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Article

Comparative Evaluation of Injectable, Oral Gavage, and Sedative-Laden Feed Administration of Diazepam–Xylazine for Sedation in Guinea Fowl (*Numida meleagris*)

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Abstract: This study evaluated the sedative effects of diazepam–xylazine administered via intramuscular injection, oral gavage, and sedative-laden feed in Guinea fowls (*Numida meleagris*). Eighteen clinically healthy Guinea fowls were randomly assigned to three groups (n = 6). Group A received diazepam–xylazine intramuscularly, Group B received the combination by oral gavage, and Group C received sedative-laden feed. Onset of sedation, duration of analgesia, sedation score, muscle relaxation score, heart rate, respiratory rate, and cloacal temperature were measured at predetermined intervals. Data were analyzed using one-way ANOVA, with significance set at $P < 0.05$. Route of administration significantly affected sedative efficacy. Oral gavage produced the shortest onset of sedation (1.33 ± 0.33 min) and the longest duration of analgesia (73.33 ± 3.84 min), whereas sedative-laden feed resulted in the longest onset (22.33 ± 5.36 min) and the shortest duration of analgesia (24.00 ± 3.06 min). Sedation and muscle relaxation scores were significantly higher in the oral gavage and intramuscular groups than in the feed-treated group during the early observation period ($P < 0.05$). Heart rate and respiratory rate decreased significantly after treatment in all groups, with the greatest reductions observed in birds receiving oral gavage ($P < 0.05$). Cloacal temperature decreased slightly after treatment but did not differ significantly among groups ($P > 0.05$). Diazepam–xylazine administered by oral gavage provided the most effective and consistent sedative profile in Guinea fowls, characterized by rapid onset, prolonged analgesia, superior sedation, and enhanced muscle relaxation. These findings indicate that oral gavage may be a practical alternative route for short-term chemical restraint in Guinea fowls.

Keywords: Guinea fowl; diazepam; xylazine; oral gavage; chemical restraint; sedation; avian anesthesia.

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1. Introduction

Guinea fowl (*Numida meleagris*) is one of the most important indigenous poultry species in Africa, contributing substantially to rural livelihoods, household income, food security, and genetic diversity [1]. Owing to its adaptability to harsh environmental conditions and relatively low production costs, interest in its commercial production, conservation, and research applications has increased considerably in recent years [2]. Routine veterinary procedures such as physical examination, blood collection, diagnostic imaging, wound management, and minor surgical interventions often require adequate restraint to ensure both animal welfare and personnel safety [3,4]. However, birds are highly susceptible to stress associated with capture and manual restraint. Excessive struggling may lead to hyperthermia, trauma, capture myopathy, physiological disturbances, and occasionally mortality. Consequently, procedural sedation has become an essential component of modern avian medicine because it minimizes stress, facilitates clinical procedures, and often serves as a practical alternative to general anesthesia for short-duration interventions [5].

Among the pharmacological agents used for avian sedation, benzodiazepines and α_2-adrenergic agonists remain some of the most widely employed because of their predictable sedative and muscle-relaxant effects [6]. Diazepam, a benzodiazepine, produces anxiolysis, sedation, and muscle relaxation primarily through enhancement of γ-aminobutyric acid (GABA)-mediated neurotransmission within the central nervous system. In contrast, xylazine exerts its effects through stimulation of central α_2-adrenergic receptors, producing sedation, muscle relaxation, and varying degrees of analgesia. The combination of these drug classes may provide synergistic effects, resulting in improved chemical restraint and greater patient compliance during clinical procedures [7]. Previous studies have demonstrated that benzodiazepine- and α_2-agonist-based protocols provide effective chemical restraint in several avian species, including parrots, pigeons, partridges, pheasants, and guinea fowl. For example, the addition of midazolam to a ketamine–xylazine protocol improved anesthetic quality and duration of analgesia in guinea fowl without significant adverse effects [8]. Similar findings in other avian species suggest that appropriate sedative combinations can enhance restraint quality while maintaining acceptable physiological stability [9].

Although numerous studies have evaluated sedative drugs and dose combinations in avian species, research has focused predominantly on pharmacological efficacy rather than drug delivery strategies. Intramuscular (IM) injection remains the conventional route because of its predictable absorption; however, it requires capture and restraint before sedation is achieved, potentially increasing stress responses [10]. Consequently, there is growing interest in alternative delivery methods that minimize handling while maintaining effective chemical restraint.

Recent advances in avian anesthesia and welfare emphasize refinement of restraint techniques, yet direct comparisons of injectable and non-invasive administration routes remain scarce [4]. Alternative drug delivery strategies may therefore improve animal welfare while maintaining adequate sedation [11]. Oral administration by gavage ensures accurate dose delivery while avoiding repeated injections, whereas incorporating sedative agents into feed offers an even less invasive strategy that could facilitate flock management, transportation, wildlife capture, and the handling of semi-domesticated or difficult-to-restrain birds.

Nevertheless, evidence regarding the effectiveness of sedative-laden feed remains limited, and direct comparisons between intramuscular administration, oral gavage (OG), and feed-mediated delivery are scarce. This represents an important knowledge gap in avian anesthesia and welfare-oriented restraint strategies.

Therefore, this study was designed to compare the sedative effects of a diazepam–xylazine combination administered through three different routes—IM injection, OG, and sedative-laden feed—in guinea fowl (*Numida meleagris*). Sedative indices, including onset of action, duration of analgesia, degree of sedation, degree of muscle relaxation, and selected physiological parameters, were evaluated to determine the relative effectiveness of each route of administration and to identify practical alternatives for clinical and flock management applications.

2. Materials and Methods

The study was conducted at the Department of Veterinary Surgery and Radiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.1. Ethical approval

The experimental protocol was reviewed and approved by the Animal Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (Approval No: MOUAU/CVM/REC/202620). All procedures involving animals were conducted in accordance with institutional guidelines for the care and use of animals in research.

2.2. Animals

Eighteen apparently healthy adult guinea fowls (*Numida meleagris*) of both sexes, weighing between 0.65 and 1.0 kg, were randomly allocated into three treatment groups (n = 6 per group) for the study. Birds were clinically examined and confirmed to be free of disease. They were acclimatized for two weeks under standard husbandry conditions, with free access to commercial feed and clean drinking water.

2.3. Sedative agents

- Xylazine hydrochloride injection (20 mg/mL; Xylased®, Bioveta Company, Czech Republic).
- Diazepam injection (10 mg/mL; Univall®, Swiss Pharma Nigeria Ltd., Nigeria).
- Diazepam tablets (5 mg; Univall®, Swiss Pharma Nigeria Ltd., Nigeria).

All drug doses were calculated individually according to body weight immediately before administration. Other materials used included disposable syringes, cotton wool, methylated spirit, hand gloves, digital thermometer, stethoscope, weighing balance, and permanent marker.

2.4. Preparation of Sedative-Laden Feed

Sedative-laden feed was prepared immediately before administration. Approximately 5 g of commercial feed pellets (Chikun®, Olam Agri. Nigeria) were weighed and crushed into a fine mash. Diazepam tablets were pulverized and thoroughly mixed with the calculated volume of xylazine solution needed to achieve the desired dose for each bird. The resulting mixture was homogenized and moulded into pellet-like forms using a sterile syringe plunger. The pellets were air-dried before administration and offered individually to birds assigned to the feed-treatment group.

2.5. Experimental Design

Following acclimatization, the birds were randomly allocated into three treatment groups (A, B, and C), each consisting of six birds:

- Group A (IM Administration): Birds received diazepam (2.5 mg/kg) and xylazine (1.5 mg/kg) administered intramuscularly into the thigh muscle using a sterile syringe.
- Group B (OG Administration): Birds received diazepam (5 mg/kg) and xylazine (3 mg/kg) orally via gavage. During administration, their necks were kept upright during tube insertion to completely clear the glottis. 2 mL of normal saline was consistently used to flush the tube to ensure no dry residue was left in the upper esophagus.
- Group C (Sedative-Laden Feed Administration): Birds in this group were separated into individual cages during the test to be able to control dosing. Each bird received diazepam (10 mg/kg) and xylazine (10 mg/kg) incorporated into sedative-laden feed.

The administration routes were selected to compare the effectiveness of conventional injectable sedation, controlled oral delivery through gavage, and voluntary consumption through sedative-laden feed as alternative approaches to chemical restraint in guinea fowl.

2.6. Evaluation of Sedative Indices

The birds were kept in sound-attenuated rooms because guinea fowl are highly sensitive to sudden noise, which can trigger an adrenaline surge and potentially override the sedative effects. The following were evaluated

2.6. 1. Onset of Sedation

The onset of sedation was defined as the interval between drug administration and the first observable signs of sedation, including reduced alertness, decreased responsiveness to external stimuli, postural relaxation, and associated behavioral changes. The onset time was recorded in minutes.

2.6.2. Duration of Analgesia

Analgesic duration was assessed using the toe-pinch reflex test. The time of disappearance of the withdrawal response following drug administration was recorded, as was the time of reappearance of the response. Duration of analgesia was calculated as the interval between loss and return of the toe-pinch reflex.

2.7. Evaluation of Sedation Scoring

Sedation and muscle relaxation were assessed using a previously described clinical scoring system. Birds were evaluated at predetermined intervals following drug administration by the same observer to minimize inter-observer variation (Table 1).

2.8. Physiological Measurements

Heart rate, respiratory rate, and cloacal temperature were recorded before drug administration (baseline) and at predetermined intervals after sedation, using a standardized procedure.

2.9. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).Differences among treatment groups were analyzed using two-way repeated-measures ANOVA, followed by Fisher's Least Significant Difference (LSD) test where appropriate. Statistical significance was accepted at $P < 0.05$.

Table 1. Clinical scoring system of level of sedation and muscle relaxation

Score	Sedation level	Muscle relaxation level
0	Standing and alert	Jaw tightly closed (full muscle tone)
1	Drooping of wings	Moderate resistance to jaw opening
2	Standing with reduced alertness	Mild resistance to jaw opening
3	Sternal recumbency	No resistance to jaw opening
4	Lateral recumbency with apparent sleep	Complete flaccid paralysis

3. Results

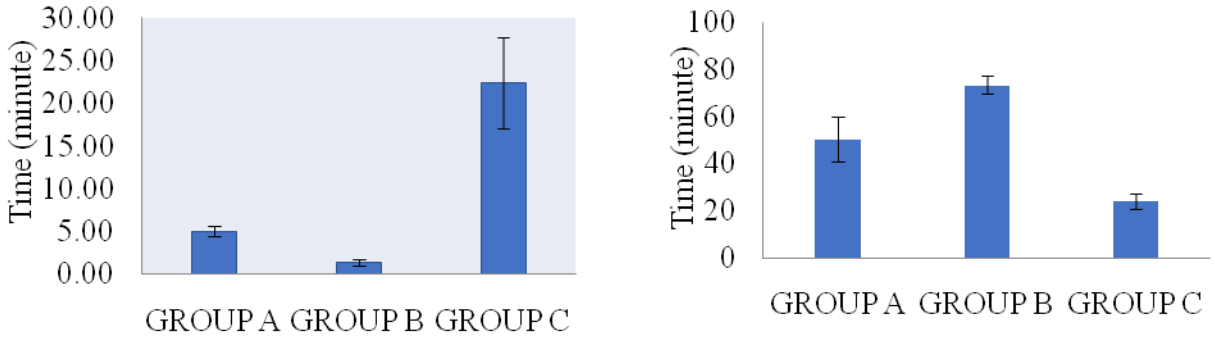
3.1. Indices of sedation

3.1.1. Onset of sedation

The route of administration did not significantly influence the onset of sedation ($P > 0.05$), although numerical differences were observed among treatment groups. However, the duration of analgesia differed significantly ($P < 0.05$), with OG producing the longest analgesic effect. Birds that received diazepam–xylazine by OG(Group B) exhibited the shortest onset of action (1.33 ± 0.33 min), whereas birds receiving sedative-laden feed (Group C) showed the longest onset time (22.33 ± 5.36 min). IM administration (Group A) produced an intermediate onset time of 5.00 ± 0.58 min. Although numerical differences were observed, no significant difference was detected among groups for onset of action ($P > 0.05$; Figure 1a).

3.1.2. Analgesic duration

Analgesic duration differed significantly among treatment groups ($P < 0.05$). OG resulted in the longest duration of analgesia (73.33 ± 3.84 min), followed by IM administration (50.33 ± 0.58 min), while sedative-laden feed produced the shortest duration (24.00 ± 3.06 min) (Figure 1b).



(**a**) (**b**)

Figure 1. Mean (±SEM) onset of sedation (min) (A) and analgesic duration (B) in Guinea fowls administered diazepam-xylazine through intramuscular injection (GROUP A), oral gavage (GROUP B) and sedative-laden feed (GROUP C).

3.2.Duration of analgesia

3.2.1. Degree of sedation

Sedation scores increased significantly following administration of diazepam–xylazine in all groups. At 10–30 min, Groups A and B maintained significantly higher sedation scores than Group C ($P < 0.05$). Maximum sedation scores of 4.00 ± 0.00 were observed in Groups A and B between 20 and 30 min, whereas Group C attained lower scores ranging from 3.00 ± 0.00 to 3.33 ± 0.33 during the same period. Sedation gradually declined after 40 min in all groups, approaching baseline values by 60 min (Figure 2a).

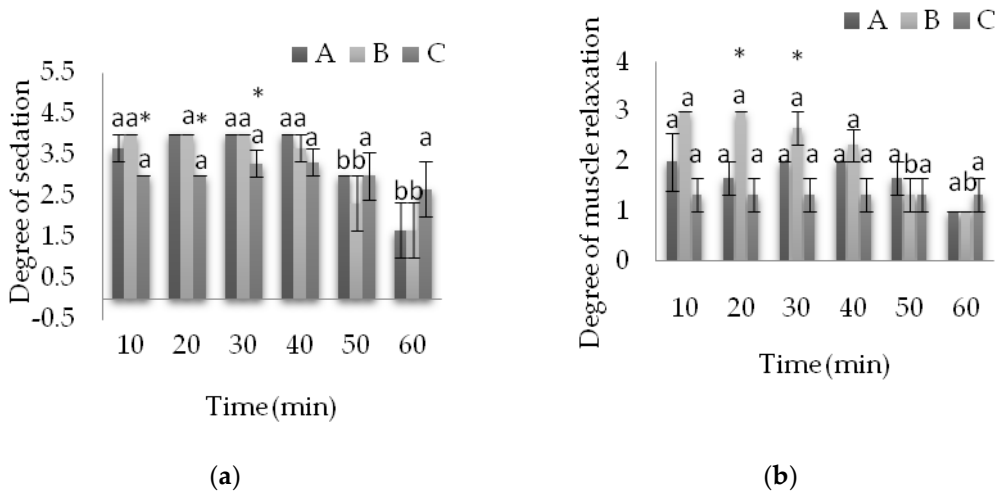


Figure 2. Mean (±SEM) Degree of sedation (a) and muscle relaxation (b) in Guinea fowls administered diazepam-xylazine via intramuscular injection (GROUP A), oral gavage (GROUP B) and sedative-laden feed (GROUP C). Different lowercase letters (a-b) indicate significant difference ($P < 0.05$) within the same group across time. An asterisk (*) indicate a significant difference ($P < 0.05$) (GROUP A), oral gavage (GROUP B) and sedative-laden feed (GROUP C) at the same time point

3.2.2. Degree of muscle relaxation

Muscle relaxation followed a similar trend. Group B exhibited the highest muscle relaxation scores, reaching 3.00 ± 0.00 at 20 min and remaining elevated at 30 min (2.67 ± 0.33). Group A showed moderate muscle relaxation with peak values of 2.00 ± 0.00 at 30–40 min, whereas Group C demonstrated minimal muscle relaxation throughout the observation period, with scores remaining between 1.00 ± 0.33 and 1.33 ± 0.33. Significant differences among groups were observed at selected time points ($P< 0.05$) (Figure 2b).

3.3. Physiological parameters

3.3.1. Heart rate

Heart rate decreased following administration of diazepam–xylazine in all treatment groups. Group B showed the greatest reduction, declining from a baseline value of 292.00 ± 18.90 beats/min to 174.67 ± 14.67 beats/min at 40 min. Group A decreased from 309.33 ± 10.73 beats/min at baseline to 236.67 ± 21.86 beats/min at 10 min, while Group C exhibited a less pronounced decline, decreasing from 299.00 ± 4.93 beats/min to 238.33 ± 19.70 beats/min at 40 min. Significant differences among treatment groups were observed at most observation periods following drug administration ($P< 0.05$). Heart rate progressively returned toward baseline values by 60 min (Table 2).

3.3.2. Respiratory rate

Respiratory rate decreased significantly following treatment in all groups ($P<0.05$). Group B showed the greatest reduction, declining from 43.67 ± 4.81 cycles/min at baseline to 13.33 ± 2.67 cycles/min at 50 min. Group A decreased from 23.33 ± 2.67 cycles/min to 12.00 ± 3.00 cycles/min, while Group C showed a comparatively smaller reduction, declining from 30.00 ± 4.73 cycles/min to 20.00 ± 5.00 cycles/min at 40 min. Respiratory rates gradually increased during the recovery period but remained below baseline values at 60 min (Table 2).

3.3.3 Cloacal temperature

Cloacal temperature decreased slightly following administration of diazepam–xylazine in all groups. The greatest reduction was observed in Group B, where temperature decreased from 42.17 ± 0.30°Cat baseline to 40.43 ± 0.33°C at 60 min. Group A showed a decline from 42.37 ± 0.23°C to 40.73 ± 0.64°C, whereas Group C exhibited minimal changes throughout the observation periods, remaining above 41°C. However, no significant differences among treatment groups were detected at any observation time ($P> 0.05$) (Table 2).

Table 2. Mean ± SEM physiological parameters of the sedative effect of diazepam-xylazine administered intramuscularly (A), by oral gavage (B), and through sedative-laden feed(C) in Guinea fowl (*Numida meleagris*)

Mean values with different superscript letters within the same column for the same parameter differ significantly at $P< 0.05$. Means with different numerals within the same row differ significantly ($P< 0.05$)

Physiological parameters	Time (minutes)	0	10	20	30	40	50	60
Heart rate (beats/minutes)	A	309.33±10.73^{c1}	236.67±21.86^{a2}	243.33±12.02^{b3}	271.67±26.19^{c4}	243.33±20.282^{c3}	274.00±13.122^{c4}	274.33±23.14^{b4}
	B	292.00±18.90^{a1}	236.00±18.90^{a5}	210.67±23.70^{a4}	186.67±24.69^{a3}	174.67±14.67 ^{a2}	242.00±21.07^{a6}	260.67±10.48^{a7}
	C	299.00±4.93^{b1}	274.33±7.22^{b5}	245.00±13.23^{b3}	248.67±30.69^{b3}	238.33±19.70^{b2}	264.67±13.92^{b4}	265.00±13.23^{a4}
Respiratory rate (cycle/minutes)	A	23.33±2.67^{a1}	15.33±2.60^{a2}	13.33±1.45^{a2}	12.00±3.06^{a2}	12.00±3.61^{a2}	12.00±3.00^{a2}	14.67±3.84^{a2}
	B	43.67±4.81^{c1}	18.00±3.06^{a2}	15.33±2.91^{a2}	15.00±3.26^{a2}	14.67±3.53^{a2}	13.33±2.67^{a2}	16.67±1.76^{a2}
	C	30.00±4.73^{b1}	24.67±6.17^{b2}	23.00±5.51^{b2}	20.33±6.01^{b2}	20.00±5.00^{b2}	21.67±4.91^{b2}	21.00±5.29^{b2}
Cloacal temperature (ºC)	A	42.37±0.23^{a1}	41.77±0.35^{a1}	41.73±0.41^{a1}	41.20±0.49^{a1}	41.07±0.38^{a1}	41.23±0.62^{a1}	40.73±0.64^{a1}
	B	42.17±0.30^{a1}	40.27±1.63^{a1}	41.07±0.38^{a1}	40.87±0.15^{a1}	40.70±0.31^{a1}	40.57±0.47^{a1}	40.43±0.33^{a1}
	C	41.77±0.23^{a1}	41.43±0.30^{a1}	41.57±0.29^{a1}	41.40±0.45^{a1}	41.40±0.40^{a1}	41.47±0.44^{a1}	41.57±0.44^{a1}

4. Discussion

The present study demonstrated that the route of administration significantly influenced the clinical response to diazepam–xylazine sedation in Guinea fowls. Although all treatment protocols produced observable sedation, OG was associated with the most favorable overall sedative profile, characterized by the shortest onset of action, the longest duration of analgesia, higher sedation scores, greater muscle relaxation, and more pronounced reductions in heart and respiratory rates. These findings suggest that the method of drug delivery plays an important role in determining the efficacy and predictability of chemical restraint in avian species.

Although the onset of sedation did not differ significantly among treatment groups ($P > 0.05$), OG had the numerically shortest mean onset time. This trend may reflect more rapid gastrointestinal absorption of the diazepam–xylazine combination under the experimental conditions, although further studies with larger sample sizes are needed to determine whether this numerical difference is biologically meaningful. In contrast, birds receiving sedative-laden feed showed a markedly delayed onset of sedation, likely due to variations in feed consumption and gastrointestinal absorption. Similar observations have been reported in avian and poultry medicine, where oral administration under controlled conditions produced more consistent pharmacological responses than voluntary drug intake through feed [12, 11]. Although IM administration is widely regarded as a reliable route for rapid systemic drug absorption, the present findings suggest that OG resulted in a numerically earlier onset of sedation. However, because this difference was not statistically significant, the observation should be interpreted with caution.

A major finding of this study was a significantly longer analgesic duration in the OG group compared with the intramuscular and feed-treated groups. Xylazine produces analgesia by stimulating central α_2-adrenergic receptors, thereby inhibiting nociceptive transmission and reducing sympathetic activity, while diazepam contributes anxiolytic and muscle-relaxant effects [7]. The prolonged analgesia observed following OG may therefore reflect more effective systemic drug availability and sustained pharmacological activity [11]. In clinical practice, prolonged analgesia is desirable because it provides a wider time window for examination and minor procedures without the need for repeated drug administration.

Sedation and muscle relaxation scores followed a similar pattern, with OG and IM administration producing significantly greater effects than sedative-laden feed during the early observation period. These findings are consistent with the known pharmacological actions of diazepam and xylazine. Diazepam enhances γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission within the central nervous system, resulting in anxiolysis and skeletal muscle relaxation, whereas xylazine produces sedation through central α_2-adrenoceptor activation. The greater muscle relaxation observed in the OG group is particularly relevant because adequate muscle relaxation facilitates handling, positioning, and clinical procedures while minimizing stress and the risk of injury [12]. The progressive decline in sedation scores after 40 min indicates gradual recovery from the drug's effects and suggests that the protocol provides temporary, reversible chemical restraint suitable for short clinical procedures.

The reductions in heart rate observed in all treatment groups are consistent with the well-documented cardiovascular effects of α_2-adrenergic agonists. Xylazine decreases sympathetic outflow and increases vagal tone, resulting in bradycardia and reduced cardiac output [13]. The greater decline observed in the oral gavage group may reflect both deeper sedation and reduced stress-associated sympathetic stimulation. Similar decreases in heart rate following xylazine-containing protocols have been reported in domestic birds and other veterinary species [14]. Importantly, heart rates gradually increased toward baseline values during recovery, indicating that the cardiovascular effects were transient and reversible.

Respiratory rate also decreased significantly following administration of diazepam–xylazine, with the most pronounced reduction occurring in birds receiving OG [15]. Sedative-induced reductions in respiratory frequency have been widely reported and are generally attributed to central nervous system depression, decreased muscular activity, and reduced metabolic demand [15, 9]. Although respiratory depression is a recognized effect of α_2-adrenergic agonists, the gradual return toward baseline values observed in the present study suggests that respiratory function remained adequately maintained throughout the observation period. Nevertheless, respiratory monitoring remains essential during avian sedation because birds possess a unique respiratory system that may be particularly sensitive to depressant drugs.

Cloacal temperature decreased slightly in all groups, although no significant differences were observed among treatments. Mild reductions in body temperature during sedation are expected because decreased muscular activity and metabolic heat production impair normal thermoregulatory processes. Similar findings have been reported in avian anesthesia studies, where sedative administration frequently re-

sults in transient decreases in body temperature [15]. Although the reductions observed in the present study were relatively small, maintenance of appropriate environmental temperature remains important during sedation to minimize the risk of hypothermia. No mortality or clinically significant morbidity was observed in any treatment group throughout the study. All guinea fowls recovered uneventfully following sedation without apparent adverse effects.

5. Conclusions

Overall, the present findings demonstrate that the route of administration influences the quality and duration of diazepam–xylazine-induced sedation in guinea fowl. While OG produced a numerically shorter onset of sedation, it significantly prolonged analgesia and provided satisfactory muscle relaxation, highlighting its potential as a practical alternative to intramuscular administration when repeated handling or injections are undesirable. Nevertheless, the absence of statistically significant differences in onset of sedation indicates that further investigations using larger sample sizes are warranted to confirm these observations and optimize sedation protocols for guinea fowl and other avian species. Although birds receiving sedative-laden feed were individually housed during feeding to minimize competition and facilitate observation, complete ingestion of the administered medicated feed could not be objectively verified. Consequently, slight variations in the amount of drug consumed may have contributed to variability in the sedative response.

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Institutional Review Board Statement: The study protocol was approved by the Animal Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (Approval No MOUAU/CVM/REC/202620).

Data Availability Statement: Research data is made available by authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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