

## The Effect of Ambient Temperature and Dietary Nucleotide Supplementation on Tonic Immobility Reaction on Broiler Chicken

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### Abstract

The purposes of this experiment were to study the effect of three different environmental conditions (hot, cool, and natural) in the chicken behavior as indicated by duration of tonic immobility, and the role of dietary nucleotide supplementation on elevate the stress. A total of 135 unsexed commercial chickens at fourteen-day of age were divided into three different environmental conditions; hot environment (H) with ambient temperature  $32 \pm 1^\circ\text{C}$ , comfortable environment (C) with ambient temperature  $23 \pm 1^\circ\text{C}$ , and natural environment (N). The chicken in every environment condition received a basal diet supplemented with three levels of nucleotide (0 g/kg) as a control group (T0), T1; 1 g/kg, and T2; 0.5 g/kg. Birds were fed *ad libitum* until slaughter at 35 days. The duration of tonic immobility was evaluated two times on age 25 day and 34 day. The result of this study revealed that chicken reared under natural environment temperature increase the duration of tonic immobility compare with chicken's rear under hot and comfortable environment condition. Based on the results of this study it was concluded that the supplementation of dietary nucleotide has no effect on reducing the stress behavior.

**Key words:** Environment, nucleotide, stress, tonic immobility

### المستخلص

هدفت هذه التجربة الى دراسة تاثير استخدام ثلاثة انظمة بيئية مختلفة (بيئة حارة، بيئة مريحة، وبيئة طبيعية) لتربية الدجاج اللحم على التنويم المغناطيسي كمؤشر للاجهاد وكذلك استخدام تاثير استخدام النيكليوتيدات كاضافة غذائية على تقليل تاثير الاجهاد. تم استخدام 135 كتكوت من الدجاج اللحم التجاري غير مجنس بعمر 14 يوم، تم تقسيم هذه الكتاكيت الى ثلاثة انظمة بيئية مختلفة كالآتي: بيئة حارة حيث كانت درجة الحرارة  $32 \pm 1^\circ\text{C}$ ، بيئة مريحة حيث كانت درجة الحرارة  $23 \pm 1^\circ\text{C}$  و بيئة طبيعية تعتمد على درجة حرارة البيئة الطبيعية. كل مجموعة من الكتاكيت في البيئات الحرارية المختلفة تمت تغذيتها على علف اساسي مضاف اليه ثلاث مستويات مختلفة من النيكليوتيدات كمضاف علفي وهي: صفر جم/كجم وهي المجموعة الضابطة، 1 جم/كجم و 0.5 جم/كجم. كانت تغذية الكتاكيت تغذية حرة خلال فترة التجربة و حتى الذبح في عمر 35 يوم. تم قياس طول فترة التنويم المغناطيسي مرتين خلال فترة التجربة في عمر 25 يوم و في عمر 34 يوم. اوضحت نتائج هذه التجربة ان الكتاكيت التي تمت تربيتها ضمن المجموعة البيئية الطبيعية اظهرت فترة تنويم مغناطيسي اطول مقارنة مع المجموعة الحارة و المريحة. بناء على نتائج هذه التجربة فان اضافة النيكليوتيدات كمضاف علفي لعلائق الدواجن ليس لديه تاثير في تقليل سلوك الاجهاد.

**الكلمات المفتاحية:** البيئة، النيكليوتيدات، الاجهاد، التنويم المغناطيسي

## Introduction

Stress happen when animals have to make extreme and/or extended physiological and behavioral adjustments in order to cope with their environment.

There are a number of factors that cause stress and are called stressors, e.g. noise, unfamiliar pen-mates or dogs. Although many animals might be able to tolerate a single stressor for a short period of time, multiple stressors over a long period of time may lead to distress and suffering.

Behavioral measurements have been shown to have a significant association with physiological measures indicating stress, and it has been proposed that some stressors, such as heat or cold, may fail to activate the hypothalamuspituitary-adrenocortical axis if the variable of emotional arousal is removed (Carnicer and Campo. 1994).

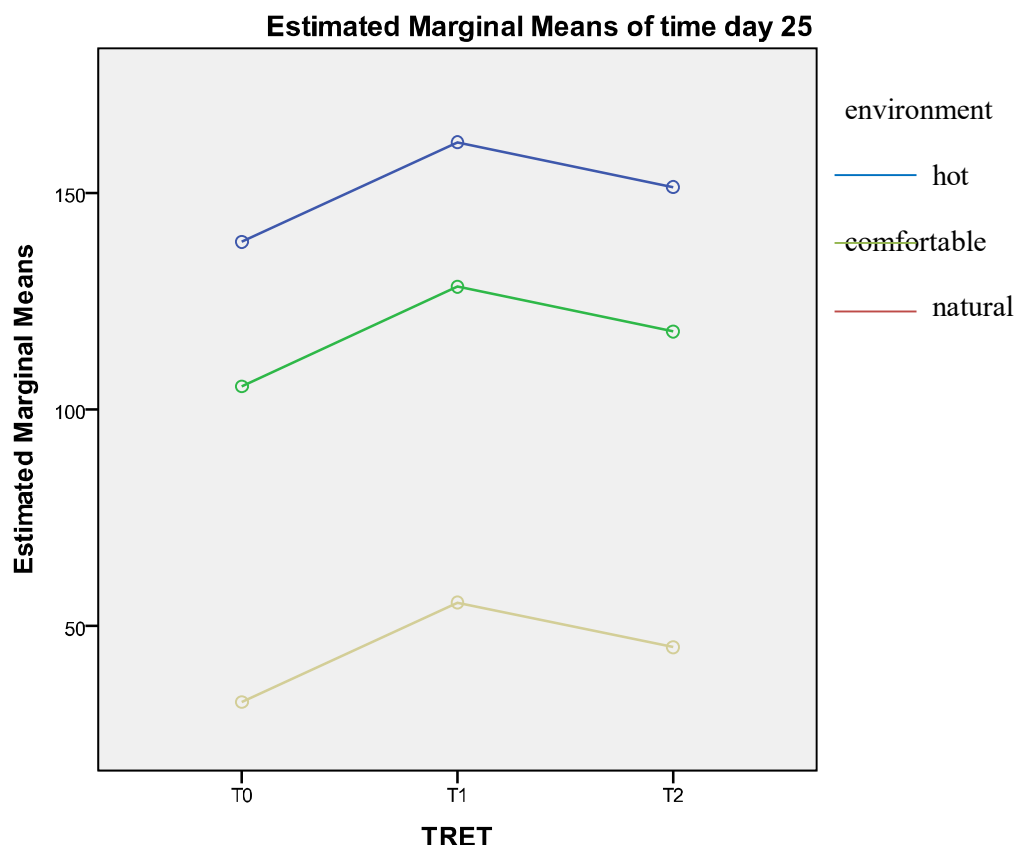
The most easily measurement of stress in chickens is the technique of tonic immobility. Tonic immobility, originally considered a form of animal hypnosis, is one of the classical behaviours that have been studied by both ethologists and behavioural geneticists alike, and was first described in the mid-1600s (Fogelholm *et al*, 2019). Poultry that are in a healthy or not stressed state can return

to their original position quickly when their body is stretched.

Nucleotides are one of biotechnology products for nutrition of farm animals, which it improves feed features through the digestive and immune systems development and function in animals (Carnicer and Campo. 1994).

However, during times of extraordinary stress, such as the periods of rapid growth, certain disease states, reproduction, environmental change, recovery from injury, limited nutrient intake or disturbed endogenous synthesis of nucleotides, their availability could limit the maturation of fast dividing tissues with a low biosynthetic capacity, such as the intestine.

The purpose of the present study was to analyze the effects of environment conditions and dietary nucleotide supplementation on the tonic immobility duration on broiler chickens. It would be hypothesized that comfortable environment and nucleotide supplementation would decrease the stress and fear levels of birds, whereas a hot environment and control diet would increase it. As far as the authors know, the relationships between environment conditions and dietary nucleotide supplementation on tonic immobility duration have not been studied yet.



## Material and methods

### Experimental Site

The study conducted at the Instructional Poultry Farm and laboratories of faculty of Animal and Agriculture Science, Diponegoro University, Semarang.

### The experimental design

This experiment designed as factorial experiment 3 x 3; the first factor was the nucleotide supplementation: the nucleotide added to the feed in three levels as (0, 0.5 and 1) g/kg feed up to 35 days of age. The second factor was the environment: the experiment was conducted under three different environmental conditions: hot environment with ambient temperature  $32 \pm 1^\circ\text{C}$ , comfortable environment with ambient temperature  $23 \pm 1^\circ\text{C}$ , and natural environment with an ambient temperature depend on the natural environment ( $24\text{--}34^\circ\text{C}$ ).

### Diet, Birds, and management

A required quantity of each feed ingredient purchased from the local market and feed prepared on the farm.

All the feed ingredients were fresh and had good quality. The nutrient requirements (ME, CP, CF, EE, Ca, P, Lysine and Methionine) were satisfied as per requirement as recommended by (NRC, 1994) as it show in the table (1). The supplement of nucleotide had been added at graded levels through feed and give to different groups.

A total of 135 unsexed birds 15 day age were divided into the three different environmental conditions, and environmental group received three different levels of nucleotide supplementations with five replications for each one with almost equal weight. Throughout the entire duration of the experiment feed and water provided *ad-libitum* and lighting provided continuous. Strict bio-security measures taken to prevent any chance of disease occurrence. Chickens were vaccinated against common viral diseases; Newcastle disease and Gumboro.

**Table 1.** Composition and nutrient content of broiler base feed

<b>Ingredients</b>	<b>%</b>
Corn	62
Soybean Meal	26.5
Rice Bran	4.0
Meat Bone Meal	3.66
Limestone Rough	0.25
DL - Methionine	0.09
Mineral	0.3
Nacl	0.2
Palm Oil	3.0
Total	100
Analyzed values	
Crude protein	18.9
ME	3145.5
Ca	0.76
P	0.32
Methionine	0.38
lysine	0.98

### Measurement of Tonic Immobility Duration

The effect of heat on the tonic immobility duration is a traditional measure of stress in poultry. Five birds from each treatment group tested for Tonic Immobility (TI) on day 23 and 34. Each individual bird was gently caught with both hands, hold in an inverted manner, and carried to a separate room (no visual contact with other birds) for TI measurements. Tonic immobility induced as soon as the bird arrived in the separate room by gently restraining it on its right side and wings for 15 sec. The experimenter then retreated approximately 1 m and remained within the sight of the bird, but made no needless noise or movement. Direct eye contact between the observer and the chicken avoided because it may prolong TI duration.

A stopwatch was started to record latencies until the bird righted itself. If

the bird righted itself in less than 10 sec, the restraining procedure repeated. If TI was not inducing after three attempts, the duration of TI was considered 0 sec (Zulkifli *et al.*, 2009).

### Statistical Analysis

All the data obtained in this study subjected to statistical analysis using the General Linear Model (GLM) univariate technique and Duncan's multiple comparison data conducted by using the SPSS software. Differences between treatment group means considered significant at ( $P < 0.05$ ).

### Results and discussion

Tonic immobility (TI) is an unlearned response characterized by a catatonic-like state of reduced responsiveness to external stimulation.

Dietary nucleotide Supplementation given for 3 weeks had no effect on the average value of tonic immobility duration tested at 25 d and 34 d as shown in table (2). This results agreement with (Carnicer and Campo., 1994) who also studied the effect of feed additives (ascorbic acid) on tonic immobility duration and he reported that there were no significant effects of ascorbic acid supplementation to diet on fearfulness of hens as estimated by tonic immobility.

The results of this study shown that the natural environment condition significantly decrease the duration of tonic immobility 44 second compared with hot and comfortable condition which recorded 150 and 117second, respectively on day 25. There was no significant difference between comfortable and natural environmental condition in terms of tonic immobility (Table 2). The housing systems or husbandry methods used can exert profound influences on a bird's behavior (Jones, 1986).

**Table 2.** The effects of dietary nucleotide and environmental conditions on tonic immobility duration

	Tonic immobility day 25 (sec)	Tonic immobility day 34 (sec)
Hot environment		
0g/kg	106.20	81.80
0.5g/kg	187.80	125.40
1g/kg	157.60	155.40
Natural environment		
0 g/kg	54.40	105.20
0.5g/kg	33.80	60.80
1g/kg	33.80	74.80
Comfortable environment		
0g/kg	115.80	65.80
0.5g/kg	154.00	110.20
1g/kg	154.00	98.00
Effect of environment		
Hot	150.50 <sup>a</sup>	120.90
Natural	44.30 <sup>b</sup>	80.30
Comfortable	117.20 <sup>ab</sup>	91.30
Effect of Nucleotide		
0g/kg	92.10	84.30
0.5g/kg	104.80	98.80
1g/kg	115.10	109.40
Probability		
Environment	0.01	0.54
Nucleotide	0.81	0.19
Env×Nuc	0.60	0.30
SEM	44.50	27.70

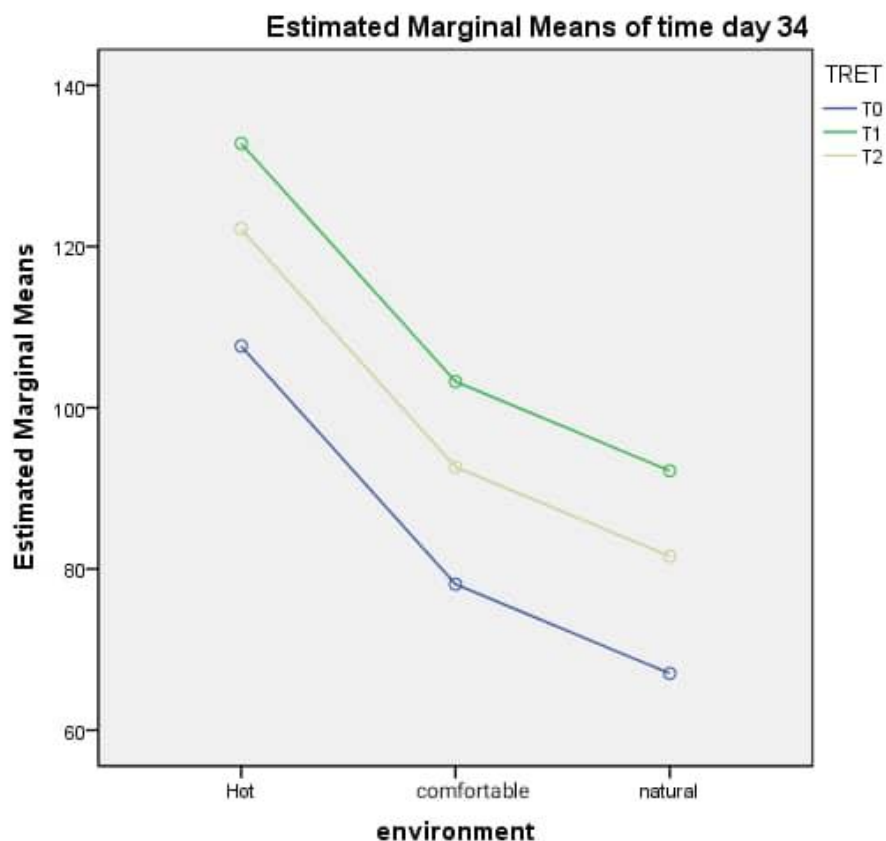
<sup>a,b</sup>Values within a column with different letters differ significantly (P< 0.05).

Env: environment, Nuc: nucleotide

Hitherto there has been no standardized method for fear measurement, because of the complexity of the concept of fear. A consistent trend towards greater adrenocortical activation was reported in high fear hens, which showed long tonic immobility reactions when compared to low-fear counterparts (Beuving *et al.*, 1989).

Heat caused a significant increase in corticosterone concentration in the plasma chicken (Jones, 1986). Similarly, plasma corticosterone

correlated positively with the duration of tonic immobility in chickens during transport and shackling (Gudev *et al.*, 2011). However, environmental conditions had no significant (P<0.05) effect on the tonic immobility duration on day 34. This may be due to regular handling or taming was thought to reduce tonic immobility, though it was later proposed that repeated elicitation of immobility, and not just handling, was responsible for reduced response durations.



### Conclusion

Dietary nucleotide Supplementation had no effect on the average value of tonic immobility duration. In addition, the natural environmental conditions significantly decrease the tonic immobility duration.

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