EFFECT OF TRANSPORTATION ON STRESS HORMONES OF INDIGENOUS CHICKEN IN MACHAKOS COUNTY, KENYA

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ABSTRACT

The objective of this study was to establish the effect of transportation on stress hormones of indigenous chicken in Machakos County, Kenya. The study was experimental with two treatments. The first batch of 4 hens were tied together and loaded on to an open roof top of the transport vehicle. The second batch of 4 hens was loaded into traditional transport cages and the cage loaded on top of the transport vehicle. The test birds acted as their own controls. Transport conditions of temperature, relative humidity, air speed, vehicle velocity was measured by use of automatic data loggers. Cortisol levels were measured for each treatment separately at the beginning and at the end of a 1 hour 59 minutes journey on a road stretch of 119.5 km (Machakos-Kitui road). Statistical t-tests at $P<0.05$ were run to determine effect of each treatment and difference between treatments. The study showed that transportation increased serum cortisol levels. The amounts were significantly higher in birds transported on the open roof top by 26.05 ng/ml. The study recommends that transportation of chicken should be done using cages designed, constructed and fitted properly to ensure sufficient floor and head space to allow the chicken to sit comfortably and evenly distributed during transportation as appropriate for the chicken size and weight. Further, transportation cages should be adequately ventilated to meet the thermoregulation conditions of the birds. The findings will be important in providing empirical evidence that helps in improving transportation conditions, and help in directing policy in the industry.

Keywords: Cortisol, Stress, Transportation, Welfare.

INTRODUCTION

In Kenya, indigenous chicken production has been an important livestock enterprise in rural households where 70% of the country's population lives (Wachira, et al. 2018). Indigenous chicken rearing in Kenya is mostly carried out in small scale and in form of free-range system across different geographical areas and transported by road over long distances to urban areas for marketing and processing. This makes transportation a crucial activity along this value chain. The modes of transportation mostly involve the placement of birds either in transport containers, which are subsequently loaded on to the available means of transport (such as vehicles, motorbikes, and bicycles), simply hung on open vehicle rooftops, bike handles, and carts, or carried by hand for transportation to their final destination. According to Mitchell and Kettlewell (2009), all procedures and practices involved in poultry transportation and the micro environments prevailing in containers and means of transportation may impose varying degrees of stress upon the birds, which may result in a compromise of their welfare status, health and productive efficiency depending upon the magnitude of the stress imposed.

Globally, to address these concerns, particular attention has been placed on animals' welfare with regard to animal transportation practices. In Kenya, the Prevention of Cruelty to Animals

Act (CAP 360) is in favor of introduction of regulations to improve the welfare of animals during transportation. The law demands that animals, while being transported should be supplied with adequate food, water and shelter in comfortable carriers (GoK, 2012). However, the standards are not explicitly defined to properly curtail transportation of chicken in substandard conditions. Multiple interactive stressors associated with aspects of transportation have been cited to be responsible for adverse effects on the physiological and biochemical status of birds on transit (Jayaprakash, et al, 2016). These effects have a direct relationship to the birds' productivity, quality of their products and welfare.

Transport related stresses are mainly triggered by the nature of bird handling (catching loading and offloading of the birds onto the transport vessel) and due to the effects of transportation microenvironment. Mitchell and Kettlewell (2009) identified major factors, which may act in isolation or in combination to impose various degrees of stress to the birds during transportation to include thermal demands of the transport microenvironment, acceleration, vibration, motion, impacts, fasting, thirst/dehydration, social disruption and noise. However, according to Nilipour (2002), thermal demands constitute the major threat to animal well-being and productivity as it may lead to imposition of thermal loads upon the birds on transit resulting in moderate to severe thermal stress and consequent reduced welfare, increased mortality due to either heat or cold stress, muscle damage and associated changes in product quality (Mitchell & Kettlewell, 2009).

The degree of these impacts however depends on the mode of transportation, speed, microenvironment, time and distances covered (Qi, et al., 2017). Stressful conditions stimulate adaptive response such as regulation of body temperature, behavioral changes and release of stress hormones from the adrenal gland, which is the main stress response organ (Vuuren, 2011). That is, stress influences parameters of bird physiological responses, like the release of corticosterone (CORT), glucose and creatine kinase (CK) from the adrenal gland (Qi, et al., 2017). These hormones are to prepare the birds by stimulating glycogenesis for release of energy into the birds' muscles and blood (Tang, et al, 2013). Quantities of these indicators in the blood stream thus acts as indicators for evaluating stress levels of the birds on transit. This is crucial in improving the management of transport conditions and care to meet the needs of the birds (Mitchell & Kettlewell, 2009).

LITERATURE REVIEW

Several studies have been carried out to establish the effect of transportation stress on chicken welfare with the major indicator of welfare concern being evaluated by the level of stress hormones in the blood (Lengkey, et al., 2013; Bulitta, 2015; Jayaprakash, et al., 2016; Arikan, et al., 2017). According to (Scanes, 2016) in poultry, two of the most common physiological parameters of stress are circulating concentrations of the adreno-cortical hormone, corticosterone (CORT) and the heterophil:lymphocyte ratio (H:L). There is conflicting conclusion regarding predominance of different stress parameters in avian species. While a review by Carsia (2015) concluded that the main avian adrenal glucocorticoid is corticosterone (CORT) with some cortisol being produced, albeit at a very low level, Kim et al. (2015) recently reported high plasma concentrations of cortisol.

According to (Scanes, 2016) the basis of this high concentration of cortisol is not readily apparent but again points to the need for close attention to assay validity. Further, most of these studies have focused on broilers and are based on regions outside Kenya. It is imperative to incorporate existing knowledge on other major types of chicken breeds such as indigenous breeds and to assess how these breeds are equipped to respond to "transportation stress" and how these characteristics are influenced by different climatic conditions in different localities as well as using different means of transport. Machakos County has unique climatic conditions whereby findings from different environmental conditions may not wholly apply. Nevertheless, this study focused on transport stress levels in indigenous chicken which have previously not been studied in Kenya

Since the level of serum cortisol and by extension welfare concerns have a direct influence on the chicken productivity and quality of their products (Nilipour, 2002), it implies that these concerns have an indirect impact on the economic contribution of the sub sector. Therefore, a study to create an understanding on the effect of transportation stress on stress hormones in indigenous chicken is significant not only in improving transportation conditions for the chicken, but also to provide information crucial in tapping opportunities of improving the earnings of rural households, whose livelihoods depend on the sub sector. Subsequently, with long-term contribution towards poverty reduction and wealth creation. The knowledge from the findings will fill the gap on the effect of indigenous chicken transportation and how the welfare can be improved during transportation and reduce stress levels in chicken.

METHODOLOGY

The study used 4 hens per group for the experiment. Hence, 4 subjects (female chicken) were used to test differences between treatments for mean cortisol levels between two groups at an α level, $p = 0.05$. In this study, the sampling unit comprised of the indigenous chicken transported by road using vehicles across Machakos County while the experimental unit comprised of specific birds on which treatments were randomly assigned for data collection.
Sampling involved purposive sampling of the transport route while sampling of the birds involved complete randomized design. Machakos-Kitui tarmac road stretch of 109.5 km was selected. Choice of the route was such that variability on vibrations between tarmac and non-tarmac roads was controlled. The total sample was randomly divided into groups and different treatments applied to the groups, one treatment for each group. The general assumption was that birds transported with and without transport cages experience different microenvironments.

Data collection involved field measurements, observations and data analysis. Data was collected through measurement of the test variables. The study model was such that transport conditions (ambient temperature, relative humidity, air speed and transport time) induce release of stress hormones (cortisol). The birds were rested, adequately fed and watered 24 hours before the experiment. Treatment birds were allowed free movement, caught and carefully loaded onto the transportation vehicle. The birds acted as their own control. The experimental units were carried either in traditional chicken transportation cages or bundled together and loaded on the means of transport as described in Table 1.

Table 1 Treatment Design

Treatment 1	Treatment 2	Control
4 birds tied together and loaded on an open roof top of the transport vehicle	4 birds loaded into transport cages and the cages loaded on an open roof top of the transport vehicle	The birds acted as their own control by comparing readings before and after

Equal number (4) of mature female indigenous chicken weighing between 1.75 and 2.4 kg were used for each treatment. Completely Randomized Design, with two levels (at the beginning, T0 and the end of the journey, T1) were used to assign the treatments. Measurements of the ambient conditions for the treatments were as shown in Table 2.

Table 2 Ambient Condition Measurements

Parameter	Point of Measurement	Frequency	Instrument
Ambient temperature	At bird level	Continuous	Automatic temperature monitor
air speed	At bird level	Continuous	Automatic Anemometer
Ambient Relative humidity g/m^3	At bird level	Continuous	Automatic relative humidity meter

Data Capture (Ambient Conditions): Continuous recording of ambient temperature, relative humidity and air speed was achieved by use of programmed automatic data capture equipment. The data capture equipment was attached to a wire frame and clipped onto the front of the cage or just mounted next to the birds. The position of the equipment was to ensure that the conditions being monitored were at bird level. Start and end times were documented to establish experiment duration and average ambient conditions in terms of temperature, air speed and humidity for the entire journey.

Table 3 Measurement of Cortisol level

Parameter	Point of Measures	Frequency	Instrument
Cortisol	Blood samples	*Time $_0$ and Time $_1$*	ELISA Kits

Data Capture (Cortisol): Blood samples, containing anticoagulants (EDTA) were placed on an icebox and transported to the lab for freezing within 2 hours for further serum analysis (hormonal).

Data Analysis Plan: Data analysis involved two models. One model testing the effect of each treatment (paired sample t-test) and the second model testing the difference between the treatments (independent sample t-test). Paired sample t-test entailed observations of the test variables before and after treatment on the same experimental units to test whether the mean difference between the two sets of observations (pre and post-treatments) is zero. The null hypothesis was that there was no difference in mean pre and post-treatments while the alternative hypothesis was that, there is a difference in mean for pre and post-treatments. Evaluation criteria involved test of significance (p- value) at 95% level of confidence. The null hypothesis was rejected if p-value was less than 0.05 indicating strong evidence that the treatment causes a change in the mean of the test variables.

The second test model was a two-sample t-test with 2 types of treatments (***Treatment 1 and Treatment 2***). The null hypothesis was that there was no difference in mean between treatment 1 and treatment 2, while the alternative hypothesis was that, there is a significant difference between the treatments.

Evaluation criteria involved test of significance (p- value) at 95% level of confidence. The null hypothesis was rejected if p-value was less than 0.05 indicating strong evidence that there was a difference between the treatments.

Ethical Consideration: The study strived to handle the chicken as humanely as possible. No bird was subjected to treatments extraneous beyond the normal practice of transportation of chicken in the county. The birds were adequately watered, fed and rested prior to the journey and were released immediately on arrival to limit further discomfort post the journey.

RESULTS

Ambient Conditions: The experiment was conducted between 13:26 pm and 15:50 pm. During the transportation period, the highest temperature was recorded at the start of the journey at 34.3 ^{0}C. The lowest temperature recorded was 26.8 ^{0}C.

Effect of Transportation Stress on Serum Cortisol: Findings in Table 4 show an increase in the mean serum cortisol level for treatment one at time T_1 by 28.1 ng/ml and a mean increase in the serum cortisol level for treatment two at time T_1 by 2.05 ng/ml.

Table 4 Means (± SE) for cortisol (ng/ml) before and after treatment for the two treatments

Parameter	Treatment 1		Treatment 2	
	T_0	T_1	T_0	T_1
Cortisol	27.1	132	28.8	47.1
	81.5	94.0	78.6	61.5
	86.3	91.7	60.5	76.3
	94.0	83.6	72.8	64.0
Mean	72.23	100.33	60.18	62.23
Stdev	17.498	21.583	15.137	11.980
SEM (Stdev/(SQRT (N))	8.75	10.79	7.57	5.99
Coefficient of Variation	24.2%	21.5%	25.2%	19.3%

Comparing the means, Table 5 indicates a statistically significant difference between treatment one and treatment two at time T_1 (t=3.0870, p=0.0273). That is, treatment one resulted in higher values of serum cortisol compared to treatment two by an average of 26.05 ng/ml. Thus, while both treatments led to an increased quantity of serum cortisol in the test subjects, on average birds transported on open roof top recorded higher values by 26.05 ng/ml compared to birds transported in the traditional cages.

Table 5 t-test for Serum Cortisol between and within groups. In bold are the means that showed statistically significant differences at P<0.05

Parameter	Paired sample t-test (Pre and Post)		Paired sample t-test (Pre and Post)		Independent T- Test (Unequal variances assumed)			
	Trt1 (T_0)	Trt1 (T_1)	Trt2 (T_0)	Trt2 (T_1)	Trt1 (T_0)	Trt2 (T_0)	Trt1 (T_1)	Trt2 (T_1)
Mean	72.225	100.325	60.175	62.225	72.225	60.175	100.325	62.225
Variance	931.520	465.810	494.460	143.520	931.516	494.456	465.809	143.516
Observations	4	4	4	4	4	4	4	4
Pearson Correlation	-0.9987		0.6109		-		-	
Hypothesized Mean Difference	0		0		0		0	
df	3		3		5		5	
t Stat	-1.0790		-0.2319		0.6382		3.0870	
P(T<=t) two-tail	0.3596		0.8315		0.5514		**0.0273**	
t Critical two-tail	3.1824		3.1824		2.5706		2.5706	

DISCUSSION

The findings of an overall increase in cortisol level on birds after transportation is in line with studies by Zhang, et al. (2009), Kang and Kuenzel (2014), Huth and Archer (2015). That is, transportation induces release of higher levels of cortisol into the chicken blood. This is an indicators of stress response. Even though the increase was not significant within both treatments, the difference between treatments was significant. This showed that transportation of chicken on an open roof top vehicle comparatively induces release of more cortisol compared to transportation using the traditional cages.

CONCLUSION AND RECOMMENDATIONS

The study objective sought to establish the effect of transportation on stress hormones of indigenous chicken in Machakos County, Kenya. Based on the findings, birds transported on open roof tops seem to be subjected to higher stress levels than those transported in the traditional cages. This study therefore indicates that birds in transit do undergo levels of stress. Nonetheless, there is need for improvement of transportation conditions. The study recommends use of cages designed, constructed and fitted properly to ensure sufficient floor and head space to allow the chicken to sit comfortably and evenly distributed during transportation as appropriate for the chicken size and weight. Further, transportation cages should be adequately ventilated to meet the thermoregulation conditions of the birds. For policy, the study recommends passing and fully enforcing of regulations on recommending standard chicken transportation equipment to safeguard on the animal welfare.

Further studies need to be conducted to indicate whether this transport induced stress affects palatability, weight of the chicken and other physiological aspects. Comparative studies need to be conducted to indicate whether time of day has an influence on the level of transportation stress since the current study was only done out around mid-afternoon. Further, the study was cross-sectional, additional studies at various times of the year would also be insightful for drawing conclusions. Moreover, the current study only focused on female indigenous chicken. Comparative studies using cocks is highly recommended.

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