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EFFECTS OF TRAMADOL, PARACETAMOL, AND DICLOFENAC ON CAPSAICIN-INDUCED PAIN IN CHICKEN (*GALLUS GALLUS DOMESTICUS*)

Joseph Muthiani Kitonyi
jkitonyi@gmail.com
School of Agriculture, Environment, Water and Natural Resources, South Eastern Kenya ` University, Department of Agricultural sciences
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ABSTRACT

*Effects of Tramadol, Paracetamol and Diclofenac in Management of Pain in Chicken (Gallus gallus domesticus) Capsaicin, an extract of hot pepper induces pain. Analgesics conventionally alleviate pain. Forty eight, five months old chicken from a hatchery in Mwala Sub County, was used within Machakos County. Objectives were to examine whether administration of tramadol has an effect on pain in chicken, to determine whether the effects of paracetamol can reduce pain in chicken and, to evaluate the effects of diclofenac on induced pain in chicken. Birds were acclimatized to laboratory conditions for one month before their use in experiments n=6. They were randomly selected, weighed, coded and cloacal temperature taken. Ethogram was developed from capsaicin (10^{-9} -10^{-2}) g/ml instillation onto either of chicken eyes was used. Capsaicin 10^{-2}ml/g was used to induce pain thirty minutes after the administration of either tramadol, paracetamol or diclofenac. Responses were recorded in blocks of five minutes in an hour. The spread 10^{-1} of dosages (Tramadol 0.3, 3, 3mg/kg, paracetamol 0.3,3,30mg/kg, and diclofenac 0.01, 0.1, 1mg/kg) was used. A dose per bird was got using a nurse calculator. GraphPad Prism 5.0 software, Turkey's statistic, and One way ANOVA analysis showed: Statistical difference ($p < 0.05$***) between the analgesics' respective levels. Paradoxical reactions of the analgesics. Confidence intervals with both ends being positive in the pairwise comparisons. At 95% confident, the standard errors were minimal. The null hypotheses for the three analgesics were rejected. The F-statistic in tramadol was large as in paracetamol where Eta squared 0.7819, F-statistic 49.29 in one way ANOVA. Berlet's test in diclofenac for equal variances indicated significant difference. Analgesics, tramadol, paracetamol, and diclofenac may not alleviate pain in chicken. They displayed paradoxical reactions, hyperalgesia. Farmers may be restricted from buying analgesics over the counter to alleviate pain. This may be made as part of livestock policy for use by veterinarians and researchers.*

Key words: Capsaicin, Pain, Analgesics, F-Statistic, hyperalgesia

INTRODUCTION

Chickens were important animal models in biomedical research by assuming that conditions or pathologies they manifested were applicable to all poultry and humans. For instance, during pain studies, similarities are reported in physiological, and behavioral

responses to pain between chickens and mammals (Gentle, 2011). The aim of this study was to use chicken for pain mechanisms studies, where the activity of some analgesics would be evaluated during capsaicin-induced pain. Alleviation of pain, like that occurring during chicken diseases, using analgesics is important to realize a profit in the poultry industry and for welfare concerns. Weight gain, productivity, and growth rate may also be negatively affected if chicken experience pain (Broom, 1993).

Pain-sensing receptors (nociceptors) have been identified, and they work by sensing painful stimuli from animal body parts. During painful experiences in animals, including chicken, there are cardiovascular and behavioral changes that occur and which can be used to assess the levels of pain being experienced. For example, physiological and behavioral experiments have shown beak trimming in chicks and feather pecking can cause acute pain (Gentle, 2011). Chronic pain, which lasts for a longer period than acute pain can also occur and is important in chicken welfare. Instances, where chronic pain can occur in chickens, include after beak trimming and orthopedic diseases in broiler chickens (Gentle, 2011).

Capsaicin (8-methyl-N-vanillyl-6-none amid), is a phenolic compound in chili peppers, and produces a burning sensation, irritation, and pain on contact, paradoxically. Capsaicin is well absorbed when administered either topically or orally (Suresh et al 2010). It can also be used to manage pain when applied at the correct dose and frequency. However, these unpleasant sensations are not felt by birds, reviewed by (Victor et al., 2016).

Capsaicin stimulates nociceptors through the activation of TRPV1 receptors when administered resulting in related pain behaviors by the target animal (Caterina et al., 1997). When capsaicin was administered in high or repeated doses, it caused analgesia through the desensitization of receptors to the offensive stimuli (Burks et al., 1985) and also through the degeneration of sensory fibers (Hiula et al., 1989). These properties of capsaicin, therefore, make it an important compound to use in pain studies.

Tramadol is an opioid drug and acts by binding to opioid receptors in the central nervous system. It inhibits the reuptake of serotonin and norepinephrine (Dayer et al., 1997).

Paracetamol (synonym: acetaminophen) was used in poultry for the treatment of painful

diseases and pyrexia. Information lacks on the dosage and duration of treatment in poultry species (committee for veterinary medicinal products 1999). In mammals (human beings), paracetamol (acetaminophen) is used to treat pain and fever. It is ineffective in intense pain and has no depressant effect on respiration, (Garry et al., 2005).

Diclofenac is an NSAID drug that offers analgesic, antipyretic and anti-inflammatory properties. Its mechanism of action involves the inhibition of cyclooxygenase pathways (Gan, 2010). It is analgesia poisonous to vultures (Moreno-Opo et al., 2021). The drug can cause nephrotoxicity in humans and animals (Wadkar & Narvekar, 2020). Broiler chicken treated with diclofenac injection 2.5 mg/kg IM depicted severe clinical signs of toxicity with high mortality and a significant increase (P<0.001) in serum creatinine and uric acid concentration (Jayakumar et al., 2010).

Statement of the Problem

In Kenya, indigenous chickens in most cases are reared under free range system of production, where they are predisposed to infectious diseases, which may lead to painful symptoms (Kingori et al 2010). During such painful diseases, chicken may also reduce appetite, emaciate, reduce production, and eventually die, and thus loss of value to the farmer (Malik: Valentine, 2018; Naser et al., 2021). Pain injuries of chicken by predators are controlled by giving first aid to the chicken, euthanize, aspirin 525mg in a gallon is given in drinking water for three days and meloxicam 1.5mg dosage which can be given orally factoring weight and egg withdrawal period, (The-chicken-chick.com: Bitchin ,2021).

Common side effects of opioid administration include sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression. Physical dependence and addiction are clinical concerns that may prevent proper prescribing and in turn inadequate pain management (Ramsin, 2008)

Opioids use in chicken may cause cardiac and/or respiratory depression, which can result to death. Opioids use in veterinary medicine is not common and not enough research has been done to the advice of their efficacy in birds (Bitchin' Chickens, 2021). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like diclofenac were commonly used in animals but studies have indicated different adverse effects to body weight

hence research is required to determine right dosage for each species.

Objectives of the Study

The overall objective of the study was to investigate the effects of selected analgesics in pain management in chicken. Specifically, the study aimed to determine whether administration of readily available pain management drugs namely tramadol, paracetamol and diclofenac.

The specific objectives of the study were: To examine whether administration of tramadol has an effect on induced pain in indigenous chicken: To determine whether paracetamol can reduce induced pain in indigenous chicken and, to evaluate the effects of diclofenac on induced pain in indigenous chicken.

The Hypotheses (H_0)

The hypotheses of study were that administration of tramadol has no effect on pain in chicken: Paracetamol administration onto the eye cannot reduce pain in chicken and, there is no effect of diclofenac on induced pain in chicken.

RESEARCH METHODOLOGY

Forty-eight chickens aged about four months and consisting of both sexes and of good health were acquired from Mwala sub-county within Machakos County. They were transported to South Eastern Kenya University where they were housed and acclimatized for one month before start of experiments. The experimental chickens were fed with grower's mash (80gms per day per chicken) until the fifth month. The feed was weighed electronically and changed from growers to layers mash at the age of five months to cater for the production age requirement of the chicken. Layer's mash (115gms per day per chicken) was given until the end of the experiments. Broken sorghum, green gram and maize grains were fed at small quantities at each stage of growth. Water was provided ad libitum. The cock hen ratio was regulated at 1:10. Treatment, isolation, de-toeing, deworming and replacement of experimental birds were done as part of the routine management. The experimental birds' room temperature and relative humidity were taken using thermo-hygrometer. Each of the experimental birds' details like body weight, temperature and code was captured before the start of every experiment. Pain in experimental birds was induced using capsaicin solution

Formulation of Capsaicin Solution

Capsaicin stock solution was prepared by dissolving 1g Capsaicin (Sigma, U.S.A) in a 100ml vehicle (10% tween 20, 10% absolute ethanol grade and 80% normal saline) as described by (Gamse et al., 1985; Kanui et al., 1990). The vehicle, (described above) was used in the preparation of stock solution and in control experiments in this study. A study was done to establish chicken pain behavior and the effective capsaicin dose for use during subsequent studies on effects of selected analgesics in pain management. The chicken behavior due to capsaicin instillation onto the eye formed the ethogram, eye closure, partial eye closure, eye scratching and head shaking.. This was done using methods explained by (Gamse et., Kanui et al., 1990 and, Kitonyi et al., 2023).

Only birds considered healthy using measurements of vital parameters were used for experiments. The experimental birds were re-used after a period of two weeks. Sick birds were isolated and treated before being used in the experiments. Drug withdrawal time was observed. The experimental birds that died in the course of experiments were replaced. The replacement birds were acclimatized before use in the experimental work.

Determination of the Effect of Tramadol on Capsaicin Induced Pain in Chicken

Three dosage levels were used in the study. The dosage levels were obtained as follows: The highest dosage of 30mg/kg was guided by existing literature from other studies (Sanchez-Migallon Guzman et al., 2012). The medium dosage was obtained by multiplying 30mg/kg by a factor of 10^{-1}. and the low dosage was got by multiplying the medium dosage with the same factor, thus (30, 3, 0.3) mg/kg were the dosages. OMNI calculator was used to calculate doses (mg/ml) of each level for a sample size of n=6 depended on the bird's body weight in the study. For instance, in level 30mg/kg, the dose for a 3.0kg bird was obtained by multiplying the dosage (30mg/kg) by the bird's weight (3.0kg) divided by the drug concentration (manufacturer's concentration indicated on the instruction flyer of the drug) as per OMNI calculator. A chicken from a sample size of n=6 per dosage (0.3, 3, 30) mg/kg was taken and parameters mentioned in 5.2 above recorded. The calculated tramadol dose was injected in the thigh muscle using a five ml syringe and a 21 and or 23-gauge needles. The chicken was immediately placed in an observation chamber described above in section 5.2.

Thirty minutes later, 2 drops of capsaicin 10^{-2}g/ml solution was instilled into one of the eyes of the sedated bird. Then the bird was immediately placed into the observation chamber (described above) for observation of behavioral responses in blocks of five minutes for one hour. The time in seconds in painful behaviors (eye closure, partial eye closure, eye scratching and head shaking) was recorded in blocks of five minutes for one hour. The same procedure was repeated for six birds in each level. Water for injection was used instead of the drug for control.

Determination of the Effect of Paracetamol on Capsaicin Induced Pain in Chicken

A chicken sample size n=6 per dosage (mg/kg) was randomly selected and coded. The experimental birds were weighed electronically (weighing balance rating 0kg to 60kg). The body temperature was taken via the cloaca using electronic thermometer. The room temperature and the relative humidity was measured with the use of thermo-hygrometer. The details were recorded in a book. A maximum dosage of 30mg/kg was chosen based on prior preliminary experiments and literature. Paracetamol doses above 30 mg/kg caused mortalities and severe side effects as per preliminary experiments and literature. The medium dose was obtained by multiplying by a factor of 10^{-1} and the medium dosage was multiplied by a 10^{-1} to obtain the low dosage. The dosage levels used in the study were 30, 3, 0.3mg/kg. In each level, doses (mg/ml) for n=6 were calculated and depended on the bird's weight in the study. In level 30mg/kg, the dose for a 3.0kg bird was obtained by multiplying the dosage (30mg/ml) by the bird's weight (3.0kg) divided by the drug concentration (manufacturer's concentration indicated on the instruction flyer of the drug) using standard omni calculator.

A calculated dose was administered through the wing vein using a ten ml syringe and a 21 and or 23 gauge needle. A stop watch and a wall clock were used to time the period the bird spent in pain behaviors. The bird was then immediately placed into the observation chamber described in section 5.2 above for thirty minutes after which 2 drops of capsaicin 10^{-2}g/ml was instilled into the eye and immediately the bird was returned into the observation chamber for observation of behavioral responses in blocks of five minutes for one hour. The time in seconds spent by the chicken while showing established painful behaviors viz eye closure, partial eye closure, eye scratching and head

shaking were then recorded in blocks of five minutes for one hour (Gamse et al., 1985 and Kanui et al., 1987). The procedure was repeated for a flock of six experimental birds per dosage (30, 3, 0.3) mg/kg. A control experiment for each dosage level was carried out by administering water instead of the drug.

Determination of the Effect of Diclofenac on Capsaicin Induced Pain in Chicken

A chicken from a sample size n=6 per dosage (mg/kg) was randomly selected and coded Three dosages were used. The highest dosage was chosen on the basis of literature and preliminary experiments. A maximum dosage of 1mg/kg was chosen. Th medium dose was obtained by multiplying by a factor of 10^{-1}. The medium dosage was multiplied by a factor of 10^{-1} to obtain the low dosage. The dosage levels used in the study were 1.0, 0.1, 0.01mg/kg. Doses In each level doses (mg/ml) were calculated as in section 5.5 above. An experimental bird from a sample size n=6 per diclofenac dosage was taken. The drug was and administered through the experimental bird's thigh muscle using a micro-fine syringe (insulin syringe). The animal, from n=6 per dosage level (1, 0.1, 0.01mg/kg) was then immediately placed into the observation chamber (as described in section 5.2) for thirty minutes and 2 drops of capsaicin 10^{-2}g/ml was introduced into the eye and immediately the bird was returned into the observation chamber for start of behavioral responses in blocks of five minutes for one hour. Responses were observed as described in section 5.4 above. Water for injection was used instead of diclofenac as control.

DATA ANALYSIS

The data was analyzed using Graph Pad Prism 5.0 statistical software. One-way Analysis of Variance (ANOVA) statistical function was used to check for statistical differences in the time spent on pain behaviors for different dosages and control. Tukey's application was used to separate means.

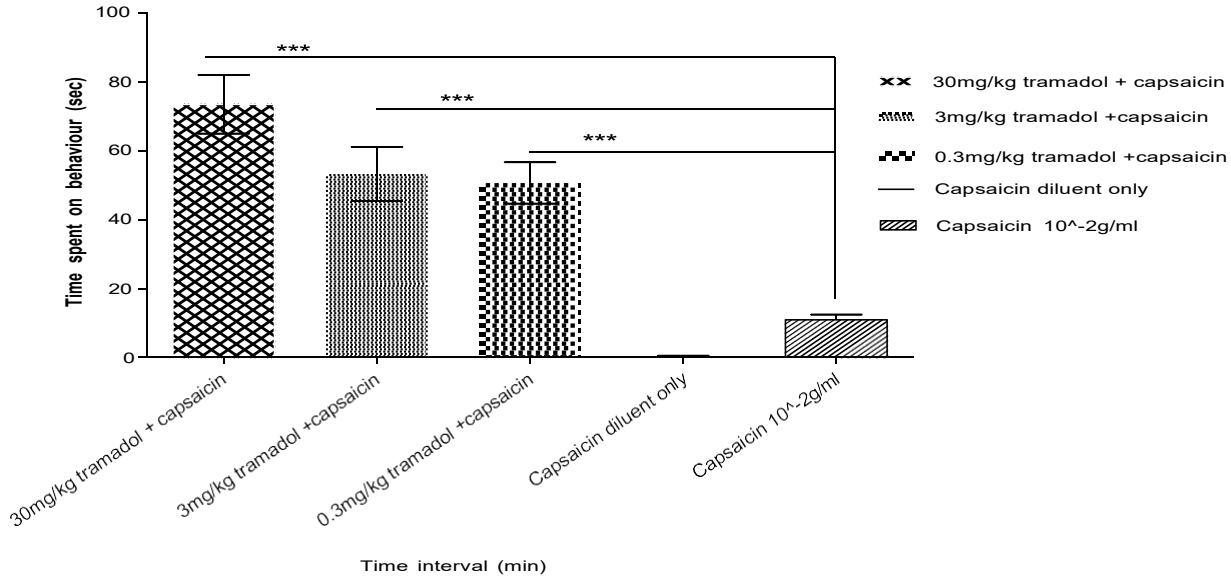
RESEARCH FINDINGS

Effects of tramadol on capsaicin pain induced behavior in chicken

Significant differences ($p< 0.05$) in time taken in pain were noted between chicken subjected to capsaicin 10^-2g/ml alone and a combination of capsaicin 10^-2g/ml with tramadol irrespective of the dosage, (Figure). Tramadol increased pain in chicken at specific dosages. The F-statistic in tramadol was large. The null hypothesis that tramadol

had no effect on pain in chicken was rejected in the study.

Figure 1 : Total mean time spent by chicken in pain behaviors due to capsaicin (n=6) alone compared to capsaicin.10^{-2}g/ml(n=6) applied thirty minutes after tramadol (03, 3, 30mg/kg, n=6)



Significant differences ($P < 0.05$) in time taken in pain were noted between chicken subjected to capsaicin 10^{-2}g/ml alone compared to capsaicin 10^{-2}g/ml applied thirty minutes after tramadol irrespective of the dosage level (Figure 4.2.1) (Mwombobia, 2018 ; Kanui, 1990). It was established that administration of tramadol had an effect in this study on pain in chicken resembling that in human (Chu et al., 2008). The effect was not the same as in red-reared slider turtles (Baker et al., 2011).

Table 1: Parameters used in tramadol analysis

ANOVA Table	SS	df	MS
Treatment (between columns)	45470	4	11370
Individual (between rows)	6938	11	630.7
Residual (random)	15820	44	359.6
Total	68230	59	
F-Statistic	31.61	1.754	
p-values	p<0.0001,		

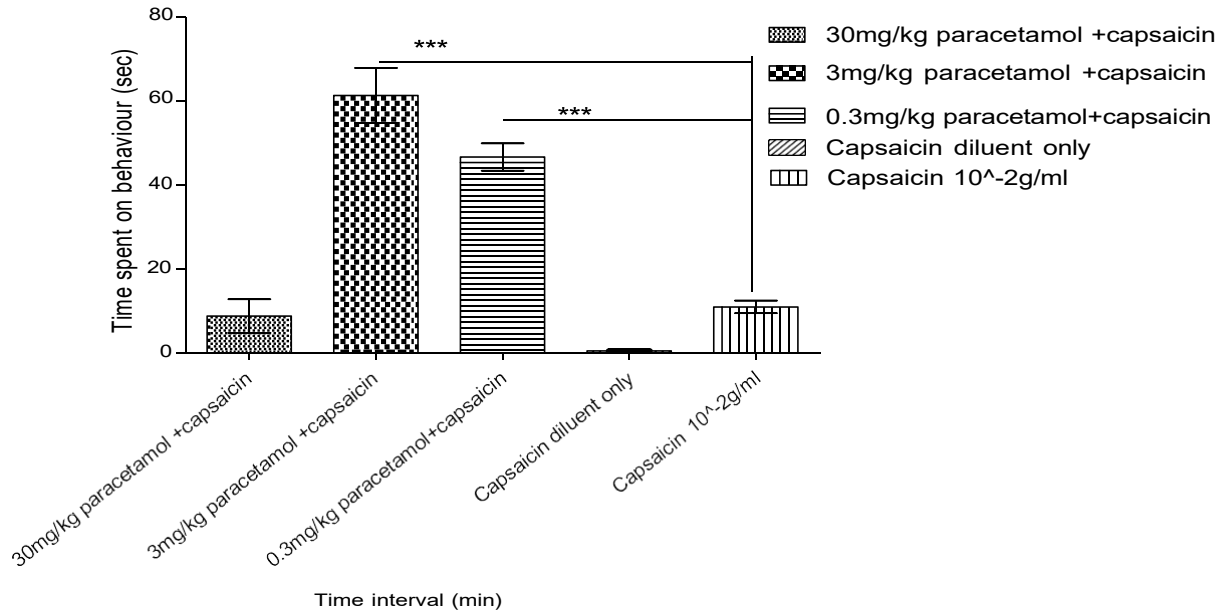
	p<0.0927	
	p<0.05	
R-values	0.7418	0.1017

The eta squared value 0.7418 was large enough to have a significant difference. Five groups were analyzed in this section using the parameters in Table 1. The groups included 0.3, 3,30mg/kg with capsaicin 10^{-2}mg/kg administered thirty minutes after tramadol at each level onto either side of the eyes, capsaicin 10^{-2}g/ml and capsaicin diluent. At p<0.0001the calculated value p<0.0927 was greater implying that the mean values were true and close to the total mean. The F-statistic 31.61 was large enough to reject the tramadol hypothesis that administration of tramadol has no effect on pain in chicken. In that respect tramadol increased pain at the increase of the dosage levels.

Effects of paracetamol on capsaicin pain behavior in chicken

Significant differences ($p < 0.05$) in time taken in pain were found between chicken subjected to capsaicin 10-2 g/ml alone and chicken subjected to a combination of capsaicin 10-2 g/ml with paracetamol at doses 3, 0.3 mg/kg. Paracetamol did increase pain in chicken agreeing with the hypothesis that paracetamol cannot reduce pain in chicken. However, 30 mg/kg paracetamol did not affect the pain behaviors expressed by chicken (Figure 2.0).

Figure 2: Total mean time spent by chicken in pain behaviors due to capsaicin(n=6) alone compared to capsaicin.10-2g/ml(n=6).applied thirty minutes after paracetamol(03,3,30mg/kg, n=6)



Five groups were used for comparison. The results parameters were the ANOVA table $p<0.0001$, $p>0.05$, Barlet's statistic (corrected) 113, R-squared (eta squared) 0.7819, F-statistic 49.29 in one way ANOVA in this study indicated a strong argument significant difference between groups. The F-statistics corresponding to alpha, $p<0.05$ implies that the means were statistically significant. The null hypothesis is rejected in this study. The value of R-squared was more than three quarters which was large enough for significant difference in mean times when the chicken showed in painful behavior (Kanui, 1990).

Effects of Diclofenac on Capsaicin Pain Induced Behavior in Chicken

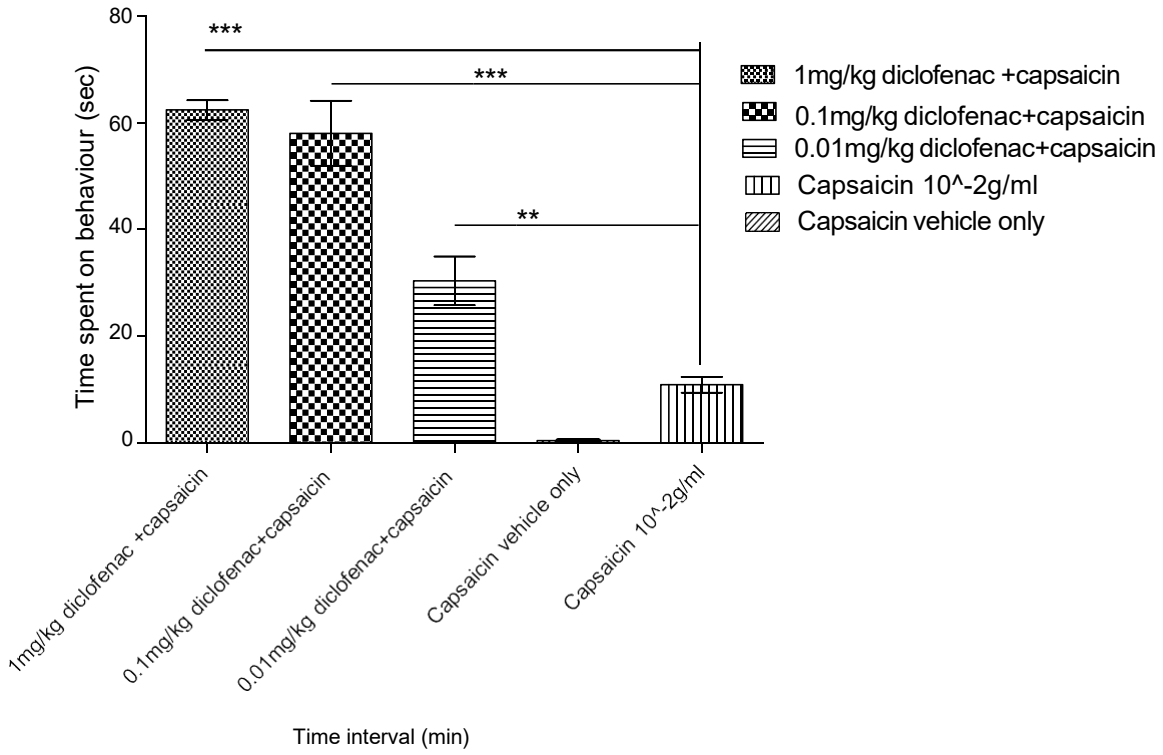
Table 2: Parameters used in diclofenac analysis

Table Analyzed	Diclofenac
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means significant different? (P < 0.05)	Yes
Number of groups	5
F	59.73
R squared	0.8129
Bartlett's test for equal variances	
Bartlett's statistic (corrected)	117.3
P value	< 0.0001
P value summary	***
Do the variances differ significant. (P < 0.05)	Yes

The parameters were used to analyze 5 groups. The F-statistic was 59.73, R-squared 0.8129 at $p<0.05$ at 95%confident. The F-statistic implied that there was a likelihood of a difference between the three levels of diclofenac dosages and the at $p<0.00001$ affirms the significant differences in pain in chicken. Even at $p<0.0001$ Barlet's test (corrected mean) for equal variances indicates a significant difference. R-squared in this study of indicated a high matching difference. Diclofenac in this study had a paradoxical effect in pain management shown in Table 2. The null hypothesis is rejected from the findings in this study and alternative is upheld.

Significant differences ($p < 0.05$) were noted when comparing the effects of capsaicin 10-2g/ml alone to a combination of capsaicin 10-2g/ml with diclofenac at doses 1, 0.1, 0.01 mg/kg (Figure 2). There was increased established effects of diclofenac at specific dosages in chicken. The hypothesis that there was no effect of diclofenac on pain in chicken was rejected.

Figure 3: Total mean time spent by chicken in pain behaviors due to capsaicin(n=6) alone compared to capsaicin.10-2g/ml(n=6).applied thirty minutes after diclofenac (0.01, 0.1 1mg/kg, (n=6)



NB: Capsaicin vehicles are same as capsaicin diluent in this study

Table 3: One way ANOVA Table for multiple comparison of diclofenac for 5 groups, n=6.

ANOVA Table	SS	df	MS
Treatment (between columns)	36520	4	9131
Residual (within columns)	8408	55	152.9
Total	44930	59	

There was a difference in pain between the diclofenac three levels in increase of pain when diclofenac was injected intramuscularly and capsaicin 10^{-2}g/ml instilled in either of the eyes thirty minutes later. This is supported by the fact that SS: MS value was large at 4 degrees of freedom.

DISCUSSIONS

Tramadol

This study also demonstrated that chicken have a functional opioid receptor pathway that also responds in a dose dependent manner. This was demonstrated by dose dependent responses exhibited following administration of tramadol at dosages 30mg/kg, 3mg/kg and 0.3mg/kg which is an opioid compound. The administration of tramadol increased pain in a dependent way in this study. The findings of the investigator were in agreement with the findings in reptiles as stated in the following statement. In reptiles, opioid drug administration has yielded unexpected results with respect to analgesia (Baker et al., 2011). However, the above contradicts the conventional use of tramadol because it has been used in the treatment of muscle pain, joint pain and wound pain (Muna et al 2019). Patients having medical history of addiction drug, head injury, alcoholism, , seizure, anabolic and catabolic disorders have more possibility of seizures when treated with tramadol (Muna et al., 2019). The finding that tramadol administered followed by capsaicin at specific doses increase the period under pain behavior indicated that it caused hypersensitivity in chicken, a condition known as hyperalgesia. Significant differences ($P < 0.05$) were noted when comparing the effects of capsaicin 10^-2g/ml alone to capsaicin 10^-2g/ml applied 30 minutes after tramadol doses 30, 3, 0.3 mg/kg. This has also been reported in rats (Abreu et a*l* 2015), mice (Frias: Merighi 2016), and humans (Chu et al., 2008). The hyperalgesia activity of capsaicin applied 30 minutes after tramadol may be explained by the fact that tramadol is an opioid compound. Opioids have been shown in some cases to cause hyperalgesia activities (Lee et al., 2011).

Paracetamol

Significant differences ($P < 0.05$) were noted when comparing the capsaicin 10^-2g/ml alone to capsaicin 10^-2g/ml applied 30 minutes after paracetamol dosages 3, 0.3 mg/kg. Paracetamol did not reduce pain in chicken but increased it at the above two dosages. In mammals, especially in human beings, Paracetamol (acetaminophen) is

popular and its used analgesic drug for the treatment of pain and fever. This was centrally to the findings of this study. Paracetamol showed no significant difference at dosage 30mg/kg which was in tandem to the findings of (Jessica et al., 2021).

Diclofenac

The findings agree with the finding by Jayakumar et al., 2010 that birds treated with diclofenac injection 2.5 mg/kg IM depicted severe clinical signs of toxicity with high mortality (Jayakumar et al., 2010). It was done in preliminary experiments. In all the three dosages1, 0.1, 0.01 mg/kg used diclofenac pain increased. This contradicts the common phenomenon that common analgesics relieve pain (Bertolini et al., 2006).

Diclofenac and capsaicin administration topically on rat, mice and human that skin has been reported to cause analgesia (Ercan et al., 2013: Predel et al., 2020). Diclofenac was well absorbed and distributed when administered through the intramuscular route (García-López, 1999). In this study it was administered intramuscularly through the thigh.

Contributions of Research Findings to the Society

The research provided knowledge that tramadol, paracetamol, and diclofenac use increase pain in chicken. Therefore, they may not be used in pain management in chicken. It may be taken as a caution to the farmers in chicken value chain not to abuse the three drugs in pain management.

CONCLUSIONS AND RECOMMENDATIONS

Tramadol

The study showed that an opioid drug, tramadol when applied at specific doses then capsaicin administered ocularly thirty minutes afterwards in chicken induced more painful responses. These responses were contrary to the normal use of tramadol in pain alleviation as in mammals (Kanui et al., 1990; Kaido et al., 2020, Palazzo et al., 201 ;Pelissier et al., 2002). It is concluded in this study that tramadol may not be good in pain alleviation in chicken.

Paracetamol

Hyperalgesia was realized at 0.3 and 3mg/kg levels in paracetamol administration after capsaicin 10^{-2}g/ml was ocularly administered thirty minutes later onto the either eye of chicken. There was no

significant difference between paracetamol 30mg/kg and capsaicin 10^{-2}g/ml. It may be not suitable to control pain in chicken using paracetamol as per this study.

Diclofenac

Diclofenac, an NSAID drug increased pain in chicken in this study when applied at specific doses and capsaicin 10^{-2}g/ml added thirty minutes later. The potentiation of painful effects of diclofenac onto either of the eye of chicken means that it may not be safe in the management of pain in chicken

It is therefore recommended that analgesics may not be sold over the counter to manage pain in chicken because of unexpected responses (hyperalgesia) that may arise as for the three selected ones.

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