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■ Keywords

Broiler production, Climatic variation, FCR, Open housing system, Stress level.

Climatic Variation: Effects on Stress Levels, Feed Intake, and Bodyweight of Broilers

ABSTRACT

Chickens tolerate a very narrow range of climatic variation, and therefore, it is essential to determine the most suitable climatic area and weather for broiler production in open-house systems. In this study, 3060 broilers were used as experimental birds to investigate the effects of seasonal differences and climatic variations on the growth performance of broilers reared in an open-house system. Birds were kept under various treatment models that differ in climate Tropic zone, sub tropic zone and temperate zones. Data were recorded during July-August as summer months and January-February as winter months. Collected data was analyzed using the least square procedure given by Harvey-1990. Significantly higher ($p < 0.05$) level of corticosteroids and H/L ratio of between the experimental birds was observed and consequent effect on feed intake was determined. The body weight and FCR at 42 day was significantly ($p \leq 0.05$) higher during winter season in subtropical zone. Subtropical climatic zone was found significantly better than other climatic zone. Efficient management of broiler farming that corresponds for effective feed intake during heat/cold stress to maximize bird's efficiency can significantly increase production efficiency and acquiesce highest profit to broiler farming.

INTRODUCTION

Physiological stress can have deleterious effects on the overall performance and body growth of meat-type poultry (Sams, 1997; Mashaly *et al.*, 2004), and this is still a challenging subject for poultry producers and academic. It is estimated that the worldwide demand for poultry products (meat and eggs) will rise exponentially in the coming decades. The human population is expected to increase in 33% by 2050 (UNO, 2015) and this will result in an increase of overall food production of 70% (Ramachandran, 2014), and hence, increase the demand of poultry meat and eggs to feed this huge human population in next few decades. Broiler production is an important sector of the poultry industry, comprising 25% of overall meat production, and has increased in 125% between 1999 and 2009 (Windhorst, 2011). Protein from chicken meat and eggs is the cheapest meat source for human consumption. However, a wide range of climatic variations greatly influences the productivity of broilers.

Broilers are challenged with infections, feedstuff variation, and climate changes, which may negatively impair the productivity of the commercial poultry industry (Shini *et al.*, 2008). Climate change and animal production are linked, and the effects of climate change on livestock and poultry production is evident all over the world (Mengesha, 2011). Stress directly affects the physiology and welfare of poultry, and result in lower profitability (Barnett & Hemsworth,



2003). Although there are many types of stressors, their overall effects are often similar. Stressors activate the sympathetic adrenomedullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes, resulting in the release of catecholamines and glucocorticoids, respectively (Belda *et al.*, 2015). Backyard farmers in developing countries rear poultry irrespective of climate and seasonal variation, and do not know its influence on overall bird performance.

A deeper knowledge of poultry management, reproduction, genetics, and nutrition is essential to meet the future demands not only to supply food to the human population, but also to provide profitability for the poultry farmers and companies, which consequently affect the global economy. Poultry production is common in subtropical zones, particularly in Asian countries, where the poultry industry is expanding (Chowdhury *et al.*, 2014). The interaction of genetics with the environment in specific geographical locations may affect broiler growth traits (Okere, 2014). At present, climatic variation is a key threat for poultry industry, especially for marginal poultry farmers in open-house systems. Poultry of different breeds and ages react differently to climatic variations (Alade & Ademola, 2013).

Broilers present optimal feed intake and weight gain when reared within the comfort zone (Al-Aqil *et al.*, 2009). Poultry growth performance is not only inherited, but it is also greatly affected by the environment (Babinszky *et al.*, 2011). Therefore, data on environmental rearing conditions and their effects on poultry production need to be generated to allow the development of common strategies to face the adverse effects of the climate change. In this study, we hypothesized that the productivity of broilers reared in different climatic zones (tropical, subtropical and temperate) would differ as a result of environmental stress level. Commercial feed formulation has evolved to supply nutrients and energy to match the bird's requirements as close as possible. In this research article, we compared total feed intake, body growth performance, water intake, mortality rates, stress hormone level (heterophil/lymphocyte ratio) of broilers

birds in three different geographical areas in two different seasons (summer and winter).

MATERIALS AND METHODS

Study period, studied area, data source and survey of the chicken production

The study period was from January 2015 to December 2015, with two important periods for weather variables having the direct effect on birds' activity. The study was conducted in three different climatic zones of Nepal: southern plains (Tarai), hills, and northern mountain belts, which are characterized by tropical, subtropical, and temperate climate, respectively (Table 1). These areas present significant differences in altitude and average weather conditions. Nepal has altitudinal variation from 75 m (meter) to 8848 m, variable land topography and climate. Environmental temperature ranges from 7°C (the northern belt) to 38°C (the southern belt). The southern part of the Nepal presents extreme heat and high humidity during the summer, whereas the northern part of Nepal, at high altitude, is characterized by severe cold and low humidity. Secondary data from government survey (MOAC, 2014) was used to choose the study area for the conduction of the research, and finally, the district with the higher broilers population in each geographic zone was chosen as the study area. The experiment was carried out in two different seasons (July-August and January-February) in each study area, which presented different temperature, rainfall and relative humidity values.

Feed Formulation and Ingredients

Feeds were based on the feed stuffs available and feed additives (Table 2). Three different feeds were formulated, according to the guidelines of the Cobb500 manual, for the following rearing phases: 1) starter (0 to 10 days of age): standard mash diet, 2) grower (11 to 22 days of age): standard pelleted diet, and 3) finisher (23 days to slaughter age): standard pelleted diet.

Table 1 – Average altitude, humidity, rainfall and temperature of the studied locations

Experimental location	Altitude (m)	Humidity* (%)		Rainfall* (mm)		Temperature* (°C)	
		Summer	Winter	Summer	Winter	Summer	winter
Tropical	Below 1000	90	62	125	32	37	22
Subtropical	1000 to 2000	77	54	88	37	29	15
Temperate	2000-3000	55	36	31	11	14	5

*average value

**Table 2** – Feed composition (units in kg)

Feeds ingredients	Starter	Grower	Finisher
Corn	500	480	500
Rice pollard	80	100	120
De-fatted rice meal	0	60	50
Soybean meal	204	140	120
Mustard meal	50	50	40
Meat and bone meal	50	40	30
Sunflower meal	32	22	30
Corn gluten (30%)	48	60	70
Limestone	0	10	0
Dicalcium phosphate	10	10	10
Molasses	20	22	24
Feed additives (Salt+ vitamins+ mycotoxin binders+ medicines+ probiotics, etc.)	6	6	6
Total (kg)	1000	1000	1000
Energy level	2950 kcal/kg	3000 kcal/kg	3100 kcal/kg
Protein level	21.8%	19.5%	18.3%

Experimental design and bird management

Treatments consisted of summer (T1), and winter (T2), which were further divided into three different climates: tropical (plains; T1R1 and T1R2), subtropical(hills; T2R1 and T2R2), and temperate (mountains, T3R1 and T3R2) as shown in Table 3. Each treatment was replicated three times.

Table 3 – Experimental treatments

	Summer (T1)			Winter (T2)		
Tropical zone	T1R1	T1R2	T1R3	T2R1	T2R2	T2R3
Subtropical zone	T1R4	T1R5	T1R6	T2R4	T2R5	T2R6
Temperate Zone	T1R7	T1R8	T1R9	T2R7	T2R8	T2R9

A total of 3060 Cobb 500 broiler hatchlings were used, with 1020 birds per study area and reared according to the Cobb 500 management guidelines (Cobb-Vantress, 2011). Hatchlings were placed in open-sided houses. In the first week, brooding temperature was maintained at 35°C and then gradually reduced at 2.5°C per week to reach 25°C house temperature in the 6th week, and maintained thereafter. A mixture of sawdust with rice husks was used as litter material, after the house floor was spread with dry limestone. At placement, water was offered to chicks to avoid dehydration. Feed and water were offered *ad libitum*, and light was provided 24 hours per day. All birds received a standard diet with the same formulation and ingredients, manufactured in the same feed mill. The feed was offered twice daily and feed residues were daily collected and weighed in the morning and in the evening. Vaccination schedule, stocking density, and number of feeders and drinkers were according to

the guidelines of Cobb 500 manual (Cobb-Vantress, 2011).

Blood collection and corticosterone levels

The stress response of the experimental birds was determined at 42 days of age. In order to minimize handling stress, each bird captured was isolated for five minutes before blood collection.

Blood was collected from the wing vein of 42-d-old experimental birds (n= 17, per replicate) in 3 mL syringes with sodium heparin as anticoagulant, and place in duly and individually identified tubes. Blood samples were refrigerated until analysis and centrifuged at 500 X g for 15 min at 5°C. Heterophil to lymphocyte ratio (H/L) and blood corticosterone level were measured as indicators of stress level.

Whole blood was smeared on slides, and examined in oil immersion under a light microscope at ×1000 magnification. One hundred white blood cells were counted to determine the frequencies of the various cell types and to calculate H/L ratios.

Blood corticosterone level was measured by enzyme immunoassay using a commercial kit (OCTEIA CORT HS, Immunodiagnostic Systems Ltd., Bolton, UK). All samples were run in duplicate and using the kit calibrators at each analysis. Absorbance was measured at 450 nm, with a reference wavelength of 650 nm, in an ELISA microplate reader (MRX® II Dynex Technologies, USA).

Data Collection

Feed offer and residues were daily weighed and recorded to calculate precise feed intake. Water was provided in water troughs using a 2-L bucket, and next morning the remaining water was collected from the troughs and measured to determine water consumption.

At 42 days of age, all birds were weighed using an electronic top-loading balance. Individual weight gain was calculated weekly for the 6-week experimental period. The comparative study was done to find out the effect of weather and season on the average feed intake and weight gain. Finally, the feed conversion ratio (FCR) was calculated by dividing total feed intake by total weight gain.

Mortality was daily recorded, and the mortality rate calculated for the total experimental period.

Statistical Analysis

The effects of treatments on growth parameters were analyzed by the least square analysis of the



data with unequal subclass numbers (Harvey, 1990). One-way analysis of variance was applied, and the unpaired Student's t-test was used to detect significant differences ($p < 0.05$) among treatments. Results are shown the mean \pm standard deviation (SD). Statistical significance is indicated as follows: ^{a/ab} $p < 0.05$; ^{a/b/c} $p < 0.01$. Data are representative of at least three independent experiments.

RESULTS

Broiler distribution and climatic variation in the study areas

We screened the secondary data of the government of Nepal and analyzed broiler distribution in the three climate zones (tropical, subtropical, and temperate). A significant difference was found in the chicken population in three climatic zones (Table 1). The tropical region of Nepal has a significantly higher poultry population than the subtropical and temperate regions.

We screened the previously reported data (MOAC, 2014) of three study areas, and detected significantly higher mean temperature, higher mean rainfall, and higher mean humidity in the tropical region compared with the other regions (Table 1).

Growth performance parameters

Daily feed intake was calculated after subtracting feed residues from feed offer. No significant feed intake differences were detected among the different

between seasons. Broilers in the temperate zone presented significantly ($p=0.04$) lower feed intake than those in the tropical and subtropical zones. However, comparing the interaction between seasons and climatic zones, the broilers reared during the winter in the temperate zone had significant lower feed intake ($p=0.031$) (Table 4).

The water intake of the experimental birds was significantly different ($p=0.04$) among climatic zones (Table 4), with broilers reared in the tropical and subtropical zones presenting higher water intake than those in the temperate zone. Moreover, water intake during the summer was significantly ($p=0.046$) higher than in winter.

Final body weight was significantly ($p=0.01$) affected by the seasons of the year, with final body weight determined in the winter than in the summer. Broilers reared in the tropical and subtropical zones were significantly ($p=0.021$) higher body mass than bird's reared at temperate zone (Table 4).

There was significant effect of season ($p=0.004$) on FCR, with better FCR obtained in winter than in the summer, and of climatic zone ($p=0.025$), with the best FCR determined in the subtropical zone. There was a significant interaction between seasons and climatic zone for FCR values ($p < 0.01$): the best FCR were obtained in broilers reared in the tropical and subtropical zones during winter (Table 4).

Total mortality was low: 4 birds in the summer experiment (temperate zone 1, subtropical zone 1, and tropical Zone =2) and 2 birds in the winter experiment

Table 4 – Broiler water intake (L), feed intake (g), total weight gain at 42 days of age (g) and FCR according to season and climatic zone.

Factor	N	Water intake (L)	Feed intake (g)	Total weight gain in 42 day (g) LS Mean \pm SE	FCR
Seasons					
Summer	1530	12.1 \pm 0.47 ^a	4018 \pm 0.43	2016 \pm 0.43 ^b	1.89 \pm 0.43 ^b
Winter	1530	11.3 \pm 0.13 ^{ab}	4121 \pm 0.43	2173 \pm 0.43 ^a	1.83 \pm 0.43 ^a
Locations					
Tropical	1020	13.4 \pm 0.43 ^a	4154 \pm 0.43 ^{ab}	2204 \pm 0.43 ^a	1.85 \pm 0.43 ^{ab}
Subtropical	1020	12.1 \pm 0.43 ^a	4165 \pm 0.43 ^{ab}	2213 \pm 0.43 ^a	1.82 \pm 0.43 ^{ab}
Temperate	1020	10.8 \pm 0.43 ^{ab}	3789 \pm 0.43 ^a	1882 \pm 0.43 ^{ab}	1.94 \pm 0.43 ^a
Interactions					
Tropical \times Summer	510	14.1 \pm 0.88 ^a	3843 \pm 0.62 ^{ab}	2020 \pm 0.62 ^{ab}	1.87 \pm 0.62 ^{ab}
Tropical \times Winter	510	13.5 \pm 0.88 ^a	4420 \pm 0.62 ^a	2361 \pm 0.62 ^a	1.81 \pm 0.62 ^a
Subtropical \times Summer	510	12.5 \pm 0.67 ^a	4255 \pm 0.62 ^a	2148 \pm 0.62 ^{ab}	1.86 \pm 0.62 ^{ab}
Subtropical \times Winter	510	11.7 \pm 0.67 ^a	4098 \pm 0.62 ^{ab}	2298 \pm 0.62 ^a	1.76 \pm 0.62 ^a
Temperate \times Summer	510	10.7 \pm 0.46 ^{ab}	3988 \pm 0.62 ^{ab}	1918 \pm 0.62 ^{ab}	1.91 \pm 0.62 ^b
Temperate \times Winter	510	9.38 \pm 0.46 ^{ab}	3692 \pm 0.62 ^b	1842 \pm 0.62 ^b	2.01 \pm 0.62 ^c

Results are shown as mean \pm standard error

^{a/ab} significant at 95% level

^{a/b/c} significant at 99% level



(temperate zone=1, tropical zone =1). Mortality was not affected by the treatments and therefore, the results are not shown.

Blood corticosterone level and H/L ratio

Blood corticosterone (CORT) levels were not affected by season ($p=0.75$). There was a significant effect of climatic zone on blood CORT levels ($p=0.006$), with broilers from the tropical zone presenting the highest levels, followed by those in the temperate zone, and the lowest levels in the subtropical zone. The highest CORT levels (3.61 ± 0.93 ng/mL) were detected in the tropical zone in the summer (Table 5), whereas the lowest (3.17 ± 0.31 ng/mL) in the subtropical zone in the winter.

The H/L ratio was highest in the birds of the tropical region during summer (0.89 ± 0.01) and lowest in subtropical birds at winter seasons (0.71 ± 0.02). Significant effect of the climate and weather on blood H/L ratio was found between experimental birds ($p=0.003$), (Table 5).

DISCUSSION

Monthly mean ranges of several climatic variables for different experiment period were taken into consideration for climatic variation as it can influence animal production (Gregory, 2010) including poultry. Since feed conversion ratio is the main factor that determines the profit/loss of the farm, and because feed accounts for more than 70% of the production costs (Osti *et al.*, 2016), we calculated the average least mean square values for 6-week body weight, feed intake, water intake and FCR. Broiler feed intake was significantly different between the summer and the winter. A similar pattern was observed among the different climatic zones. The results revealed that the climate of the tropical zone was mostly humid during both the seasons and that the environmental temperature was higher than the comfort zone, whereas lower average temperatures were found in the temperate zone was found. Feed intake increases when broilers are maintained within the suitable

Table 5 – Blood corticosterone levels and heterophil to lymphocyte (H/L) ratios of broilers according to season and climatic zone.

Factor	N	Blood corticosterone level	Blood H/L ratio
Seasons			
Summer	153	3.49 ± 0.93	0.89 ± 0.93
Winter	153	3.61 ± 0.93	0.81 ± 0.93
Locations			
Tropical	102	3.52 ± 0.13^a	0.85 ± 0.13^a
Subtropical	102	3.28 ± 0.43^c	0.74 ± 0.43^b
Temperate	102	3.37 ± 0.19^b	0.77 ± 0.73^b
Interactions			
Tropical \times Summer	51	3.61 ± 0.93^a	0.89 ± 0.01^a
Tropical \times Winter	51	3.43 ± 0.62^{ab}	0.81 ± 0.62^{ab}
Subtropical \times Summer	51	3.32 ± 0.62^{ab}	0.76 ± 0.62^b
Subtropical \times Winter	51	3.17 ± 0.31^c	0.71 ± 0.01^b
Temperate \times Summer	51	3.33 ± 0.62^{ab}	0.79 ± 0.62^b
Temperate \times Winter	51	3.45 ± 0.62^{ab}	0.83 ± 0.62^{ab}

^{a/b} significant at 95% level

^{a/b/c} significant at 99% level

comfort zone. Comfort zone of broilers was defined as that with temperature between 15 to 25°C and 60-65% humidity (El Boushy & Van Marle, 1978), where the birds are able to regulate their heat balance relatively well and do not spend much energy on activity (Syafwan *et al.*, 2011). The mean temperature and humidity of the subtropical zone was close to the comfort zone, and this may explain the higher feed intake of broilers reared in that zone. This finding is supported by a previous report (Al-Aqil *et al.*, 2009), that state that optimal feed utilization and weight gain can be achieved by broilers when the environmental

temperature is about 23°C. In addition, long-term excessively high or low environmental temperatures have adverse effects on bird's physiology. In vertebrates, when females experience stress in the early stage of reproduction, their body hormonal balance can change, and the consequent effect is the epigenetic modification of the offspring. Exposure to stress activates the hypothalamic-pituitary-adrenal axis (HPA), with a subsequent increase of blood glucocorticoid levels. In avian species, the main glucocorticoid is corticosterone (CORT). Corticosterone acts mainly on the metabolism, and regulates energy intake, for



instance (Sapolsky *et al.*, 2000). At the peak of the hormonal effect, there is change in animal behavior. (Sapolsky *et al.*, 2000; Tilgar *et al.*, 2010).

Corticosterone is the main adrenocortical hormone present in the peripheral blood. Elevated blood corticosterone levels and heterophil to lymphocyte (H/L) ratios are well-known physiological indicators of welfare (Rogers *et al.*, 2015; Alm *et al.*, 2016), and are used as a stress indicators in poultry (Delezie *et al.*, 2007; Cirule *et al.*, 2012). Corticosteroids contribute for the reestablishment of homeostasis via negative feedback mechanisms that act on the hypothalamus and/or pituitary structures, decreasing HPA axis activation (Canoine *et al.*, 2002). They promote adaptive behavioral responses by providing the metabolic requirements for flight or fight responses (Sapolsky *et al.*, 2000). The significant increase in blood corticosteroid levels of the broilers reared in the tropical zone during the summer clearly indicate these birds were stressed due to climate and weather. This result was supported by the significantly higher H/L ratios determined in those birds.

During severe heat stress, corticosterone is released, and consequently, its blood levels are increased in broilers (Zulkifli *et al.*, 2009). Table 1 clearly indicates the extreme heat in the tropical zone may have induced heat stress in the broilers. It is well established that during heat stress, heat dissipation occurs by evaporative cooling, which, however, may be hindered if relative humidity is high. Therefore, in the tropical zone, the birds may have failed to cope with the hot and humid environment. Heat tolerance in birds helps to increase the level of heat shock protein (HSP) (Liew *et al.*, 2003) as HSP modify protein folding and aids the body to handle with proteins affected by heat and other stressors (Gething & Sambrook, 1992). During extremely hot weather, severe reduction in feed intake has been previously reported (Austic, 1985; Howlider & Rose, 1987), which affects metabolism and results in low body growth (Morêki, 2008; Quinteiro-Filho *et al.*, 2010). Particularly, protein digestion is hindered during chronic heat stress, leading to low feed intake (Larbier *et al.*, 1993). When the temperature is above 32 °C, feed intake is reduced by 5% for each degree of temperature increase (Balogun *et al.*, 2013).

On the other hand, chickens are able to increase their body temperature when it falls below 20 °C (Dozier Iii *et al.*, 2003). However, when the environmental temperature is low, chickens need to use much of their energy to warm their body, therefore, deviating feed energy from growth. Oxygen requirements,

cardiac output, and blood flow are higher during cold environmental temperatures, resulting in excessive pulmonary arterial pressure in the right ventricle and consequent development of ascites in broilers (Gleeson *et al.*, 1986; Yahav *et al.*, 1997). The heat gained or lost through radiation or conduction depends on the environmental temperature and its difference relative to body temperature. Birds try to dissipate heat during extreme heat and try to conserve heat during extreme cold temperature; however, in both cases birds need to expend a lot of energy to maintain their bodies within the comfort zone. This phenomenon is also correlated with water intake in poultry, as respiration rate severely increases in both during hot and cold stress (Morêki, 2008).

In conclusion, the tropical plains and the temperate mountains are relatively less favorable for broiler farming than the subtropical hills in the study area, as shown by their high blood corticosteroid levels and H/L blood ratios and worse growth performance. We concluded the subtropical climatic zone causes relatively less stress in broilers as it is close to the comfort zone of broilers. Climatic stress plays a critical role on the physiology of broilers and impacts their final body weight. A combination of sound environmental and nutrition management will greatly improve production efficiency and yield maximum profitability.

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CONFLICTS OF INTEREST

The authors have no conflict of interest regarding the publication of this article.

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