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Comparative efficacy of Diazepam, Ketamine, and Diazepam-Ketamine combination for sedation or anesthesia in cockerel chickens

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ABSTRACT

The comparative efficacy of Diazepam, Ketamine, and Ketamine-Diazepam combination was assessed in adult chickens. The chickens (n=30) were divided into three equal groups (G-1, G-2 and G-3), and were administered with Diazepam dosed at 0.5 mg/kg body weight (b.wt.), Ketamine HCL dosed at 20 mg/kg b.wt., and Ketamine HCL (dosed at 10 mg/kg b.wt.) combined with Diazepam (dosed at 2 mg/kg b.wt.) through intramuscular (IM) route. The means of induction period, duration of sedation or anesthesia, full recovery period and duration of analgesia were significantly ($p \leq 0.05$) differed among the groups. Also, the clinical and hematological parameters measured before and after the sedation or anesthesia within the groups were found to be differed significantly ($p \leq 0.05$) from each other. It was concluded that Diazepam dosed at 0.5 mg/kg b.wt. (IM) can be used in cockerels. However, combination of Ketamine (at 10 mg/kg IM)-Diazepam (at 2 mg/kg b.wt. IM) is preferably recommended as this combination is comparatively safer, and minimizes the pains elicited from the surgical procedure of using Diazepam alone.

Keywords

Anesthesia, Cockerel chicken, Diazepam, Ketamine, Ketamine-Diazepam combination, Sedative

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INTRODUCTION

Various sedatives and tranquilizing agents are used as pain killers and/or chemical restraints while animals undergo minor or major surgeries. These drugs are needed in veterinary practice and are indispensable as they help in overcoming resistance of the animals during examination, maintaining depth of anesthesia, reducing the amount of anesthetic agents and increasing margins of safety (Habib et al., 2002). For these purposes, the commonest drugs used in Nigeria are Ketamine, Diazepam and Xylazine.

Ketamine is considered as a less potent dissociative anesthetic agent because it lacks cardio-pulmonary depression effect (Durrani et al., 2008). Ketamine induces amnesia and anesthesia of stages I and II but not stage III anesthesia (Booth, 1988), and is a potent inhibitor of Gamma amino butyric acid (GABA) binding. This agent is rarely used alone because of its association with poor muscle relaxation, muscle tremors, myotonic contractions, opisthotonus and rough recoveries. Recently, depending on the species involved, the drug is commonly used in combination with diazepam, alpha-2-adrenergic drugs or azaperone, (Valvered et al., 1993).

Since, drugs manifested different effects when these were used separately or in combination, changes that occurred during use of combined-drug should be understood and recognized (Ozkan et al., 2010). In humans, addition of a sedative-hypnotic drug to ketamine was commonly practiced. In animals, it was also frequently used for animal studies in augmenting ketamine's anesthetic effects, decreasing side effects, and to provide necessary depth of anesthesia for surgical comfort (Muir et al., 2000). To minimize the unwanted effects, ketamine is usually administered in combination with drug groups such as benzodiazepines, and alpha-2 agonists (Ozkan et al., 2010).

Diazepam is a potent hypnotic-sedative that causes muscle relaxation. It is a long-acting drug as it is metabolized slowly, and it has relatively weaker cardiovascular effects as compared to other sedative drugs (Koshy et al., 2003). In combined use with ketamine, diazepam alleviates unwanted cardiovascular effects of ketamine, and demonstrates anticonvulsive, amnestic and muscle relaxant effects (Koshy et al., 2003).

Various sedative and anesthetic agents have been used and analyzed in birds (Christensen et al., 1987; Curro, 1998; Durrani et al., 2008). None of these studies analyzed their efficacies in cockerel chickens, probably because these birds are usually kept for meat purposes. However, with the recent gradual improvement in awareness of surgical procedures in cockerels (i.e., canonization) (Mahmud et al., 2013), the use of these drugs as either sedative and/or anesthetic agents became indispensable for performing surgical procedures with maximum precision and sagacity (Muhammad et al., 2009).

In view of the sparse literature reports about the use of anesthetics in chicken, this study was designed to compare clinical, sedative/anesthetic and hematological efficacies of Ketamine, Diazepam, and combination of Ketamine and Diazepam in cockerel chickens.

MATERIALS AND METHODS

Bird management and experimental design: The present study was conducted at the Poultry Unit, Livestock Farm of Niger State College of Agriculture, Mokwa. Mokwa is located at latitude $9^0 17' 38''$ North and longitude $5^0 3' 16''$ East (Google Maps, 2014). Apparently healthy adult cockerel chickens (n=30) aging 12 months were purchased from the College Farm, Mokwa, Nigeria, and were randomly divided into three experimental groups with ten cockerel chickens in each group; the Diazepam (alone) injected group (G_1), the Ketamine (alone) injected group (G_2) and the Ketamine-Diazepam (combined) injected group (G_3). Body weights of the birds ranged from 2-3 kg. All the cockerels belonged to same flock. The birds were physically examined and found apparently healthy and active. The birds were given water *ad libitum* and fed commercial grower diet (Animal Care®).

Drug administration: ***G_1***: Diazepam (Wuhu Kangqi Pharma Co. Ltd., China, 2 mL ampoule, 10 mg/mL) at 0.5 mg/kg live body weight intramuscularly (IM) was used. ***G_2***: Ketamine HCL injection (Rotex Medica, German, 10 mL ampoule, 50 mg/mL) at 20 mg/kg live b.wt., IM was used. ***G_3***: A combination of Ketamine HCL at 10 mg/kg IM and Diazepam at 2 mg/kg IM was used.

Post-treatment monitoring: In post-treatment monitoring, the procedures used by Durrani et al. (2008) were followed. Briefly, after treatment, birds of all groups were kept under close observation. Induction period, duration of sedation/anesthesia or analgesia, recovery period, body temperature, respiratory rate, and heart rate were recorded. Respiratory rate was recorded from sternal movements, while heart rate was recorded by stethoscope from left costal area and per rectum temperature was noted using digital thermometer.

Hematological parameters: Blood samples (n=2) of 2 mL each were collected from wing-web vein of each experimental bird 15 min prior to administration of the drugs and 15 min after sedation and anesthesia. Immediately after collection, the blood samples were transferred in a sterile test tube containing Ethylene Diaminetetra Acetic Acid (EDTA) as anticoagulant. It was used within 2 h after collection to determine Packed Cell Volume (PCV), White Blood Cells (WBC), Hemoglobin Content (HBC), Mean Corpuscular Hemoglobin Concentration (MCHC) and Differential Leukocyte Counts (DLC) according to the procedures of Orpet and Welsh (2002).

Statistical analysis: The recorded data were expressed as Mean±SEM (Standard Error of Mean) and subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) version 17.0**.** Paired-Samples T-Test was used to compare clinical and hematological parameters taken before and after the administration of the drugs for each group. One-Way Analysis of Variance (ANOVA) at 95% confidence interval (CI) was used to determine the level of significant difference in mean values among the three groups. Where there was difference in means, it was separated as per Turkey's Honestly Significant Difference (HSD) (Kaps and Lamberson, 2004). Values of $p \leq 0.05$ were considered as significant.

Table 1: Mean (±SEM) induction period, duration of sedation/anesthesia, recovery period and duration of analgesia of thirty cockerels in minutes.

Parameters	G1	G2	G3
Induction period	11.574±0.048^{c}	3.300±0.156^{a}	5.986±0.734^{b}
Duration of sedation/anesthesia	306.000±0.882^{c}	91.000±1.000^{a}	131.000±2.246^{b}
Recovery period	26.160±1.269^{a}	46.516±0.767^{b}	53.078±0.281^{c}
Duration of analgesia	9.808±0.107^{a}	104.400±1.447^{b}	141.200±0.892^{c}

$^{a, b, c}$ Means within the same row without the same superscript letters are significantly different (P≤0.05) from one another.

Table 2: Mean (±SEM) temperature (T) (^{0}C), respiratory rate (RR) (cycles/min) and heart rate (HR) (beats/min) after the sedation and anesthesia in cockerels of among the three groups.

Parameters	G_1	G_2	G_3
Temperature	41.400±0.427	41.600±0.371	41.400±0.371
Respiratory rate	41.800±0.692	41.00±0.856	41.800±0.629
Heart rate	162.400±3.789	167.200±0.987	163.300±1.732

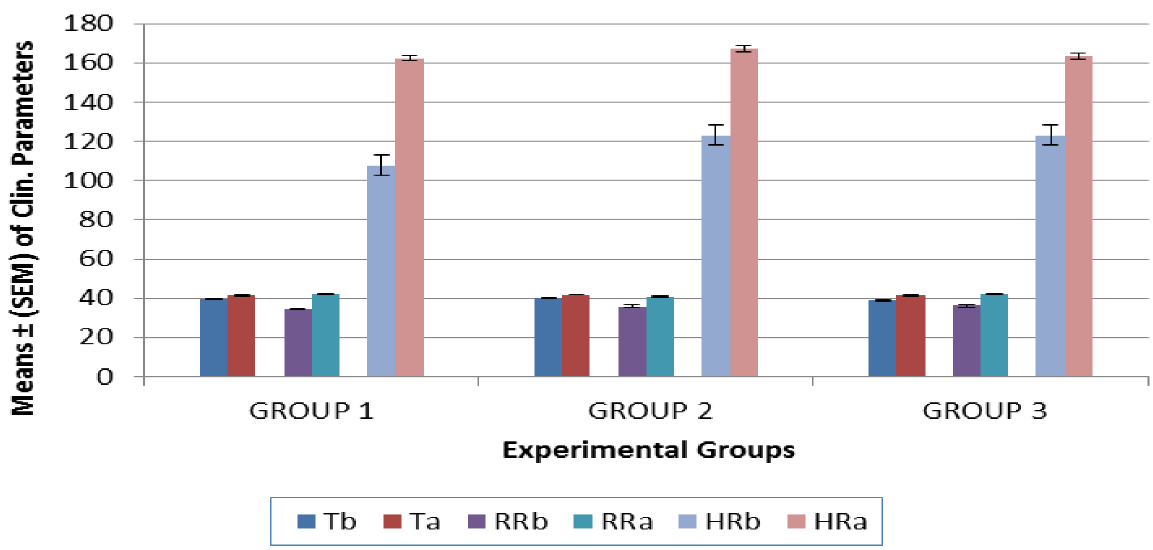

Figure 1: Mean±(SEM) temperature (T) (^{0}C), respiratory rate (RR) (cycles/min) and heart rate (HR) (beats/min) taken before (b) and after (a) the sedation/anesthesia in cockerels of the same groups.

RESULTS AND DISCUSSION

The means (±SEMs) of induction periods, duration of sedation/anesthesia, full recovery period and duration of analgesia were presented in **Table 1**. The results indicated that the means were significantly differed (p≤0.05), and on further separation by Tukey's Honestly Significant Difference (HSD), these were statistically different (p≤0.05) from one another. The highest induction period observed in Diazepam (alone) injected group (G_1) (**Table 1**) could be explained on the ground that diazepam could induce slight sedation with slight muscle relaxation (Koshy et al., 2003). The fastest onset of anesthesia was seen in Ketamine (alone) injected group (G_2), which might be due to rapid distribution of ketamine into all body tissues primarily adipose tissue, liver, lung and brain (Lanning and Harmel, 1975). The highest duration of sedation (306.000±0.882) was observed in the birds of G_1. This might be due to wide-distribution of diazepam in the body, as because diazepam is highly soluble in lipid and can be redistributed into muscles and adipose tissues (Bateson, 2002). Furthermore, diazepam is a long-acting drug due to its slow-metabolism in the body as compared to the other sedatives (Koshy et al., 2003). The results obtained here were in line with the findings of Azizpour and Hassani (2012) who performed general anesthesia in pigeons using a combination of ketamine HCL and diazepam.

A mild-rough and smooth recoveries were observed in the birds of G_2 and G_3, respectively. These findings

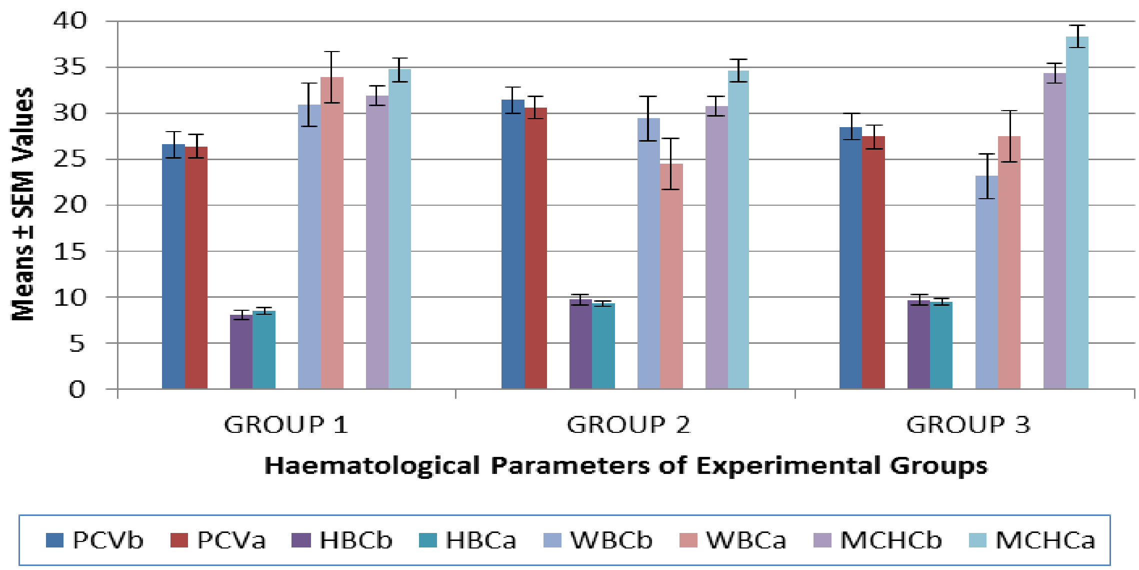

Fig. 2: Means ± SEM packed cell volume (PCV), hemoglobin content (HBC), white blood cells (WBC) and mean corpuscular hemoglobin concentration (MCHC), taken before (b) and after (a) the sedation/anesthesia in cockerels of the same groups.

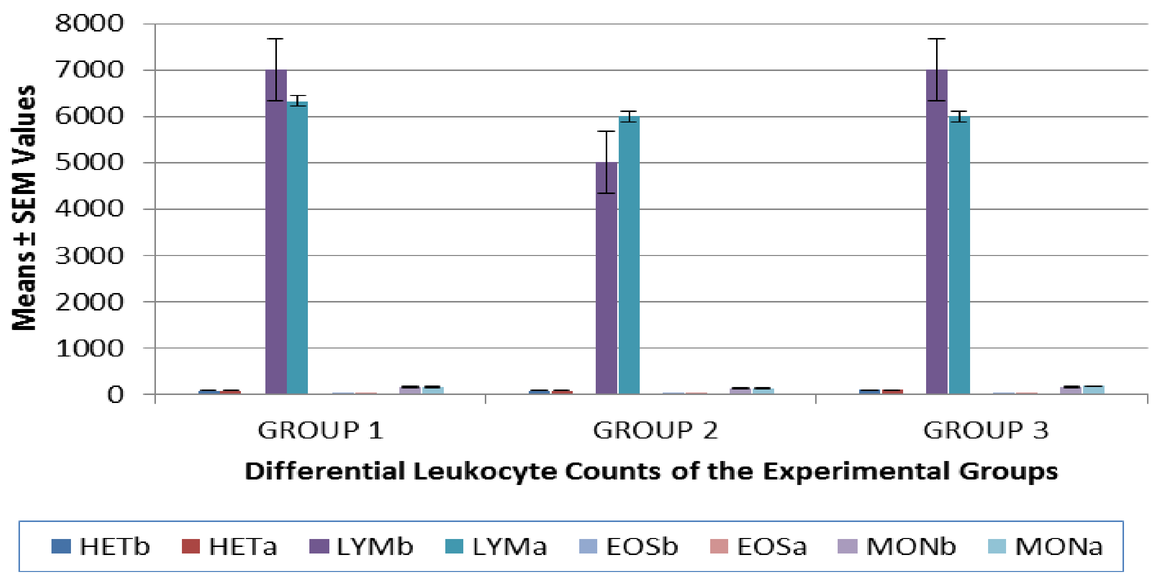

Fig. 3: Means ± SEM Differential Leukocyte Count [heterophils (HET), lymphocytes (LYM), eosinophils (EOS) and monocytes (MON)] taken before (b) and after (a) the sedation/anesthesia in cockerels of the same groups.

were in line with some earlier studies in pigeons (Durrani et al., 2008; Lumeij and Deenik, 2003). The longest recovery period was observed in the birds of G_3, which was desirable since diazepam could augment ketamine's anesthetic effects decreasing its side effects; thus, it provided necessary depth and duration of anesthesia for surgical comfort (Muir et al., 2000). These could answer the question- "*why combination of ketamine and diazepam or xylazine is routinely used for general anesthesia in birds?*" (Varner et al., 2004; Patrick, 2005; Durrani et al., 2009). Similar observations were reported by Durrani et al. (2008) and Azizpour (2012); where, they compared the efficacies of detomidine, ketamine and detomidine ketamine cocktails in pigeons. In our study, mild-rough recovery of wing fluttering was seen in G_2, which might be due to dissociative characteristics of ketamine anesthesia (Hall et al., 2001; Azizpour et al., 2012). Similar observations were also made in pigeons by Atalan et al. (2002) and Azizpour et al. (2012).

Diazepam was known to produce little or no analgesia (Abou-Madi, 2001) while ketamine induced visceral analgesia (Durrani et al., 2008). Combination of

Table 3: Means (±SEM) packed cell volume (PCV), hemoglobin content (HBC), white blood cells (WBC), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count (DLC) measured after the sedation/anesthesia in cockerels of the three groups.

Parameters	G_1	G_2	G_3
PCV (%)	26.370±0.597^a	30.600±0.229^c	27.4300±0.249^b
HBC (g/dL)	8.473±0.020^a	9.304±0.019^b	9.488±0.015^c
WBC(×10^{3}g/µL)	33.885±0.400^c	24.490±0.015^a	27.480±0.015^b
MCHC(g/dL)	34.673±0.008^a	34.642±0.012^a	38.310±0.048^b
HETEROPHILS (×10^{3}g//µL)	74.620±0.051^a	83.330±0.021^b	85.685±0.027^c
LYMPHOCYTES(×10^{3}g//µL)	6333.52±0.070^b	5999.933±0.145^a	5999.882±0.133^a
EOSINOPHILS(×10^{3}g//µL)	3.655±0.023^c	3.014±0.013^b	2.670±0.027^a
MONOCYTES(×10^{3}g//µL)	163.530±0.070^b	126.510±0.084^a	170.260±0.052^c

$^{a, b, c}$ Means within the same row without the same superscript letters are significantly different (p≤0.05) from each other.

Ketamine and diazepam induced a synergistic action producing a deep analgesia for long duration. Similar observations were also reported in pigeons using ketamine-diazepam, and ketamine-detomidine combinations (Lumeij and Deenik, 2003; Durrani et al., 2008).

The means (±SEMs) of temperature (^{0}C), respiratory rate (cycles/min) and heart rate (beats/min) recorded before and after sedation/anesthesia of cockerel chickens of same group and among the three groups were illustrated in **Figure 1** and **Table 2**. There was a significant difference (p≤0.05) between the temperatures recorded before and after sedation/anesthesia. The increments seen in the temperatures of birds of the same group were in contrary to the reports of the earlier studies in pigeons (Sandmeier, 2000; Metehan et al., 2003; Azizpour and Hassani, 2012). However, the results of the present study were in line with the findings of Durrani et al. (2008). The difference seen in this study might be due to environmental, species or genetic differences that existed between the pigeons and chickens.

The significant increment was seen in the heart rate between the cockerels of G_1 and G_2; this might be explained on the ground that ketamine could increase heart rate (Branson, 2001). The results obtained in this study were in agreement with the earlier studies of Machin and Caulkett (1998), Metehan et al. (2003) and Durrani et al. (2008).

The results of hematological parameters measured before and after sedation/anesthesia in birds of the same group were significantly differed (p≤0.05) from each other. Although the results were found within the physiological limits as outlined by Wakenell (2010), PCV, HB, WBC, HTRP, ESNP and MNC values of the three different groups were significantly different (p≤0.05) from each other (**Figures 2, 3** and **Table 3**). However, the results obtained in our study did not agree with the earlier studies conducted in ruminants using ketamine and medetomidine or their combinations (Hossain and Shahriari, 1989; Dehgani et al., 1991; Dilipkumar et al., 1997; Chitale et al., 1998; Bhattacharya and Samanta, 1998; Kilic, 2008; Gweba et al., 2010; Umar and Wakil, 2013). The opposite effects seen in this study might be due to species and/or genetic variations. However, on further separation of means, the MCHC values G_1 and G_2 were not significantly differed (p>0.05) from each other. However, MCHC values of G_3 were significantly differed (p≤0.05) as compared to the other two groups. The LPC value of G_1 was significantly differed (p≤0.05) from those of Ketamine (alone) injected group (G_2) and Ketamine-Diazepam (combined) injected group (G_3); whereas, G_2 and G_3 were not significantly differed (p>0.05) from each other.

CONCLUSIONS

In cockerel chickens, Diazepam alone can be used for less painful surgical procedures and handling. However, in the cases of more painful surgical procedures that may take quite a long time, use of ketamine-diazepam combination dosed at 10 mg/kg IM and 2 mg/kg IM respectively, is safer and strongly recommended.

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