^{(2)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$	1 $1^{(5)}$ - $2^{(3)}$
	IN	$2^{(8)}$	1 $0^{(1)}$ - $1^{(4)}$ - $2^{(3)}$	1 $0^{(3)}$ - $1^{(4)}$ - $2^{(1)}$	0.5 $0^{(4)}$ - $1^{(4)}$	0 $0^{(5)}$ - $1^{(3)}$	0 $0^{(5)}$ - $1^{(2)}$ - $2^{(1)}$	1 $0^{(1)}$ - $1^{(4)}$ - $2^{(3)}$
P	IM	$2^{(8)}$	1 $0^{(6)}$ - $1^{(2)}$	0 $0^{(6)}$ - $1^{(2)}$	0 $0^{(6)}$ - $1^{(2)}$	0.5 $0^{(1)}$ - $1^{(7)}$	1 $1^{(8)}$	1 $0^{(1)}$ - $1^{(7)}$
	IO	$2^{(8)}$	1 $1^{(7)}$ - $2^{(1)}$	0 $0^{(2)}$ - $1^{(6)}$	0 $0^{(8)}$	0.5 $0^{(3)}$ - $1^{(5)}$	1 $0^{(2)}$ - $1^{(6)}$	0 $0^{(4)}$ - $1^{(4)}$
	IN	$2^{(8)}$	1 $0^{(2)}$ - $1^{(4)}$ - $2^{(2)}$	1 $0^{(3)}$ - $1^{(5)}$	0 $0^{(6)}$ - $1^{(2)}$	0 $0^{(5)}$ - $1^{(3)}$	0 $0^{(6)}$ - $1^{(2)}$	1 $0^{(2)}$ - $1^{(4)}$ - $2^{(1)}$
BB	IM	$2^{(8)}$	1 $0^{(2)}$ - $1^{(5)}$ - $2^{(1)}$	0 $0^{(1)}$ - $1^{(7)}$	1 $0^{(3)}$ - $1^{(5)}$	1 $0^{(3)}$ - $1^{(5)}$	1 $1^{(8)}$	1 $0^{(1)}$ - $1^{(6)}$ - $2^{(1)}$
	IO	$2^{(8)}$	0.5 $1^{(7)}$ - $2^{(1)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$	1 $1^{(8)}$	1 $1^{(7)}$ - $2^{(1)}$
	IN	$2^{(8)}$	1 $0^{(2)}$ - $1^{(5)}$ - $2^{(1)}$	1 $0^{(2)}$ - $1^{(6)}$	1 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(4)}$ - $1^{(4)}$	1 $0^{(5)}$ - $1^{(2)}$ - $2^{(1)}$	1 $0^{(1)}$ - $1^{(6)}$ - $2^{(1)}$
K	IM	$2^{(8)}$	0 $0^{(2)}$ - $1^{(6)}$	0 $0^{(3)}$ - $1^{(5)}$	1 $0^{(5)}$ - $1^{(3)}$	1 $0^{(2)}$ - $1^{(6)}$	1 $1^{(8)}$	2 $1^{(8)}$
	IO	$2^{(8)}$	1 $0^{(2)}$ - $1^{(6)}$	0 $0^{(6)}$ - $1^{(2)}$	0 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0.5 $0^{(3)}$ - $1^{(5)}$
	IN	$2^{(8)}$	0.5 $0^{(2)}$ - $1^{(4)}$ - $2^{(2)}$	0.5 $0^{(4)}$ - $1^{(4)}$	0.5 $0^{(4)}$ - $1^{(4)}$	0 $0^{(6)}$ - $1^{(2)}$	0.5 $0^{(4)}$ - $1^{(4)}$	1 $1^{(7)}$ - $2^{(1)}$
I	IM	$2^{(8)}$	1 $0^{(4)}$ - $1^{(2)}$ - $2^{(2)}$	0 $0^{(7)}$ - $1^{(1)}$	1 $0^{(5)}$ - $1^{(3)}$	1 $0^{(3)}$ - $1^{(5)}$	1 $0^{(3)}$ - $1^{(5)}$	1 $1^{(7)}$ - $2^{(1)}$
	IO	$2^{(8)}$	0.5 $0^{(3)}$ - $1^{(3)}$ - $2^{(2)}$	0.5 $0^{(3)}$ - $1^{(5)}$	0 $0^{(4)}$ - $1^{(4)}$	0 $0^{(3)}$ - $1^{(5)}$	0 $0^{(4)}$ - $1^{(4)}$	0.5 $0^{(3)}$ - $1^{(5)}$
	IN	$2^{(8)}$	1 $0^{(3)}$ - $1^{(3)}$ - $2^{(2)}$	0 $0^{(4)}$ - $1^{(4)}$	0 $0^{(5)}$ - $1^{(3)}$	0.5 $0^{(4)}$ - $1^{(4)}$	1 $0^{(6)}$ - $1^{(1)}$ - $2^{(1)}$	1 $0^{(1)}$ - $1^{(5)}$ - $2^{(2)}$

Explanations: Pm – parameter; Gr – group; IM – intramuscular; IO – intraosseous; IN – intranasal; G – eyelid gap; P – pedal reflex; BB – head and neck posture; K – wing withdrawal reflex; I – response to needle prick; sedation score (number of pigeons); T0 – pre-injection, baseline; T5, T10, T15, T20, T30, T40 – 5, 10, 15, 20, 30, 40 min, respectively, after T0. The total sedation scores were presented as medians at the top of the scoring, including the number of pigeons in each group.

Tab. 4. Effects of IM, IO, and IN midazolam-butorphanol (4 mg/kg and 2 mg/kg) on sedation onset time and duration

Gr	Time to sedation onset (min) p = 0.006	Duration of sedation (min) p = 0.01
IM	4.86 ± 0.9 5	34.53 ± 3.4 35
IO	4.22 ± 0.7 4	37.23 ± 3.21 39
IN	4.37 ± 0.89 4.5	30 ± 1.21 29

Explanations: Gr – group; IM – intramuscular; IO – intraosseous; IN – intranasal. Data are presented as mean ± SD and median.

biochemical evaluations, as findings obtained from young and old animals exhibit notable differences. Consequently, it has been emphasized that caution should be exercised when evaluating results from avian species of similar age ranges (28, 29). In comparable studies involving pigeons, it has been reported that the findings obtained demonstrated significant differences, particularly in liver enzymes. For example, a study comparing IM administration of ketamine, ketoprofen, ketoprofen/ketamine, and midazolam/ketamine combinations reported a significant decrease in ALP exclusively in the midazolam/ketamine group (12). In another study, midazolam(IM)/propofol(IV) and metamizole(IM)/propofol(IV) groups were compared, and a significant increase in AST and ALP values was reported in the midazolam/propofol group (25). The fact that the ALP value in the present study not only did not increase, but decreased significantly across all methods may be attributed to the effect of propofol administered intravenously (IV) in another study or may be a result of the doses used and the interaction between the drugs. The most notable finding was the significant decrease in WBC, RBC, HCT, HGB, and CREA values, observed particularly for the IM method. In light of these findings, the use of the IM method should be carefully considered when selecting the administration route. This difference in the IM method may be explained by the effect of the dose or by the first-pass effect, wherein the drug initially undergoes hepatic metabolism, resulting in decreased efficacy and indirect entry into the circulation. Conversely, the IO method is not affected by this phenomenon, whereas the IN method may be associated with direct entry of the drug into the circulation (9, 15). However, further comprehensive studies are necessary to elucidate these differences.

Several studies have compared the sedative efficacy of midazolam and butorphanol combinations administered via different routes (IM, IN, IO) in various avian species. In one study, intramuscular administration of diluted MID (4 mg/kg) and BUT (2 mg/kg) in pigeons resulted in no significant difference between groups in terms of sedation duration or depth (23). Another study comparing IM and IN administration of ketamine/midazolam in pigeons reported that the IN route provided a faster onset of sedation, while the IM route produced more prolonged effects. However, regurgitation was observed in two pigeons (6). In kakudas, intranasal administration of midazolam alone versus a midazolam/butorphanol combination revealed that the combination produced a more effective sedation (8). Similar routes have also been evaluated in quails. For example, midazolam/ketamine was administered via IM, IN, IO, and oral routes, whereas xylazine/ketamine was administered via IO and IM routes (17, 37). These studies consistently demonstrated that IO administration achieved the most rapid and profound sedation, while IN administration was less effective. In the present study, the sedation depth and efficacy of the midazolam-butorphanol combination was satisfactory in all treatment groups. This effect may be attributed to the synergistic action of butorphanol's analgesic properties (31). Notably, the IN route induced a more rapid onset of sedation compared to the IM route, which may be due to the water-soluble formulation of butorphanol resulting in faster delivery to the brain via olfactory neurons and enhanced transmucosal absorption (7). The most prominent finding of this study was that IO administration resulted in a significantly faster and deeper sedation than either IN or IM routes. This can be explained by the direct vascular access afforded by IO administration. Furthermore, no adverse effects, including regurgitation, were observed in any of the groups, which contrasts with some previous reports.

One of the most significant limitations of this study was the insufficient number of investigations on hematological and biochemical evaluations of IM, IO, and IN application methods in avian species and pigeons. A comprehensive literature search of the Web of Science and Scopus databases between 1980 and 2024 clearly demonstrates this deficiency. Consequently, it is challenging to obtain robust results to distinguish significant differences between the parameters. Given these limitations, we posit that this study will serve as foundational work and contribute to future research.

In conclusion, No complications were observed when comparing IM, IO, and IN administration of the midazolam/butorphanol combination in pigeons. While the IN method is preferred in clinical practice, its safety profile makes it particularly suitable for short-term procedures in pigeons with compromised health. For long-term procedures, the IO method may be more appropriate, although it poses a higher risk for pigeons with respiratory issues. Given its impact on blood values, the IM method should be used with caution.

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