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SIMULATED TRANSPORTATION STRESS: ITS EFFECT ON THE PRODUCTIVITY AND SELECTED BIOCHEMICAL AND HAEMATOLOGICAL INDICES OF LAYING HENS

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ABSTRACT

In the Philippines, ready-to-lay (RTL) hens are often sold to poultry layer farms as new stocks or replacement. These stocks are transported from the supplier farm via trucks which can last from hours to days. This study aimed to determine the effect of various transportation durations using a mechanical vibration simulator on the productivity and selected haematological and biochemical indices of laying hens. Results revealed that increasing vibration (stressor) duration exposure elevated the total white blood cell count, and heterophil-to-lymphocyte ratio (H/ L) of the hens, an indicator of stress levels. Even with the shortest vibration exposure of 2 hours, H/ L values were still elevated until the 2nd week. Blood glucose level was found significantly different between treatments, but with no correlation with feed intake. No significant difference was observed on plasma protein values and egg production among treatments. The stressor did not affect egg production but had an effect on the animal's feed intake.

Keywords: blood sugar, heterophils, layer chickens, lymphocytes, stress

INTRODUCTION

Ready-to-lay (RTL) hens are being sold by commercial farms to other poultry layer farms that lack space, facility nor expertise to brood or grow their own layers. For convenience and less risk to morbidities and mortalities brought about by brooding and growing, some farm owners prefer to purchase RTL hens. However, when purchased RTL hens are transported from the supplier farm, this subjects the animals to physiological stress that might affect their productivity (Mumma *et al.*, 2006; Altan *et al*., 2003).

The intensity, specialization and market demand of poultry have necessitated transportation of these animals from production areas to end users in different locations all over the world (Minka and Ayo, 2009). Transportation distance, duration and modes as well as practices prevailing in containers and vehicles may cause varying degrees of stress on the birds (Nicol and Scott, 1990; Mitchell *et al.,* 1998; Carlisle *et al.*, 1998), which can compromise their welfare status, health and production efficiency. It is believed that the transmission of vibratory movements can create uncomfortable conditions to the animal (Gebresenbet *et al.,* 2011), leading to stressful conditions, possible lesser egg productivity, possible death, and consequent profit losses (Mitchell *et al.,* 1998; Mashalay *et al.*, 1984).

The ultimate goal of animal researchers studying welfare issues is to find new ways to eliminate the negative effects of husbandry stress that impair the health and well-being of farm animals while maintaining acceptable levels of productivity from animals. The stress effects of vibrations in poultry transport were investigated in several studies (Carlisle *et al.,* 1998; Randall *et al.,* 1997; Warris *et al.,* 1997; Abeyesinghe *et al.*, 2001; Garcia *et*

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al., 2008). However, related studies in layer have been limited. Although the effects of simulated long transport on behavioural characteristics in two strains of day old chicks were already studied (Valros *et al.*, 2008), the effect of simulated transportation stress in relation to haematological indices in ready-to-lay pullets has not yet been explored. While transportation stress can apparently have an effect on the productivity of RTL hens, no recent studies have been conducted under Philippine settings. Moreover, physiological stress experienced by laying hens in transit may dictate its productivity during its useful years. An investigation dealing with simulated transportation stress is therefore important. Hence, this study was conducted.

METHODOLOGY

Individual experimental cages (0.30 m x 0.24 m x 0.30 m) were made of bamboo slats, coconut lumber and screens. Each cage had its own waterer and feeder. Two weeks prior to the conduct of the study, the area and cages were cleaned and disinfected.

Forty 16-week-old Dekalb White RTL pullets from a commercial layer farm in Cebu Island were used. These animals were certified to be completely vaccinated against common poultry diseases in the Philippines. Upon arrival in Leyte island, the birds were randomly distributed into four treatment groups (T0 = 0 hour, T1 = 2 hours, T2 = 4 hours and T3 = 6 hours of vibration duration) with four replicates per treatment and one bird per replicate following completely randomized design (CRD). The pullets were given glucose monohydrate supplementation in drinking water (5 g per 25 l of water) to minimize transport stress. Acclimatization lasted for 3 weeks, where chick starter mash feed ration were given for the first 2 weeks and was gradually shifted to layer mash at the start of the third week with the following ratio: 25:75 on the 1st day, 50:50 on the 2nd day, 75:25 on the 3rd day and 100% layer mash on the 4th day. Feeding was done twice a day (7 a.m. and 3 p.m.). Access to fresh drinking water was provided at all times. Cleaning of the area was performed twice a day.

The vibration simulator (Fig. 1) consist of a table on soft springs with actuating system that included adjustable weights on two counter rotating shafts (counterweights), that revolves in opposite directions at the centre of gravity of the table and its load, providing vertical vibration. Because the frequency of the vibration simulator table is counterweights, the frequency of the table was obtained based on the number of revolutions of the electric motor (Shahbazi *et al.,* 2010).

Figure 1. Vibration simulation set-up of the experimental birds consists of a table on soft springs with actuating system that included adjustable weights on two counter rotating shafts (counterweights), that revolves in opposite directions at the centre of gravity of the table and its load, providing vertical vibration. Because the frequency of the vibration simulator table is counterweights, the frequency of the table was obtained based on the number of revolutions of the electric motor.

Prior to the experiment, the birds were deprived of feed for 7 hours. The pullets were placed inside a crate (1.0 m x 0.6 m x 0.3 m). Each crate has a capacity of 10 birds, similar to the one used in transporting birds. The crates were then placed on top of the simulator. The customized simulator with 1 horsepower and 1800 rpm was designed to mimic that of a cargo truck transport (Figure 1).

Blood samples (0.25-0.5 ml) were collected after the simulation procedure (1 hour, 20 hours, 1 week and 2 weeks). Collection site was disinfected prior to sample collection from the brachial vein of the chicken wings using a 1-ml sterile syringe. Collected samples were placed in an EDTA (ethylenediaminetetraacetic acid) microtainer, and were immediately processed.

Haematological and biochemical values obtained included packed cell volume (PCV), total WBC count, differential leukocyte counts, blood glucose and plasma protein. The initial and weekly body weights of the laying hens were monitored for 4 weeks after the simulation procedure. The feed intake, egg production, egg weight, and FCR were also monitored for 28 days.

Data gathered were analysed using One-way ANOVA (repeated measures) with post-hoc analyses (Tukey's Honesty Significant test, least significant denominator). Variables were subjected to multivariate analyses using multiple ANOVA (MANOVA). Statistical analyses were performed using Statistical Packages for Social Science (SPSS®) v.22 (IBM, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Haematological and biochemical indices

Significant differences on PCV were observed between observation times [F (3,34) = 3.70, p-value = 0.021], but not between treatments. Similarly, transportation stress was observed to have no significant effect on PCV in pigeons (Scope *et al*., 2002) (Table 1).

A highly significant difference was observed across observation times [F (3,34) = 75.20, p-value = 0.000]. An interaction between observation time and treatment was observed [F (9,82.90) = 5.67, p-value = 0.000]. There was also a highly significant difference observed between treatments [F (3,36) = 29.39, p-value = 0.000]. This indicates that WBC count can be affected by vibration duration. WBC counts have been used to generally assess stress levels in animals (Kegley *et al*., 1997). However, the heterophil to lymphocyte ratio was shown to be more meaningful in assessing stress in birds (Gross and Siegel, 1983).

Heterophil and lymphocyte levels appeared to be in the normal range for the control group across all observation times. Heterophil counts were higher than the normal for T2

Table 1. Mean packed cell volume (%) of layers subjected to varying vibration duration.

Group	1 hour	20 hours	1 week	2 weeks	Over-all Mean
Control	32.00 ± 4.78	29.80± 6.09	25.90 ± 4.53	28.40 ± 2.32	29.03
T1	26.90 ± 5.84	28.40 ± 3.24	26.20 ± 3.33	26.13 ± 4.73	26.91
T2	25.50 ± 6.19	30.40 ± 4.01	26.60 ± 3.31	25.20 ± 3.71	26.93
T3	33.30 ± 7.41	25.10 ± 4.23	25.80 ± 3.99	26.10 ± 5.80	27.58

T1=2 hour, T2=4 hours, T3=6 hours

and T3 in the 1st hour of observation, and for T1 to T3 in the 20th hour of observation (Table 2). Statistical analyses showed significant differences between animals, treatments and observation times (p<0.001). However, heterophil and lymphocyte counts are not useful indicators for stress when interpreted individually, and must be converted to ratio to be meaningful (Gross, 1990).

The eosinophil count was high in birds subjected to 6 hours vibration duration (T3) and low in the control group (T0) during the first hour of observation, however during the 20th hour, the eosinophil count in T3 dramatically decreased until after two weeks of observation. Jain (1986) reported that a reduction in relative eosinophil numbers is more often a stress reaction than a response to disease. This suggests that eosinophil could be used as a reliable indicator of stress in layer chickens. Statistical analysis shows significant difference between treatments (p<0.01) and in the interaction between the treatments and time (p<0.001). The basophil number of this study shows that layer hens stressed for several hours (T3 and T2) have higher number of basophils as compared to unstressed animals (T0). Difference between the four treatments were highly significant from each other (p<0.001) and an increase in basophil numbers has been suggested as a method of detecting stress in birds (Melesse, 2011). The results of this study revealed that the number of monocyte decreased with an increase in stress exposure which coincides with study of Joseph *et al.* (1991) in albino rats as exposed to chronic heat stress. Statistical analysis on the monocyte level revealed significant difference between the four treatments (p<0.1).

Table 2. Average counts of heterophil (H) (%) and lymphocyte (L) (%) of layers subjected to vibration stress at different observation times.

Group	1 hour		20 hours		1 week		2 weeks	
	H	L	H	L	H	L	H	L
Control	24.1±8.1	48.7±10.1	30.3±9.1	49.0±8.3	25.3±7.1	58.4±10.4	24.0±7.5	61.4±6.7
T1	45.2±10.3	36.7±11.4	53.6±3.4	24.1±6.5	35.8±10.7	41.3±12.2	23.5±6.2	58.5±6.2
T2	51.8±7.3	22.4±5.7	55.3±7.4	20.9±5.1	34.9±7.8	40.0±9.9	30.6±6.3	49.9±9.4
T3	58.5±9.8	22.1±6.7	68.7±5.5	17.9±3.4	44.3±11.7	30.3±10.1	40.4±11.7	47.2±10.0

Normal reference range: 15-50% for heterophils, 29-84% for lymphocytes (Tharall *et al*., 2013); T1=2 hour, T2=4 hours, T3=6 hours

Table 3. Heterophil to lymphocyte ratio and stress level (SL) of layers exposed to different vibration durations.

Group	1 hour SL				20 hours SL				1 week SL				2 weeks SL				Over-all mean
	L	O	H	Ave	L	O	H	Ave	L	O	H	Ave	L	O	H	Ave	
Control	-	-	10	0.53 ± 0.25	-	8	2	0.66 ± 0.27	-	5	5	0.46 ± 0.21	-	10	-	0.40 ± 0.16	0.52
T1	-	-	10	1.44 ± 0.77	-	2	8	2.40 ± 0.79	-	-	10	0.98 ± 0.49	-	3	7	0.41 ± 0.16	1.31
T2	-	-	10	2.50 ± 0.93	-	-	-	2.86 ± 1.07	-	-	10	0.95 ± 0.41	-	3	7	0.65 ± 0.23	1.74
T3	-	-	10	2.95 ±1.21	-	-	-	4.00 ±0.98	-	-	10	1.72 ± 0.94	-	2	8	0.97 ±0.66	2.41

L=low(=<0.2), O=optimum (0.21-0.79), H=high(0.8 and above) (Gross and Siegel, 1993); T1=2 hour, T2=4 hours, T3=6 hours

H/ L ratio has been used as a reliable stress indicator in birds. Higher ratio values were observed in animals that were treated with longer vibration durations. An hour after the stressor (vibration) was introduced, all treatments showed high levels of stress as shown by their H/ L ratios. The increase of H/ L ratio in the first hour after stressing the birds is similar to the findings of Jones *et al*. (1991) in Japanese quails and Marin *et al*. (2001) in domestic chicks exposed to a stressor. Peak values were observed on the 20th hour. H/ L ratio has been shown to peak in the said observation time post-stress exposure (Gross, 1990). T3 consistently showed the highest ratio values. An increasing trend on the values was also seen in each observation time where controls had the lowest value (Table 3). Statistical analyses revealed that there was a significant difference between the animals [F (2.30,82.64) = 61.51, p-value < 0.001], treatment groups [F (3, 36) = 45.81, p-value < 0.001] and collection times [F (3,34) = 59.97, p-value < 0.001]. Treatment and collection times of the different animals were found to have highly significant interaction on its effect on H/ L values (p-value < 0.001). Post-hoc results showed significant differences among treatment groups (p-values < 0.001 against T0, T1 and T3, p-value < 0.05 against T2). Multivariate analyses revealed that vibration duration revealed significant differences on the H/ L ratio between groups (almost all observation times have p-value < 0.001, except at 2nd week where H/ L ratio had p-value = 0.005; R2 values were 0.57, 0.70, 0.35 and 0.24 for 1st hr, 20th hrs, 1st week and 2nd weeks, respectively). Results indicated that the animals were initially stressed, but later adjusted as the H/ L ratio lowered in the 1st and 2nd weeks post-vibration exposure. Maxwell (1998) showed that for 7 days, heterophil becomes unrecognizable, and lymphocyte counts gradual increases as the production of lymphocyte inhibiting corticosterone is also decreased. The high H/ L ratio values in stressed birds were in agreement with the results of previous studies (Gross and Seigel, 1983; Maxwell, 1993; Ots *et al*., 1998; Aguirre *et al*., 1995). Birds that are exposed to

Table 4. Average blood glucose (mg/dL) values of layers subjected to varying vibration stress at different collection periods

Group	1 hour	20 hours	1 week	2 weeks	Over-all mean
Control	253.50 ±27.62	257.50 ±24.05	224.90 ±17.27	239.80 ±22.73	243.93
T1	233.90 ±17.09	252.50 ±22.80	224.80 ±16.94	228.20 ±11.77	234.85
T2	233.80 ±22.06	252.10 ±16.43	224.00 ±21.01	228.00 ±16.38	234.48
T3	208.90 ±13.23	219.40 ±73.37	228.50 ±18.11	222.30 ±19.97	219.78

T1=2 hour, T2=4 hours, T3=6 hours

Table 5. Mean plasma protein level (g/dL) of layers for two weeks as exposed to different vibration duration for the four collection periods using vibration simulator.

Group	1 hour	20 hours	1 week	2 weeks	Over-all mean
Control	10.03±1.40	10.32±0.90	10.38±1.25	10.44±1.27	10.29
T1	9.33±1.57	10.58±1.88	10.94±2.56	10.81±2.88	10.42
T2	9.21± 1.46	10.76±1.31	9.54±1.39	10.56±1.39	10.02
T3	9.08±1.00	9.80±1.16	9.94±0.91	10.30±0.88	9.78

T1=2 hour, T2=4 hours, T3=6 hours

stress are expected to have elevated H/ L values (Zulkifli *et al*., 2002).

Werner *et al*. (2007) reported that not only long transport time, but also short journeys can affect the welfare of the animals with increased mortalities and pathological findings in pigs. A study conducted by Huff (2005) further indicated that transport stress increased the heterophil percentage and decreased the lymphocyte percentage. Plasma corticosterone is elevated following a road journey (Scholtyssek and Ehinger, 1976; Freeman *et al*., 1984; Satterlee *et al*., 1989), and apparent activation of the hypothalamo-adenohypophyseal-adrenocortical axis is consistent with post-transport increases in heterophil:lymphocyte ratios (Satterlee *et al*., 1989; Mitchell *et al*., 1992; Maxwell, 1993). The response of poultry to the specific noise encountered during transportation has not been assessed, though it is generally thought that vibration is likely to be more aversive than noise (Grandin, 2004).

Average blood glucose levels were consistently shown to be highest for control and lowest for T3 (Table 4). Blood glucose levels between animals in each observation period [F (2.01,72.36) = 4.45, p-value = 0.015] and between treatments [F (3,36) = 4.95, p-value = 0.006)] were found to be significantly different. Post-hoc analyses revealed that T3 was significantly different with T0 (p-value = 0.001), T1 (p-value = 0.023) and T2 (p-value = 0.026). Pronounced stress in most species can result to changes in the glucose concentration (Everds, 2013). Simon (1984) also found out that animals stressed with corticosterone had increased glucose levels. Serum glucose can change rapidly in response to stress depending on the condition of the animal. However, blood glucose is not a very reliable indicator of the stress intensity in animals as its levels can be affected by various factors (Scholz, 1990; Everds, 2013).

Plasma protein values in each observation time were found to be significantly different (p-value < 0.000) but there was no significant difference between treatments (Table 5). This shows that plasma protein is not a good measure of stress in birds. Contrary to other animals, an increase in the plasma total protein was found in pigs (Brown *et al*., 1999) and calves (Knowles *et al*., 1999) that were stressed after long transportation duration. Parker *et al*. (2003) added that plasma total protein increases when an animal suffers from dehydration as a result of prolonged transit time.

Table 6. Weekly average feed intake (grams) of layers subjected to different vibration duration.

Group	1 week	2 weeks	3 weeks	4 weeks	Over-all mean
Control	94.70±4.37	95.46±2.03	96.06±1.90	94.99±4.68	95.30
T1	93.11±2.82	95.81±1.79	95.14±2.34	94.81±3.42	94.72
T2	90.39±3.43	97.51±1.48	95.19±2.12	95.87±2.27	94.74
T3	89.03±2.59	92.49±6.04	91.31±7.53	92.74±5.26	91.39

T1=2 hour, T2=4 hours, T3=6 hours

Table 7. Weekly average body weight (kg) of layers subjected to different vibration duration.

Group	Initial	1 week	2 weeks	3 weeks	4 weeks	Over-all mean
Control	1.47±0.11	1.48±0.10	1.50±0.11	1.50±0.11	1.50±0.08	1.49
T1	1.56±0.10	1.51±0.11	1.47±0.20	1.52±0.15	1.46±0.14	1.50
T2	1.49±0.15	1.50±0.16	1.52±0.15	1.56±0.15	1.49±0.16	1.51
T3	1.48±0.11	1.48±0.12	1.50±0.23	1.38±0.21	1.36±0.24	1.44

T1=2 hour, T2=4 hours, T3=6 hours

Production Parameters

T3 appeared to consistently have the lowest average weekly intake per bird (Table 6). Feed intake was found significantly different between each observation day [F (27,10) = 9.27, p-value = 0.000] and birds [F (8.08,290.82) = 9.16, p-value = 0.000), while treatment was seen to have an effect on the values across daily observations [F (81,30.79) = 5.32, p-value = 0.000]. A significant difference was also observed between the treatments [F (3,36) = 3.90, p-value = 0.016)]. Post hoc analyses revealed that T3 was significantly different from T2 and T1 (p-value = 0.013), and from T0 (p-value = 0.004). Results implied that vibration stress of 6 hours may cause significant reduction of feed intake of layers for at least 4 weeks. Stress, especially if chronic, is known to cause adverse effects on the feed intake of birds (Ferket and Gernat, 2006).

Except in the 2nd week, T3 was found to have the lowest average body weight (Table 7). A significant difference was observed between the 4th week and initial weight (p-value = 0.18), and 1st and 4th week weights (p-value = 0.026). However, no significant difference (p value = 0.557) was found between treatments, which implies that vibration duration until 6 hours did not have an effect on the body weight of birds. In a related study by Bergoug *et al*. (2013), transportation duration was shown to affect the body weight of birds only until day 21. However, the transportation duration was until 10 hours and the experiment was done in day-old broiler chicks. In another study about the progression on transportation duration in broilers, respective body weight losses of 1.3, 2.3 and 3.1% of the animal's body weight after journey times of 1.5, 3 and 4.5 hours were observed (Scholtyssek *et al*., 1977). Further studies are needed to clarify the effect of longer transportation duration to the body weight of layers.

No trend concerning the average egg weights of the different treatment could be seen (Table 8). Moreover, no significant difference was found between treatments and observation time. Total egg weight per treatment was also found not significantly different from each other. Vibration stress of up to 6 hours appears to have no effect on the average egg weight of layers. Studies detailing effects of transportation stress or transportation have been limited. However, some studies cited another factor (heat stress) to cause a significant decrease in the egg weight (de Andrade *et al*., 1976).

Table 8. Weekly average egg weight (g) produced by birds subjected to different vibration duration.

Group	1 week	2 weeks	3 weeks	4 weeks
Control	55.23	55.74	52.21	57.11
T1	55.41	55.36	55.53	56.81
T2	53.45	55.33	55.43	55.89
T3	54.19	55.00	55.69	56.18

T1=2 hour, T2=4 hours, T3=6 hours

Table 9. Weekly average egg production of birds subjected to different vibration duration.

Group	1 week	2 weeks	3 weeks	4 weeks	Over-all mean
Control	91.43±9.99	91.43± 7.38	85.71±13.47	88.21±5.06	89.20
T1	90.00±11.76	85.71± 17.82	81.43±26.13	87.86±13.28	86.25
T2	92.86±10.10	91.43± 12.05	75.71±21.35	87.86±9.10	86.96
T3	84.29 ± 19.58	74.29± 37.37	74.29±26.77	81.79±18.78	78.66

T1=2 hour, T2=4 hours, T3=6 hours

T3 was consistently observed to have the lowest average weekly egg production but with the highest deviation among replicates (Table 9). Weekly observations were found significantly different [F (3,34) = 3.64, p-value = 0.022], especially between week 4 and week 3 (p-value = 0.048), week 2 (p-value = 0.002), and week 1 (p-value = 0.007). However, there was no significant difference observed between treatments (p-value = 0.617). It shows that vibration duration of not more than 6 hours does not have a significantly direct effect on egg production performance, and other factors must be explored to demonstrate possible effects, including feed intake and body weight (Everds *et al*., 2013).

No effect of vibration duration on FCR was observed. This result is expected to be similar to that of egg weight, since FCR is a function of the egg weight. As other studies found out that stressors can affect egg production (Talukder *et al*., 2010; Kocaman *et al*., 2006), further studies are needed to verify the impact of vibration stress on FCR of laying hens. Feed efficiency has been generally linked to feed intake (Mashaly *et al*., 2004).

Multivariate analyses

Multivariate analyses revealed that treatment appears to have an effect on the total WBC counts, differential WBC counts (heterophil, lymphocyte, basophil, monocyte and eosinophil counts) and H/ L values at different observation times. Glucose level was observed significant only in the 1st hour post-treatment exposure. Although feed intake (1st hour, 20th hour, 1st week average, 2nd week average, 4th week average, over-all average) was found significant at different observation periods, FCR and total feed intake per egg produced was found significant only at the 1st week. Results imply that effects of vibration stress are mainly on the haematological and biochemical indices of the affected birds, which may be associated with a chronic effect on different production parameters, including feed intake. Low feed intake can have long term effects on egg production (Ivy and Gleaves, 1997) and body weight (Mashaly *et al*., 2004).

This study demonstrated the effects of vibration duration of up to 6 hours in layers. As vibration stress can have detrimental effects to layer productivity, exposure to similar stress should be avoided or minimized. Further studies are needed to determine the long-term effects of longer vibration duration, or actual transportation stress on layers which can be longer than 6 hours.

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