UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF KETAMINE XYLAZINE ANAESTHESIA IN MALAYSIAN VILLAGE CHICKEN (GALLUS GALLUS DOMESTICUS) CHICKS

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THE EFFECTS OF KETAMINE XYLAZINE ANAESTHESIA IN MALAYSIAN VILLAGE CHICKEN (*GALLUS GALLUS DOMESTICUS*) CHICKS

BY

SALINA ESTHER SAMUEL

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It is hereby certified that we have read this project paper entitled "The Effects of Ketamine Xylazine Anaesthesia in Malaysian Village Chicken (*Gallus gallus domesticus*) chicks" by Salina Esther Samuel and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement of the course VPD 4999-Final Year Project.

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DEDICATION

I DEDICATE THIS DISSERTATION WORK TO MY MOM, DAD AND MY BELOVED FAMILY AS WELL AS MY ESTEEMED LECTURERS, ADVISORS, MY BUDDY, AND DEAR FRIENDS FOR GIVING ME THEIR UNWAVERING SUPPORT AND ENCOURAGEMENT THROUGHOUT THIS JOURNEY.

I AM DEEPLY GRATEFUL TO HAVE ALL THESE PEOPLE IN MY LIFE

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Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF ABBREVIATIONS/ SYMBOLS

KX	Ketamine-Xylazine
MVC	Malaysian Village Chicken
i.e.	That is
wk	Week
n	Number of animals
mg/kg	Milligram per kilogram
ml	Millilitre
min	Minutes
°C	Degree celsius
RR	Respiratory Rate
HR	Heart Rate
SD	Standard Deviation
ANOVA	Analysis of Variance
IM	Intramuscular
>	More than
<	Less than
%	Percentage

ABSTRACT

An abstract of the project presented to the Faculty of Veterinary Medicine in partial fulfillment of the requirement of the course.

The Effects of Ketamine-Xylazine Anaesthesia in Malaysian Village Chicken (*Gallus gallus domesticus*) Chicks

By

SALINA ESTHER SAMUEL

December 2023

Supervisor: **Dr. Awang Hazmi Awang Junaidi**

Co-supervisor: **Dr. Mazlina Mazlan & Prof. Dr. Jalila Abu**

General anaesthesia is essential for birds before invasive surgeries to prevent pain and remain immobile during the procedure. Although inhalation anaesthesia is the preferred choice, when it's not possible, injectable anaesthesia, like Ketamine-Xylazine (KX), is used for its practicality, safety and effectiveness. To date, no prior research has explored the effect of anaesthesia in Malaysian Village Chicken (*Gallus gallus domesticus*); MVC. This study was conducted with the objective of investigating the effects of different dosages of KX on the stages of anaesthesia and physiologic parameters in MVC. Fifteen MVC chicks were randomly selected from Orilui Neutral Farm, Hulu Langat, Selangor. The chicks were divided into three different age groups: < 1 week old, 2 weeks old, and 4 weeks old (n=5/ age group). In each group, three different doses of Ketamine; 10 mg/kg, 20 mg/kg, and 40 mg/kg, with a combination of constant dosage of Xylazine; 2 mg/kg, were

examined. The KX was administered intramuscularly into the pectoralis muscle. The anaesthesia stages; induction time, duration of anaesthesia, recovery time, physiological parameters; respiratory rate, heart rate, and cloacal temperature, of MVC chicks were monitored closely and recorded. Overall, the dosage of KX has significant effects ($P<0.05$) on the anaesthesia stages. Increasing the dosage of ketamine results in a longer induction period, anaesthesia period, and recovery time in the MVC chicks, regardless of their age. Meanwhile, the age of chicks has significant effects ($P<0.05$) on all the physiological parameters during the induction. In conclusion, KX at the dosage of 10-40/2 mg/kg is safe as anaesthesia for performing surgical procedures in MVC chicks.

Keywords*: anaesthesia, ketamine, xylazine, Malaysian Village Chicken, chicks*

ABSTRAK

Kesan Anestesia Ketamin-Xylazine Terhadap Anak Ayam Kampung

(*Gallus gallus domesticus*)

Oleh

SALINA ESTHER SAMUEL

Disember 2023

Supervisor: **Dr. Awang Hazmi Awang Junaidi**

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Anestesia am adalah penting untuk burung sebelum menjalani pembedahan invasif bagi mengelakkan kesakitan dan memastikan pesakit tidak bergerak sepanjang prosedur. Walaupun anestesia inhalasi adalah pilihan utama, jika tidak mengizinkan, anestesia suntikan seperti Ketamin-Xylazine (KX) akan digunakan untuk praktikaliti, keselamatan dan keberkesanannya. Sehingga kini, tiada kajian terdahulu yang mengkaji kesan anestesia terhadap anak ayam kampung (*Gallus gallus domesticus*). Kajian ini dijalankan dengan objektif untuk menyiasat kesan dos KX yang berbeza terhadap peringkat anestesia dan parameter fisiologi dalam anak ayam kampung. Lima belas anak ayam kampung dipilih secara rawak dari Orilui Neutral Farm, Hulu Langat, Selangor. Anak ayam kampung dibahagikan kepada tiga kumpulan umur yang berbeza; < 1 minggu, 2 minggu dan > 4 minggu (n=5/ setiap kumpulan umur). Dalam setiap kumpulan, tiga dos Ketamin yang berbeza; 10 mg/kg, 20 mg/kg, dan 40 mg/kg dengan kombinasi dos tetap Xylazine; 2 mg/kg telah diperiksa. KX disuntik secara intramuskular ke dalam otot pektoral. Peringkat anestesia; masa induksi, tempoh anestesia, dan masa pemulihan, dan parameter fisiologi;

kadar pernafasan, kadar degupan jantung, dan suhu kloaka anak ayam kampung dipantau dengan teliti dan direkodkan. Secara keseluruhannya, dos KX memberikan kesan yang signifikan ($P<0.05$) ke atas peringkat anestesia. Peningkatan dos ketamin menghasilkan jangka masa induksi, tempoh anestesia, dan masa pemulihan yang lebih lama pada anak ayam kampung tanpa mengira umur. Sementara itu, umur anak ayam kampung memberikan kesan yang signifikan ($P<0.05$) ke atas semua parameter fisiologi semasa induksi. Kesimpulannya, KX pada dos 10-40/2 mg/kg adalah selamat sebagai anestesia bagi prosedur pembedahan untuk anak ayam kampung.

***Kata kunci**: anestesia, ketamin, xylazin, Ayam Kampung, anak ayam*

1.0 INTRODUCTION

Powerful or convenient general anaesthesia is required prior to all minor and major surgeries in birds (Makky et al., 2017 & Nazhvani et al., 2019). General anaesthesia in birds can be achieved by giving either injectable or inhalant anaesthetic agents, which both possess positive and negative effects (Hall et al., 2001). Birds require anaesthesia during procedures involving substantial restraint, including surgery, wound care, physical therapy, bandage changes, and at times the initial examination may impose anaesthesia to reduce stress in birds (McMunn, 2022).

Avian species are very sensitive animals and any mismanagement in the dosage of an anaesthetic drug can lead to shock and death (Kaya et al., 2013). Hence, the selection of the safest anaesthetic agent is crucial. Injectable anaesthesia is relatively cheap, has fast action, and requires less equipment (Mandelker,1988). Inhalant anaesthethic agents, on the other hand, have the benefit of rapid changes in the depth of anaesthesia, but they need more care and close observation in birds than other species due to the anatomical and physiological differences (Hall et al., 2001).

Most avian patients encountered in a clinical environment are prey species, making them particularly susceptible to stress when subjected to handling (Lierz & Korbel, 2012). Sample collections, wild bird rehabilitation, and pain management in the field require effective anaesthesia to ease the procedures (McMunn, 2022). Thus, the use of injectable anaesthesia in a field setting is preferred when inhalant anaesthesia is not readily available and practical to be used in the field (Sontakke et al., 2007). The minimal requirement for technical equipment, rapid induction, ease of administration, and cost-effectiveness makes

injectable anaesthesia to be the first choice in field conditions.

Researchers have been recommending Ketamine, a dissociative anaesthetic, as a suitable drug for anaesthesia of different bird species (Hall et al., 2001). Generally, ketamine is less likely to be used as a single agent, due to its poor ability in muscle relaxation, inducing muscle tremors, opisthotonos, myotonic contractions, and rough recoveries (Gunkel & Lafortune, 2005). Therefore, ketamine is always used in combination with alpha-2 adrenergic agonists or benzodiazepines, such as xylazine or diazepam, to provide the best anaesthesia throughout the procedures with minimal effects, improve the relaxation of muscles, and maintain the depth of anaesthesia (Paul-Murphy & Fialkowski, 2001; Durrani et al., 2008).

Malaysian Village Chicken (MVC), locally known as 'Ayam Kampung' in Malaysia, is known to inherit the genetics of red jungle fowls, wild birds, and domesticated chickens (Lokman et al., 2015). Owing to this characteristic, MVC can be a potential model for studying anaesthesia and surgical protocol in wild bird species (McGrew, 2022). To date, no study has been conducted on the effects of anaesthesia using the MVC model.

In this study, the effects of ketamine-xylazine (KX) anaesthesia were studied in MVC chicks of different age groups (*i.e.*, <1 wk, 2 wk, and 4 wk). The anaesthesia stages (*i.e.*, induction time, anaesthesia, and recovery period) and the physiological parameters (*i.e.*, heart rate, respiratory rate, and cloacal temperature) after the induction of KX (*i.e.*, 10:2 mg/kg, 20:2 mg/kg and 40:2 mg/kg) were evaluated. This preliminary study provides useful data to improve our understanding of the appropriate and safe dosages of anaesthesia for MVC or *Gallus gallus domesticus* chicks.

1.1 Objectives

The general objective of this study is to evaluate the effects of KX anaesthesia in MVC (*Gallus gallus domesticus*) chicks. The specific objectives are:

1. To evaluate the effects of KX dosage on the anaesthesia stages and physiological parameters of the MVC (*Gallus gallus domesticus)* chicks.
2. To assess the effects of age on the KX anesthesia in MVC (*Gallus gallus domesticus*) chicks.

2.0 LITERATURE REVIEW

2.1 Anaesthesia in Avian Species

General anaesthesia involves inducing and sustaining a state of unconsciousness with the absence of deep pain sensation (Hyunchul, 2008). The goal of anaesthesia is mainly for immobilization, safe examination, and treatment (Forbes, 1998). In avian medicine, anaesthesia is widely employed for diagnostics and surgical procedures (Noha, 2001). The period of anaesthesia can vary according to the procedures to be performed (Raftery, 2013). Besides, birds also possess distinctive anatomical and physiological traits that influence the whole anaesthesia process (Wyles et al., 1983). Hence, anaesthethic protocol is a very crucial and challenging aspect of avian medicine and surgery. To decide on which anaesthetic protocols that are particularly appropriate, it is imperative to have the knowledge and comprehension of the avian anatomy, physiology specifically on the cardiorespiratory system and its characteristics as well as their response towards the anaesthesia (Dzialowski & Crossley, 2015). When the birds are in a stressful condition, they can have a more pronounced impact during anaesthesia by releasing endogenous catecholamines (Perry & Capaldo, 2011). Hypoxia, severe hypercapnia, and anaesthetic drugs can further depress the avian cardiovascular system (Chan et al., 2013). Anaesthesia could be given in different routes in avian, it can be either inhalant or injectable anaesthesia (Dutton et al., 2010). However, the most preferred approach for both induction and maintenance of anaesthesia is through the use of inhalant anesthesia (Ludders, 2001).

2.2 Inhalant Anaesthesia

The utilization of inhaled anaesthethics offers several advantages during induction and recovery stages and can make rapid and frequent adjustments in the anaesthesia depth, minimal cardiorespiratory side effects or organ toxicity at clinically relevant doses (Lewis, 2004). All these characteristics render most inhaled anaesthethics well suited for anaesthesia of birds with compromised liver or kidney function (Elliott et al., 2014). Some of the examples of inhalation anaesthethics are isoflurane, halothane methoxyflurane, oxygen, and nitrous oxide (Leslie, 1994). Mask induction is commonly used followed by the intubation of the bird and the endotracheal tube is securely attached to the lower beak (Mcdonald, 2013). Isoflurane is administered at 5% for induction, while for maintenance is usually maintained at 2.5% (Elliott, 2004). Chamber induction is also a viable approach. It is proven that isoflurane anaesthesia is the best choice of anaesthethics in all situations due to its high therapeutic index, minimal physiological alterations and effective restraint for various procedures (Leslie, 1994). However, a recent comparative study conducted between isoflurane and sevoflurane in avian patients proved that sevoflurane is the best anaesthesia among all anaesthethic protocols due to 100% of birds showed excellent and safe recovery (Anjana et al., 2021).

2.3 Injectable Anaesthesia

Injectable anaesthethics are more practical to be used in field conditions where inhaled anaesthesia is not available (Samour et al., 1984). Injectable anesthetic agents come with

several inherent drawbacks with the most prominent ones including substantial variations in response between species, risk of cardiopulmonary depression, prolonged and rough recovery periods as well as the challenge when administering a safe and efficacious dose (Lierz & Korbel, 2012). On the other hand, the advantages of injectable anaesthesia are very limited which is primarily linked to cost and easy administration (Murphy & Fialkowski, 2001). Nevertheless, disadvantages prevail over the advantages. Some of the injectable anaesthethics that have been used in avian species are ketamine, diazepam, medetomidine, and alfaxalone (Kaya et al., 2019). There are several anaesthesia studies using ketamine in avian species. Most of the research focused on the effect of the dosage and the combination of the drugs. Ketamine alone or in combination has been studied in several avian species such as pigeons (Durrani et al., 2009), parrots (Paula et al., 2013), ring-necked pheasants (Kaya et al., 2019), domestic fowls (Elowni et al., 2020), domestic chicken chicks (Amjed & Ahmed, 2021) and ducks (Nordheim et al., 2023). A combination of ketamine and diazepam was used in parrots (*Amazona aestiva*) showed that ketamine alone or in combination with diazepam promotes a good quality of sedation (Paula et al., 2013). Moreover, a study that used a combination of Alfaxalone with ketamine and Alfaxalone with xylazine in a group of *Gallus gallus domesticus* chicks proved that Alfaxalone was effective and safe anaesthethic for a short duration of surgical procedures in chicks (Amjed & Ahmed, 2021). Besides, Alpha 2 – adrenergic agonists including xylazine and medetomidine have sedative and analgesic properties (Giovannitti et al., 2015). The effect can be significant on the cardiopulmonary system of the patient which includes bradyarrhythmia, second-degree heart block and has possibility of increased susceptibility to catecholamine induced cardiac arrhythmias

(Lumeij & Ritchie, 1994). In a recent study conducted, Pekin ducks (*Anas domesticus*) were given xylazine and a combination of xylazine and ketamine that exhibited hypoxemia and hypercapnia (Ludders et al., 2023). When used in conjunction with ketamine, xylazine's sedation and analgesic effects are intensified (Degernes et al., 2020).

Ketamine is a phencyclidine derivative with analgesic and anesthetic properties that are effective in both mammalian and avian species (Elowni et al., 2020). It is commonly recommended as an appropriate injectable general anaesthesia for broad avian species (Flecknell, 2009), and suitable for chemical restraint during minor surgical and diagnostic procedures (Ludders and Matthews, 2007). A study in pigeons (*Columba livia*) using KX combinations with the dosage of 30: 8 mg/kg showed that combinations of both drugs are suitable for painful procedures (Durrani et al., 2009). Despite the extensive use and dependability, research concerning its effectiveness on small or young birds is lacking (Ludders et al.,2007).

2.4 Avian Respiratory System

Disparities in avian and mammalian respiratory systems can significantly influence the effectiveness of anaesthesia (Miller & Buitrick, 1999). Generally, the avian respiratory system consists of two main parts, ventilation, and gas exchange (Ludders, 1998). The differences between avian and mammalian respiratory systems are quite significant (Brown et al., 1997). In contrast to mammals, birds have a highly efficient gas exchange system (Powell, 2015). Anaesthesia negatively impacts the avian lung's capacity for

efficient gas exchange and the duration for which birds can sustain apnea is influenced by their restricted functional residual lung volume (Seamon et al., 2017). Birds have air sacs that store and pump air through stationary lungs, unlike in mammals, air flows in only one direction through bird lungs (Currumbin, 2015). With the help of the air sacs, birds can take in oxygen even during exhalation (Codd, 2010). Under the influence of inhalant anaesthesia, birds exhibit more efficient gas exchange approximately ten times more than mammals (O'Malley, 2008). Consequently, there is an increased risk of gaseous anaesthethic overdose in birds (Yayla et al., 2018). The caudal air sacs function as a reservoir, holding inspired air with a high concentration of anaesthethic gas even after the induction concentration has been decreased at the vaporiser and the depth of anaesthesia will increase as a result of the retained volume of concentrated anaesthethic gas in the air sacs (Forbes, 1998).

2.5 Avian Cardiovascular System

The avian's heart consists of four fully separated chambers and is situated midway within the thoracic cavity (Lumeij & Ritchie, 1994). The avian cardiovascular system has several notable distinctions which include a proportionally larger heart, greater stroke volumes, high cardiac output as well as increased blood pressure and a lower heart rate compared to mammals (Gunkel & Lafortune, 2005). These elements contribute to an improved circulatory and oxygen transport system essential for sustaining flight (Lumeij & Ritchie, 1994). However, anaesthesia can have a significant effect on this system where it causing decreased heart rate in birds under the influence of anaesthethic drugs (Lumeij & Deenik, 2003). This is mainly due to the effects of anaesthethic drugs on the

heart's electrical activity (Celik et al., 2020). The extent of the decrease in heart rate highly depends on the dosage and type of anaesthesia used. Inhalant anaesthesia tends to have a less pronounced effect on heart rate of avian species compared to injectable ananesthesia (Jaensch et al., 1999).

2.6 Ketamine- Xylazine Pharmacokinetics

Ketamine is a widely used drug in both human and veterinary medicine to induce anaesthesia during invasive procedures (Kohtala, 2021). It achieves its hypnotic and analgesic effects by inhibiting the central nervous system (Kurdi et al., 2014). This is primarily accomplished through its antagonistic impact on the N-methyl D-aspartate (NMDA) receptor, which is a type of glutamate receptor (Allweiler, 2023). Glutamate serves as the principal excitatory neurotransmitter in the brain, and the NMDA receptor plays an important role in various physiological functions such as pain transmission, learning and memory (Pal, 2021). Ketamine is believed to inhibit the NMDA receptor, thereby impeding glutamate from signaling at this receptor (Jewett & Thapa, 2022). This inhibition can induce a state of dissociation, wherein the bird becomes immobilized yet remains conscious (Struck et al., 2011). The overall outcome is the induction of a state of deep sleep and alleviation of pain. The impact of ketamine varies among different avian species (Miller & Buitrick, 1999). In mammals, ketamine is metabolized by the liver and excreted by the kidneys (Lemieux et al., 2013), however no studies have been conducted to look into the metabolism of the drug in birds (Pan & Fouts, 1979). On the other hand, xylazine, an alpha 2 adrenergic agonist activates central presynaptic adrenoceptors, this activation hinders the release of norepinephrine at the nerve terminal,

resulting in the induction of sedation and analgesia (Degernes et al., 2020). In most of the avian species, xylazine does not induce sufficient immobilization for surgical procedures (Uzun et al., 2006). Xylazine's side effects include the occurrence of bradycardia and respiratory depression hence, close monitoring is necessary when inducing anaesthesia with xylazine (Rehman et al., 2020). It is not advisable to be used as a sole ananesthethic agent (Lumeij & Deenik, 2003). A combination of both xylazine and ketamine proved to be effective in both mammals and in avian species (Degernes et al., 2020). The synergistic action of xylazine and ketamine leads to smooth induction and recovery, enhancing muscle relaxation and analgesia (Horr et al., 2023).

2.7 Malaysian Village Chicken

Malaysian Village Chicken more commonly known as Ayam Kampung can be characterized as a hybrid that has become evident through the spontaneous crossbreeding between the Malay fowl, jungle fowl, and different exotic breeds introduced during the periods of European colonization (Jajere et al., 2019). The red jungle fowl serve as the progenitor of domestic chickens, possess a diverse and intricate genetic profile that holds significant importance for conservation efforts (Wilson, 2021). With an estimated genome size of 1.2 billion base pairs, it aligns closely with the genomic dimensions of other avian species (Hata et al., 2021). The species features 78 chromosomes (Sharma et al., 2023). Genetic analysis uses microsatellites for parentage and population studies, single nucleotide polymorphisms for identifying genes associated with specific traits and mitochondrial DNA for tracing maternal lineages and evolutionary history (Phuc & Berres, 2018). Red jungle fowl exhibits notable genetic diversity, evident within and

between populations spread across distinct geographical regions (Lawal et al., 2018). In comparison to domestic chickens, they share approximately 71-79% of their DNA, reflecting their ancestral influence (Lawal et al., 2020). Village chickens are mostly raised in the backyard and primarily serve as supplementary income and protein sources for local households (Padhi, 2016). MVC are commonly raised for their meat and eggs. The MVC's are hardy, disease-resistant and calm animals that are easily available and most importantly affordable (Jajere et al., 2019). In addition, their anatomy and physiology are similar to the Red Jungle Fowls (Lokman et al., 2015), thus making them an ideal model for studying surgical techniques and anesthesia protocols that can be used in the field. The Red jungle fowls are known to be wild ancestors of domestic chicken and a threatened species (Board, 2022). Thus, it has also been identified as a potential model for anesthesia and surgery in wildlife owing to the research conducted (McGrew, 2022).

3.0 MATERIALS AND METHODS

3.1 Animals

A total of fifteen healthy MVC chicks were used in this study. The chicks were obtained from Orilui Neutral Farm, Hulu Langat, Selangor. The chicks were randomly selected regardless of their sex and transported to the Animal Research Facility (ARF), Faculty of Veterinary Medicine, Universiti Putra Malaysia. The chicks were acclimatized for 48 hours, kept in cages at a temperature of 32-35°C, with continuous lighting. The chicks had access to drinking water *ad libitum* and fasted for 8 hours prior to the experiment. All procedures involving animals were reviewed and approved by the Universiti Putra Malaysia's International Animal Care and Use Committee (IACUC): UPM/IACUC/AUP-U 009/2023.

3.2 Experimental Design

The chicks were divided into three different age groups; <1 week old, 2 weeks old and 4 weeks old (*n*=5/age group). The effects of three doses of ketamine hydrochloride (Narketan 10; 100 mg/ml, Vetoquinol UK Limited, Buckingham, UK); 10 mg/kg, 20 mg/kg or 40 mg/kg and xylazine hydrochloride (Ilium Xylazil-100; 100 mg/ml, Troy Laboratories Pty Limited, Glendenning, Australia); 2 mg/kg combination (KX) will be examined on each age group (Table 3.1). A 24-hour washout period was given between different dosage trials.

3.3 Drugs Preparation and Administration

The chicks were weighed and the average body weight for each age group were determined. The volume of ketamine and xylazine for the preparation of 10 ml working solution was calculated based on the dosage (Table 3.1) and average body weight of chicks of each age group. The ketamine and xylazine were mixed in a 15 ml sterile conical centrifuge tube and Dulbecco's phosphate buffered saline (DPBS) was added to the KX mixture until 10 ml volume is reached. The mixture was homogenized using the vortex mixer. The injection site was disinfected using the 70% alcohol swab. The KX was administered intramuscularly into the pectoral muscle, using the 1 ml syringe and 23-gauge needle.

3.4 Anesthesia Stages

The period following the administration of KX was divided into three phases: induction, anaesthesia and recovery (Table 3.2 and Fig. 3.1). The occurrence of these three phases, on each chick, was monitored and the duration for each phase was timed and recorded.

3.5 Physiological Parameters

Respiratory rate, heartbeat and cloacal temperature were recorded before the induction of anaesthesia, during the anaesthesia and the recovery. The respiratory rate (RR; breaths/min) was taken by counting the number of times the chest rises over one minute. The heart rate (HR; beats/min) was determined by auscultation of the heart sounds using the stethoscope. The cloacal temperature (°C) was measured using a medical grade digital thermometer, with a temperature range 32°C to 42.9° and resolution of 0.1°C.

3.6 Statistical Analyses

All data are presented as mean ± standard deviation (SD). Unless stated otherwise, the data were analyzed using a two-way analysis of variance (ANOVA) as appropriate. The level of significance was set at $P< 0.05$. Data were analyzed using the Statistical Package for Social Sciences (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp).

Table 3.1 Experimental Design.

Age (week/s)	*n*/group	Ketamine (Dosage; mg/kg)	Xylazine (Dosage; mg/kg)
<1	5	10	2
		20	
		40	
2	5	10	2
		20	
		40	
4	5	10	2
		20	
		40	

Table 3.2 Anaesthesia Stages.

Stages	Description of event
Induction	The period from administration of KX until the chick demonstrated ataxia or incoordination and sank from normal posture to progressive drooping of head.
Anaesthesia	The time of involuntary recumbency; either sternal or lateral recumbency and not responsive to tactile or auditory stimuli.
Recovery	The period when the chicks started to gain their consciousness and until they are able to maintain a sitting posture

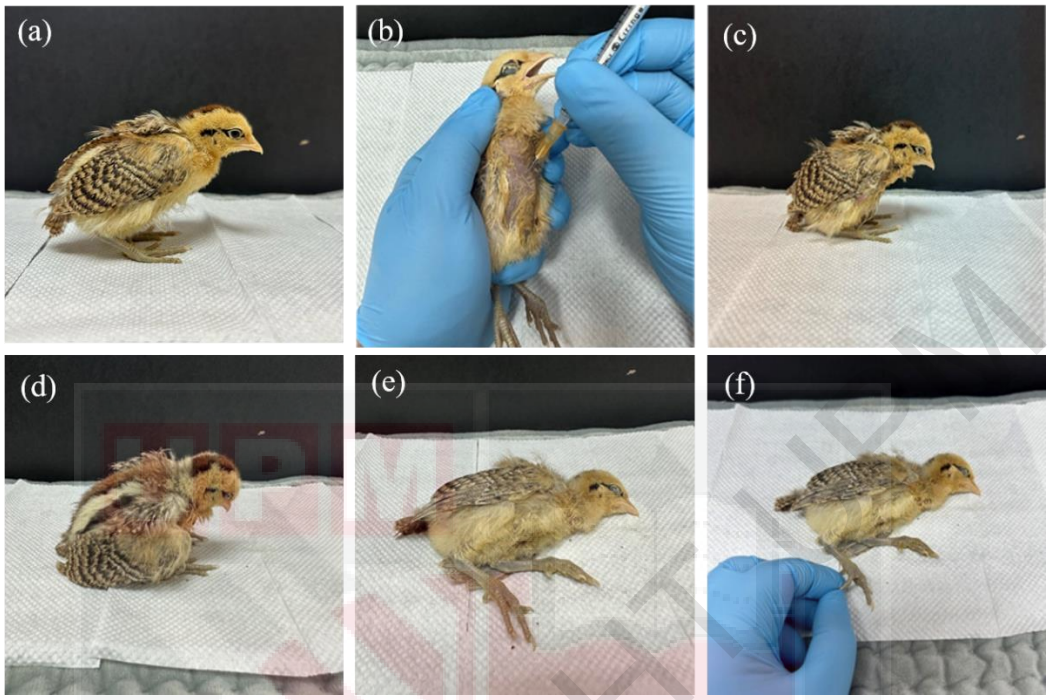

Fig. 3.1 Representative images showing the stages of anaesthesia in the Malaysia Village Chicken (MVC) chicks. (a) The MVC chick at conscious state prior to the anaesthesia, (b) Administration of ketamine-xylazine (KX) performed via the intramuscular injection at the pectoralis muscle, (c) Ataxia and incoordination of chick demonstrated during the induction stage, (d) Progressive head dropping demonstrated before the chick entering anaesthesia stage, (e) The chick on lateral recumbency during the anaesthesia stage, and (f) Pinching of the toes performed to assess the withdrawal reflex or respond to auditory or tactile stimuli.

4.0 RESULTS

4.1 Anaesthesia Stages

Induction Time

The age of MVC chicks and dosage of KX have significant effects ($P<0.05$; $P=<0.001$ and $P=0.042$, respectively) on the induction time (Figure 4.1a). Older MVC chicks demonstrated longer induction time than the younger MVC chicks. The induction time in the 4-week-old chicks was significantly longer ($P<0.05$) in 4-week-old MVC, than the < 1-week-old MVC when 10/2 and 40/2 mg/kg KX were administered ($P=0.04$ and $P<0.01$, respectively). Meanwhile, increasing the dosage of ketamine resulted in a shorter induction time within the age group. The induction time in the 4-week-old MVC was significantly shorter ($P<0.05$; $P=0.05$) when the KX dosage was increased from 10/2 mg/kg to 20/2 mg/kg (Table 4.1).

Duration of Anaesthesia

The KX dosage has a significant effect ($P<0.05$; $P=<0.01$) on the duration of anaesthesia in the MVC chicks (Figure 4.1b). Regardless of the age of the chicks, the duration of anaesthesia was longer as the ketamine dosage increased. The duration of anaesthesia was significantly longer ($P<0.05$; $P=0.02$ and $P=<0.01$, respectively) in 2-week-old and 4-week-old MVC, when 20/2 mg/kg and 40/2 KX dosage were used (Table 4.2).

Recovery Time

The KX dosage has a significant effect ($P<0.05$; $P<0.01$) on the recovery time in the MVC chicks (Figure 4.1c). Regardless of the age of the chicks, the recovery time was longer as the ketamine dosage increased. The recovery time was significantly longer ($P<0.05$) in the chicks anaesthetized with 40/2 mg/kg of KX, compared to the 20/2 mg/kg and 10/2 mg/kg in 2-week-old and 4-week-old MVC ($P=0.02$ and $P=<0.01$, respectively), and 10/2 mg/kg in <1 week-old MVC ($P=0.017$) (Table 4.3).

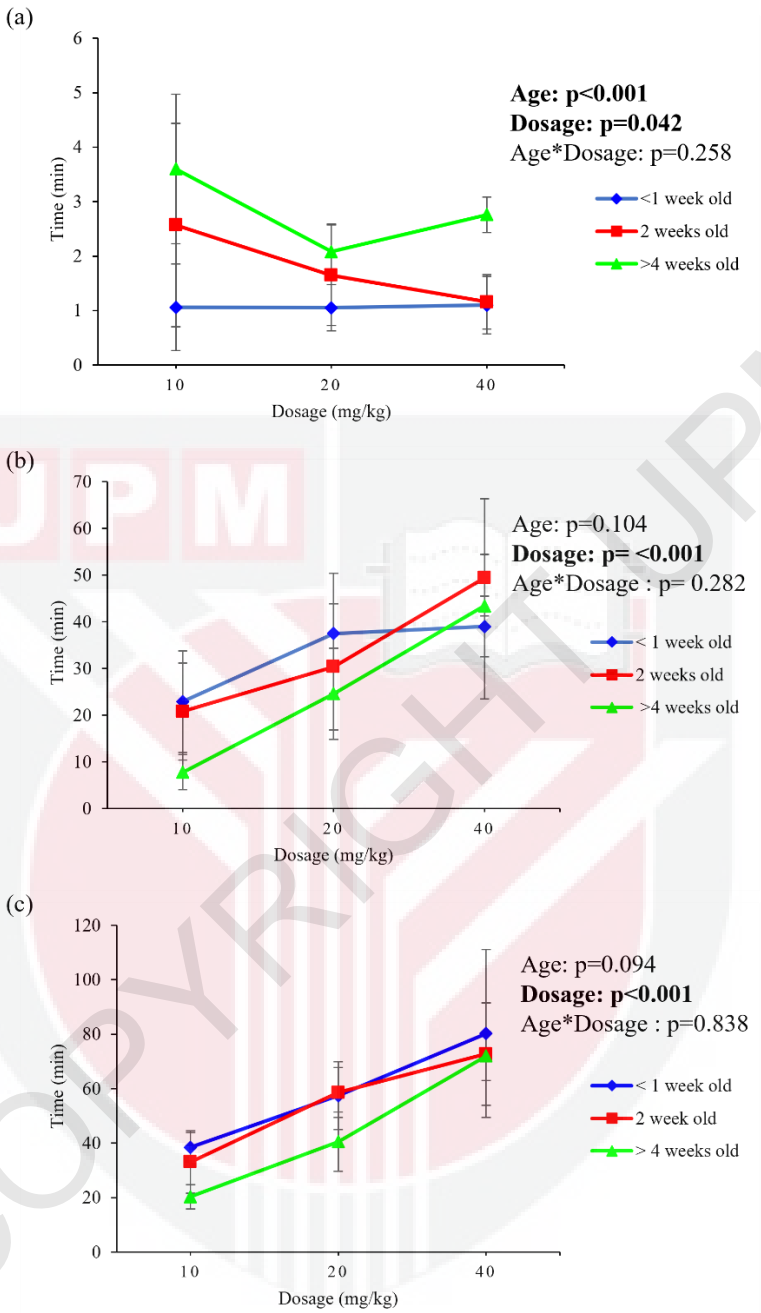

Figure 4.1 Effects of Ketamine-Xylazine (KX) anaesthesia over time on the (a) induction time, (b) duration of anaesthesia, and (c) the recovery time in the Malaysian Village Chicken (MVC) chicks. The chicks from three different age groups; <1 week old, 2 weeks old, and 4 weeks old (n=5/age group) were subjected to three doses of KX anaesthesia: 10 mg/kg, 20 mg/kg, or 40 mg/kg of ketamine, with a constant dosage of xylazine; 2 mg/kg. Data are presented as mean ± SEM.

Table 4.1 Effects of Ketamine-Xylazine anaesthesia on the induction time in MVC chicks.

MVC age (wk)	Ketamine/Xylazine dosage (mg/kg)		
	10/2	**20/2**	**40/2**
<1	1.06 ± 0.8^{a}	1.05 ± 0.4	1.10 ± 0.5^{a}
2	2.57 ± 1.9^{ab}	1.65 ± 0.9	1.16 ± 0.5^{a}
4	$3.60 \pm 1.4^{b, x}$	2.08 ± 0.5^{y}	$2.76 \pm 0.3^{b, xy}$

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same column and row are significantly different (P<0.05)

Table 4.2 Effects of Ketamine-Xylazine anaesthesia on the duration of anaesthesia in MVC chicks.

MVC age (wk)	Ketamine/Xylazine dosage (mg/kg)		
	10/2	**20/2**	**40/2**
<1	22.91 ± 10.9	37.49 ± 12.9	38.97 ± 15.5
2	20.79 ± 10.4^{x}	30.38 ± 13.5^{xy}	49.4 ± 16.9^{y}
4	7.80 ± 3.7^{x}	24.62 ± 9.8^{y}	43.39 ± 2.1^{z}

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same row are significantly different (P<0.05)

Table 4.3 Effects of Ketamine-Xylazine anaesthesia on the recovery time in MVC chicks.

MVC age (wk)	Ketamine/Xylazine dosage (mg/kg)		
	10/2	**20/2**	**40/2**
<1	38.45 ± 5.4^{x}	57.45 ± 12.4^{xy}	80.29 ± 30.9^{y}
2	33.11 ± 11.5^{x}	58.61 ± 9.1^{y}	72.72 ± 18.7^{y}
4	20.32 ± 4.5^{x}	48.53 ± 10.9^{y}	72.02 ± 9.0^{z}

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same row are significantly different (P<0.05)

4.2 Physiological Parameters

Induction Time

The age of MVC chicks has significant effects ($P< 0.05$; $P<0.001$, $P=0.001$, $P= 0.002$, respectively) on the physiological parameters (respiratory rate, heart rate, and cloacal temperature) during the induction time. The respiratory rate and heart rate during the induction time of 2-week-old chicks are significantly higher ($P< 0.05$) than > 4-week-old chicks when 20/2 and 40/2 mg/kg KX were administered. The cloacal temperature during induction time is significantly higher ($P< 0.05$) in > 4-week-old chicks than in 2-week-old chicks when 10/2 and 20/2 mg/kg KX were administered (Table 4.4).

Duration of Anaesthesia

The age of MVC chicks and KX dosage have a significant effect ($P< 0.05$; $P<0.001$ and $P=0.021$ respectively) on the respiratory rate during the duration of the anaesthesia. The respiratory rate on the duration of anaesthesia of 4-week-old chicks is significantly higher than the 2-week-old chicks when 10/2 and 20/2 mg/kg KX was administered. The age of chicks has a significant effect ($P<0.05$; $P=0.004$) on the cloacal temperature during the duration of anaesthesia. Meanwhile, the heart rate and cloacal temperature showed no significant difference in the duration of anaesthesia when compared among groups (Table 4.5).

Recovery Time

The age of MVC chicks has significant effects ($P<0.05$) on the respiratory rate and heart rate of recovery time ($P=0.033$ and $P=0.001$ respectively). The respiratory rate on the

recovery time of 2-week-old chicks is significantly higher than the < 1-week-old chicks when 20/2 and 40/2 mg/kg KX were administered. Moreover, the heart rate on the recovery time of 4-week-old chicks and 2-week-old chicks has an increasing trend when the dosage of KX administered increases respectively. Meanwhile, the cloacal temperature showed no significant difference in the recovery time when compared among groups (Table 4.6).

Table 4.4 Effects of the Ketamine-Xylazine anaesthesia on the physiological parameters during the induction stage in MVC chicks.

Physiological parameters	Age (wk)	Duration of Anaesthesia		
		Ketamine/Xylazine dosage (mg/kg)		
		10/2	20/2	40/2
RR (Breath/min)	<1	60 ± 13	47 ± 13^{a}	52 ± 9^{a}
	2	69 ± 23	70 ± 13^{b}	82 ± 8^{b}
	4	70 ± 10	57 ± 9^{ab}	60 ± 11^{a}
HR (Beat/min)	<1	157 ± 7	136 ± 21^{a}	127 ± 27^{a}
	2	167 ± 7	160 ± 21^{ab}	182 ± 9^{b}
	4	156 ± 28	178 ± 25^{b}	160 ± 32^{ab}
CT (°C)	<1	37.66 ± 1.3	37 ± 1.0^{a}	37 ± 1.1
	2	39.20 ± 1.1	37.12 ± 1.3^{a}	38.72 ± 0.6
	4	39.38 ± 0.7	39.82 ± 2.0^{b}	39 ± 2.3

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same column significantly different (P<0.05)

Table 4.5 Effects of the Ketamine-Xylazine anaesthesia on the physiological parameters during the anaesthesia stage in MVC chicks.

Physiological parameters	Age (wk)	Duration of Anaesthesia		
		Ketamine/Xylazine dosage (mg/kg)		
		10/2	20/2	40/2
RR (Breath/min)	<1	26 ± 4[a]	18 ± 5[a]	29 ± 9
	2	34 ± 11[ab]	31 ± 8[b]	37 ± 9
	4	40 ± 3[b]	34 ± 4[b]	39 ± 6
HR (Beat/min)	<1	128 ± 21	126 ± 20	121 ± 13
	2	124 ± 9	129 ± 19	138 ±13
	4	126 ± 11	132 ± 13	134 ± 15
CT (°C)	<1	39.70 ± 0.9	38.52 ± 1.1	38.66 ± 0.7
	2	38.70 ± 1.2	39.06 ± 1.7	38.84 ± 1.6
	4	40.10 ± 0.3	40.18 ± 1.2	40.34 ± 0.8

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same column significantly different (P<0.05)

Table 4.6 Effects of the Ketamine-Xylazine anaesthesia on the physiological parameters during the recovery stage in MVC chicks

Physiological parameters	Age (wk)	Duration of Anaesthesia		
		Ketamine/Xylazine dosage (mg/kg)		
		10/2	20/2	40/2
RR (Breath/min)	<1	37 ± 3	30 ± 7	40 ± 7
	2	42 ± 10	48 ± 17	48 ± 21
	4	35 ± 7	35 ± 6	43 ± 8
HR (Beat/min)	<1	123 ± 13	115 ± 16[a]	104 ± 22[a]
	2	142 ± 16	146 ± 13[b]	170 ± 19[b]
	4	148 ± 17	154 ± 17[b]	162 ± 13[b]
CT (°C)	<1	40.60 ± 0.8	40± 0.5	40 ± 0.9
	2	38.64 ± 2.5	40.80 ± 1.2	38.14 ± 2.9
	4	39.80 ± 1.3	40.12 ± 1.3	40.72 ± 0.9

Data are mean ± SD. n=5/age group
MVC Malaysian Village Chicken; wk week(s)
Data with different letters within the same column significantly different (P<0.05)

5.0 DISCUSSIONS

There is wide and considerable individual variation in response to ketamine (Steffey et al., 2015). The widest variation can be observed in the degree of analgesia produced, ranging from the subtle levels produced in nonhuman primates to the inappreciable level in small rodents. Among various animal species, such as pigs, sheep, and cats, the highest degree of analgesia is notably evident in birds (Green et al., 1981). Meanwhile, ketamine is not recommended to be used alone due to its prolonged recovery time and inadequate sedation (Azizpour & Hassani, 2023). The analgesia effect needs to be refined by the administration of either diazepam or xylazine. The administration of xylazine balanced out the effect of ketamine on muscular tone, it is either unaffected or markedly enhanced.

The study reported here is the first detailed study on anaesthesia induced by a combination of ketamine-xylazine in (MVC) chicks. In this study, three different doses of ketamine xylazine ranging from 10-40/2 mg/kg were used in different age groups of chicks, and appeared to be well tolerated by young chicks of all age groups and the dosages used were safe. The experience with ketamine reported here is in general agreement with the dosage used in the study conducted by (Green et al., 1981).

In our experiment, intramuscular injection was used in chicks and the results of the study indicate that intramuscular injection of sedative agents can provide reliable sedation in chicks. IM route drug delivery was well tolerated by the chicks regardless of the age of the chicks, and no complications were detected. Nevertheless, all age groups of MVC chicks had a smooth recovery from sedation and had no signs of respiratory distress throughout the sedation period. Our data suggests that IM administration of KX can

provide adequate sedation for diagnostic and minor therapeutic or surgical procedures in MVC chicks. Meanwhile, in another study conducted by Rehman et al., (2020) who worked on isoflurane an inhalant anaesthethic drug reported that isoflurane induces rapid and smooth anaesthesia in pigeons, achieving anaesthesia within 2-3 minutes and can maintain a desirable duration throughout the procedure. These different routes of administration of drugs clearly show that the results in our study are suitable for minor surgical procedures for a shorter period.

Moreover, in our study a range of young age groups of MVC chicks were selected due to this study will be very helpful in establishing a model for future research, including cryopreservation and implantation, where young chicks are commonly used as a recipient (Song & Silversides, 2007). Moreover, as the development of the cardiopulmonary system in chickens progresses after birth, it might have effects on the efficiency of anaesthesia. As a result of these developmental changes, young chicks can exhibit increased vulnerability to hypoxemia and hypercapnia (Haron et al., 2021). Hence, close monitoring of the physiological parameters is needed to avoid respiratory depression and hypoxia (Curro, 1998)

Miller & Buitrick, (1999) found out that the onset of anaesthesia after (IM) administration usually will be 3-5 minutes when the dosage used was 10-30mg/kg. Our findings are not in agreement with this study which shows an average of 1-3 minutes in each age group of chicks when 10-20mg/kg was used. This might be due to the variation in factors such as breeds, and individual and experimental settings, Thus, this is why establishing the right dosage is important before anaesthesia in birds.

The results of the present study indicate that deep anaesthesia with complete muscle relaxation and a wide safety margin can be achieved by suitable combination doses of KX. Administration of KX resulted in anaesthesia of the MVC chicks and was assessed by loss of reflex of chicks. The loss of reflex of the chick was tested by pinching the toe to evaluate deep pain sensation in birds (Kaya et al., 2019). In our study, two age groups of chicks achieved anaesthesia despite differences in recovery time. This could be due to individual variation (Curro, 1998). Meanwhile, three out of five 4-week-old chicks were in the light anaesthesia plane, although they were in dorsal recumbency and looked immobile, the withdrawal reflex was still present. It is very crucial to ensure chicks are in a deep plane of anaesthesia throughout the procedure to ensure chicks are not in pain and remain immobile (Curro, 1998).

Mousa et al., (2021), stated that age plays an important role in the efficacy of the anaesthesia in birds, increasing as age progresses. This finding also agrees with the present study, MVC chicks of all age groups experimented showed significant results during induction time and duration of anaesthesia. In our study, we took into consideration the research done before and experimented on the chicks starting from 10/2 mg/kg which were able to produce anaesthesia (Krajca & Juranova, 2023). Nevertheless, it varies within the age of the group of chicks, > 4 weeks old MVC chicks produce light anaesthesia when administered with 10/2 mg/kg of KX. This can be observed when chicks still have their reflexes present, but there is no voluntary movement. The induction time markedly decreased, and the recovery time also became shorter.

Miller & Buitrick (1999) suggested that the dosage of 10-30 mg/kg of ketamine and a

ratio of both ketamine-xylazine combination is 10:1 is the suitable dosage for avian species. Miller & Buitrick (1999) also found out that the standard duration of anaesthesia ranges from 10 to 30 minutes, with recovery time varying significantly based on the dosage administered and it may extend over several hours. This finding is in agreement with our study, when 40 mg/kg is administered, the duration of anaesthesia, as well as the recovery period of chicks, was longer, approximately 2 hours in chicks.

Anaesthesia results in a state of unconsciousness and basic principles of anaesthethic management that applies in mammalian anaesthesia should apply to birds as they possess different anatomic and physiological differences (Curro, 1998). Apart from evaluating the anaesthesia stages, the physiological parameters of the MVC chicks were also assessed. Respiratory rate, heart rate, and cloacal temperature were assessed continuously throughout the sedation period to ensure chicks were stable under the influence of anaesthesia (UFO Themes, 2015). All the parameters will reduce as the chicks achieve deep surgical anaesthesia. Monitoring continuously is crucial as birds are more predisposed to experience anesthesia-related complications with greater susceptibility and rapid onset (Curro,1998). Ketamine has a cyclohexamine compound that induces a cataleptic state, it will have effects on the vitals of the chicks, especially the cardiovascular system and respiratory systems. Degernes et al., (1988) discovered that the effect of xylazine was to lower the respiratory rate in birds anaesthesized with KX combination. This finding is also in agreement with this study which shows significantly lower respiratory rate in chicks during the induction time.

The cloacal temperature was taken due to chicks becoming hypothermic under the

influence of ketamine. According to Miller & Buitrick, (1999), birds experience more challenges in efficiently regulating their body temperature than mammals. Consequently, they undergo rapid changes in body temperature when subjected to anaesthesia, loss of heat during anaesthesia can significantly affect anaesthetic recovery (David et al., 1996). Heart rate can be a good indicator of the depth of anaesthesia, when the chicks have stable heart rate, it is safe to proceed with surgical procedures. Yıldırım Çelik et al., (2020) stated that chicks may experience a higher rate before anaesthesia probably due to the effect of stress due to handling. Our study is in agreement with Yıldırım Çelik et al., (2020), where all the chicks' heart rate was high before the anaesthesia.

6.0 CONCLUSIONS

In conclusion, the effects of ketamine xylazine anaesthesia on the MVC chicks are dependent on the dosage and age. Meanwhile, the effects of ketamine xylazine on physiological parameters in MVC chicks during anaesthesia is age dependent. Ketamine xylazine at the dosage of 10-40/2 mg/kg is safe as anaesthesia for performing surgical procedures in MVC chicks.

7.0 RECOMMENDATIONS

This study has successfully demonstrated the safest anaesthethic protocol to be used in MVC chicks for performing surgical procedures. In future studies, we propose delving into the impact of sex on anaesthesia in chicks. This research could provide more information on whether male and female chicks exhibit distinct responses to anaesthesia, in terms of induction time, the depth of anaesthesia, and recovery time, and also to determine whether anaesthethic drugs are potentially influenced by factors like metabolism, body composition, or hormonal influences. Braithwaite et al., (2023) state that female has a higher occurrence of consciousness during general anasthesia and they recover faster from anaesthesia than men. As research has only been conducted on humans and not on chicks, we aim to explore the gender characteristics of chicks in future studies.

Further research to examine the potential influence of environmental factors on the efficacy of sedation is crucial to explore how external elements may impact the effectiveness of anaesthesia. To fully comprehend the applicability and precision of anaesthesia protocols in MVC chicks, further investigations into the drug's toxicity are crucial, particularly in situations requiring prolonged procedures or additional drug administration. Analyzing the threshold and toxic effects of KX usage in chicks will yield valuable data for future reference.

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