

## COMPARATIVE EFFECTS OF LOW ENERGY DIETS ON BLOOD PARAMETERS AND LIVER HSP70 AND iNOS GENE EXPRESSIONS AMONG TANZANIAN LOCAL CHICKEN ECOTYPES

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### ABSTRACT

*A study comparing effects of low energy diets on liver HSP70 and iNOS gene expressions and blood indices of three Tanzanian chicken ecotypes was conducted. Four weeks old hens belonging to Kuchi (KU), Ching'wekwe (CH) and Morogoro medium (MM) ecotypes were allocated to 9 pens in a 3 x 3 factorial design, with three replicates. They were fed 3 diets containing 40, 55 and 0% less energy than prescribed. Only KU 55% restriction groups had marked up-regulations ( $p < 0.05$ ) of HSP70 after 3 weeks. After 7 weeks, expression levels of HSP70 for KU and CH 55% restriction groups increased, while those for MM restriction groups remained unaltered. The iNOS levels were notably up-regulated for KU and CH 55% restriction groups after 3 and 7 weeks, respectively. Significant elevations ( $p < 0.05$ ) of serum corticosterone were only noted for KU restriction groups after 1 and 3 weeks. Serum uric acid was significantly increased ( $p < 0.05$ ) in all ecotypes and groups but triglycerides were markedly reduced as determined after 1, 3 and 7 weeks. There were no significant differences between controls and restricted groups in Hb and Hct levels except for CH ecotype, which showed lower ( $p < 0.05$ ) Hb and Hct levels after 5 and 7 weeks for both restricted groups. Results of this study show that low energy diets induced stress in chickens, and ecotype-specific tolerance was manifested through changes in liver HSP70, iNOS and blood parameters, with MM showing better tolerance at lowest energy levels and KU being least tolerant.*

**Keywords:** Corticosterone, Dietary energy, Ecotypes, Haemoglobin, Restriction, Stress, Tolerance, Haematocrit

### INTRODUCTION

Local chickens are an important food resource and a source of income for rural households in many developing countries (Wilson, 2015). In Tanzania like in many other tropical African countries local chickens have been managed under traditional free ranging management systems (Sanka and Mbagha, 2014) where they

are left to scavenge for whatever is available and also left to die under severe feed scarcities or preceding disease attacks. The existing local chicken ecotypes or strains have mostly evolved in part due to natural genetic selection compounded with human developmental need for cheap protein sources. Over the past few years, studies on Tanzanian local chickens have been done mainly with emphasis on

determination of their disease resistance, genotype variation and production potentials (Msoffe *et al.*, 2001; 2005; Lwelamira, 2012; Guni *et al.*, 2013; Mayardit *et al.*, 2016). Findings from these studies have shown variations in disease resistance and production potential within local chicken populations, indicating the possibility of improving the genetic potential through selective breeding within and between local chicken populations. According to Guni *et al.* (2013) Kuchi (KU) from Mwanza, Morogoro medium (MM) and Chingw'eke (CH) from Morogoro are some of the most prospective local chicken ecotypes under traditional production systems.

Dietary energy interferes with basal metabolic rate and plasma levels of different metabolic hormones in animals (LeBlanc *et al.*, 1986; Gabriel *et al.*, 2000). Research information on the extent of tolerance to low dietary energy in Tanzanian local chickens is scarce. A diet containing 18 % CP: 2800 kcal ME/kg is sufficient for rearing Ugandan local chickens in early growth phase (Nakkazi *et al.*, 2015), and similarly Miah *et al.* (2014) showed that 2800 kcal ME /kg was required by Bangladesh Desi local chickens to achieve a targeted weight of 950 g at 14 weeks of age. Stress, such as induced by low dietary energy, modifies the development of the hypothalamic-pituitary axis response thereby affecting the growth and behaviour of the chicken, and it may exert a negative impact on physiological processes and pose many health problems, including disturbances of immune processes and antioxidative defenses (Ognik and Sembratowicz, 2012).

Commonly used physiological indices of stress following feed deprivation or restriction are plasma corticosterone, glucose and heterophil/lymphocyte (H/L) ratio (Zulkifli *et al.*, 2006). In addition blood parameters, including hemoglobin (Hb), hematocrit (Hct), CO<sub>2</sub> levels, saturated O<sub>2</sub> and pH are potential biomarkers of stress tolerance (Lamont *et al.*, 2015). Moreover, the biochemical parameters of the blood such as uric acid and triglycerides reflect the physiological state of an animal, and metabolic changes due to stress conditions can be tracked through assessment of these

parameters. On the other hand, when living organisms are exposed to various stress conditions, such as energy depletion, the synthesis of most proteins is retarded, but heat shock proteins (Hsps) are rapidly synthesized (Kregel, 2002; Al-Aqil and Zulkifli, 2009; Zhao *et al.*, 2013). HSP70 is one the Hsp families and is highly inducible (Kregel, 2002) and the most extensively studied because of its prominent response to diverse stressors, and increased synthesis of these proteins is involved in the protection of stressed cells and organisms (Gabriel *et al.*, 2002; Zhao *et al.*, 2013). Inflammation is an important indicator of animal tissue damage due to stress conditions and one of the most pivotal enzymes involved in maintaining inflammation is inducible nitric oxide synthase (iNOS), which is responsible for the production of nitric oxide (NO) (Surh *et al.*, 2001; Zhao *et al.*, 2013).

Lack of access to quality feed and failure to balance between energy and protein requirements is still a huge challenge for local chickens' production sector (Sonaiya, 2007; Mutayoba *et al.*, 2012). The chemical composition of feeds eaten by rural scavenging chickens of Tanzania is generally below the nutritional requirements and varies with season, climate and age of birds (Mwalusanya *et al.*, 2010). Since it is already established that there are ecotype-differences in some aspects of productive performance and disease resistance among various local chickens (Msoffe *et al.*, 2002; Lwelamira, 2012), it could be of great interest to determine if the chickens' responses to low energy diets will show similar differences. The apparent differences would be beneficial in making informed recommendations for selection in the future breeding programs. The current study, therefore, was designed to compare the physiological responses of KU, MM and CH local chicken ecotypes to low dietary energy levels. It was hypothesized that low energy diets would affect the performance of the local chicken ecotypes differently and this would be reflected in the blood physiological parameters and gene expressions of HSP70 and iNOS in the liver.

**MATERIALS AND METHODS****Experimental Design and Chicken Rearing:**

The experimental design, chicken rearing and feed formulation are as described in Khondowe *et al.* (2017). Briefly, about 720 (240 of each ecotype) day-old local chicks from the three ecotypes, CH, KU and MM were supplied feed and water *ad libitum*. They were fed the same diet consisting of 18 % crude protein and 2,864 kcal ME/kg up to the 4<sup>th</sup> week and were vaccinated routinely against Newcastle Disease, Infectious Bursal Disease (Gumboro), and Fowl pox. At four weeks old after sexing, a total of 351 (117/ecotype) female chicks were weighed and randomly allocated to 9 pens in a 3 x 3 (3 ecotypes and 3 types of diets) factorial design, with 3 replicates. Each pen had on average an area of 2 m<sup>2</sup> floor space per 13 birds, and the study was conducted at the prevailing cyclic ambient temperatures ranging from 21.6 to 34.3° C. The birds were fed 3 types of iso-nitrogenous (18 % crude protein) diets formulated to contain 40, 55 and 0 % (control) less energy than prescribed by the NRC (1994) for layer chickens for seven weeks. The pens were artificially lit using a 12 hour light: 12 hour dark cycle light auto regulator. All procedures used in this study were in compliance with the Sokoine University of Agriculture's guidelines for care and use of animals in research.

**Tissue Collection:** After 3 and 7 weeks of dietary energy restriction, 5 chickens from each pen were randomly selected and humanly decapitated. Liver samples were quickly collected after chicken dissection and were immediately iced before transferring them to the refrigerator at -80° C.

**RNA Extraction and Quantitative Real-Time PCR:** Total RNA was extracted from liver samples (50 mg) using the Quick-RNA™ MiniPrep Plus kit (Zymo Research) following manufacturer's instructions of preparation and purification. The integrity of the isolated RNA was examined using 1.2 % agarose gels containing 0.1 % ethidium bromide. First-strand complementary DNA was synthesized from 5 µg of total RNA according to manufacturer's

instructions in a 20 µL reaction volume by using RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific) following the manufacturer's instructions. Predesigned primers for HSP70 and iNOS (Table 1) were used according to Xie *et al.* (2014) and Zhao *et al.* (2013). The quantitative real-time PCR (qPCR) was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) on an ABI 7500 (Applied Biosystems USA). Reactions were performed in a 25 µL reaction mixture. The cycling protocol included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 60 seconds. A dissociation curve was run for each plate to confirm the production of a single product. The relative expression levels of the genes tested were calculated using the 2<sup>-ΔΔCt</sup> method (Livak and Schmittgen, 2001) and were normalized to the mean expression of GAPDH.

**Blood Sampling and Analysis:** Whole blood was collected via the wing vein at similar times of the day (between 10:00 and 12:00 hours) using syringes into ethylene diamine tetracetic acid (EDTA) containing and/or plain vacutainers at intervals of 1, 3, 5, and 7 weeks of energy restriction. The sampling procedure lasted for about less than 1 min per bird. For serum preparation, blood samples (in plain vacutainers) were allowed to clot, serum separated, and stored at -20°C until analysis. The corticosterone levels were measured by ELISA using commercially available kits (Sunlong Biotech Company Limited., Hangzhou, China) and measurements were calibrated by Multiskan EX Primary EIA V. 2.3 Reader (Applied Biosystems). Sera levels of uric acid, triglycerides and glucose were determined colorimetrically according to instructions provided with the commercial kits (Erba Diagnostics Mannheim, Germany). The differential white blood cell count and all other haematological indices were determined using the MS4S automated haematological analyser (Melet Schloesing Laboratories, Germany).

**Table 1: Target gene primers used in determining effects of low diets on liver HSP70 and iNOS gene expressions**

Gene	Primer set	Product (bp)	Temperature (°C)
HSP70	F 5'-CGGGCAAGTTTACCTAA-3'	250	58
	R 5'-TTGGCTCCACCCCTATCTCT-3'		62
iNOS	F 5'-CCTGGAGGTCCTGGAAGAGT-3'	82	64
	R 5'-CCTGGGTTTCAGAAGTGGC-3'		62
GAPDH	F 5'-CTTTGGCATTGTGGAGGGTC-3'	128	60
	R 5'-ACGCTGGGATGATGTTCTGG-3'		60

**Statistical Analysis:** One-way analysis of variance (ANOVA) was used to analyze differences among all treatments data. Significantly different means were separated using Dunnett t-test and LSD test with significance statements based on  $p \leq 0.05$ . All data were analyzed using SPSS version 23.

## RESULTS

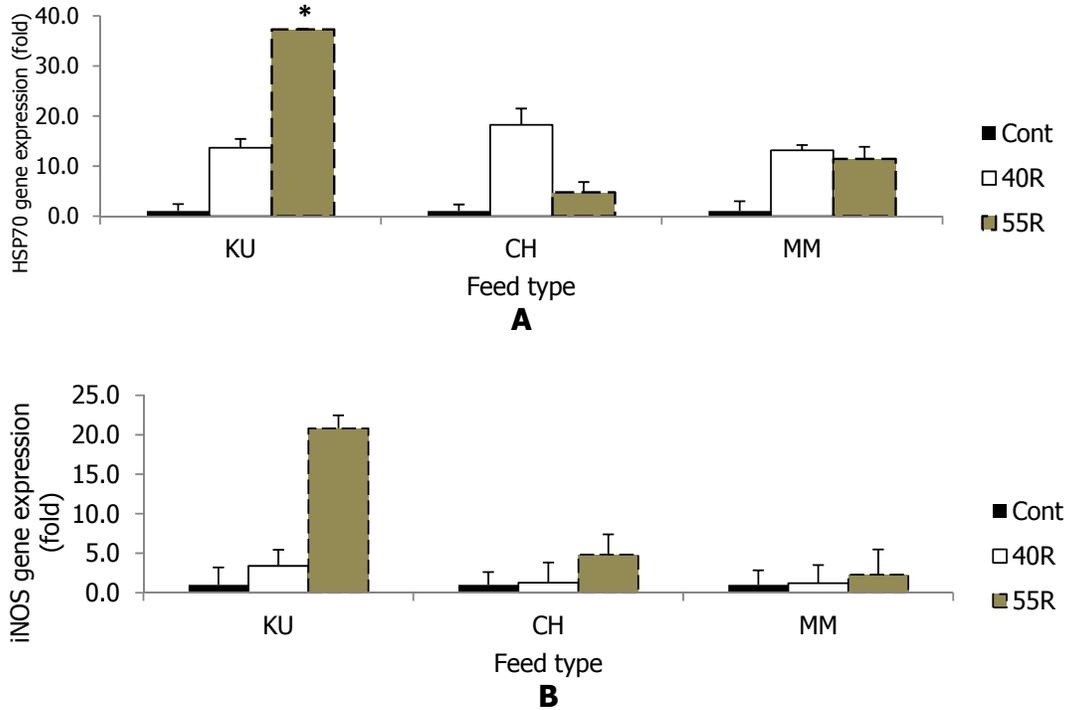
### HSP70 and iNOS Relative Gene Expression:

Results for liver HSP70 and iNOS relative gene expression are presented in Figures 1 and 2. Low energy diets induced up-regulation in liver HSP70 relative gene expression (Figure 1 A) after 3 weeks in both restriction groups for all the chicken ecotypes but levels were only significant ( $p < 0.05$ ) for the KU 55 % restriction group. After 7 weeks of the study, both groups of dietary energy restriction for KU and CH 55 % restriction group had up-regulated levels of HSP70, and the levels in KU 40% restriction group were markedly higher ( $p < 0.05$ ) than in CH. While the levels in all other restriction groups remained unaltered after 3 weeks of the study, the liver iNOS relative gene expression levels were notably up-regulated, though not markedly, for the KU 55% dietary energy restriction group (Figure 1 B). On the other hand HSP70 gene expression levels for both restriction groups of MM and CH 40 % restriction group were not up-regulated (Figure 2 A).

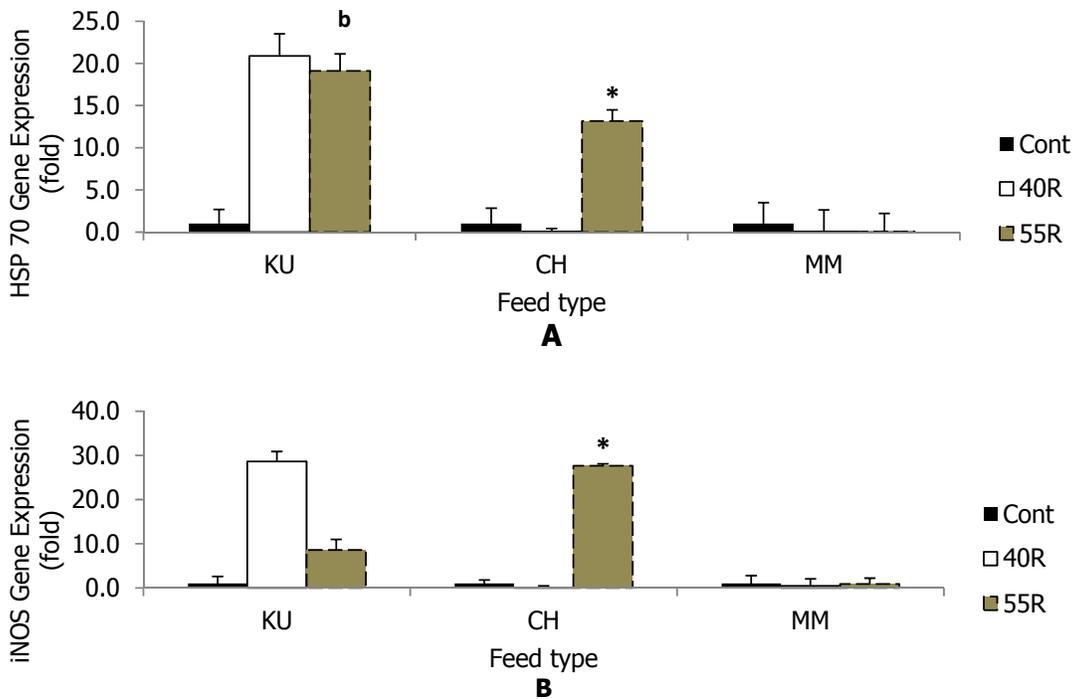
The iNOS relative gene expression levels were up-regulated for CH 55 % restriction group and both restriction groups of KU after 7 weeks of the study, and the up-regulation in CH 55 % was significant ( $p < 0.05$ ) (Figure 2 B). Both dietary energy restriction groups for MM and CH 40 % restriction group were not altered by low energy diets.

**Corticosterone:** Results for serum corticosterone concentrations are presented in Figure 3. After a week of feed restriction, significant elevations ( $p < 0.05$ ) of serum corticosterone levels were noted for KU whilst MM recorded a drop. Ecotype-specific differences in the levels were notable, with KU 40 % restricted group showing a significantly higher level than CH 40 %. Apparently, MM had the least amount of serum corticosterone ( $p < 0.05$ ) for the 55 % restricted groups. After 3 weeks, the low energy diet induced markedly elevated corticosterone levels ( $p < 0.05$ ) only for the KU 55 % restricted group. Among controls MM had higher ( $p < 0.05$ ) levels of serum corticosterone than KU and CH. The MM 40% restricted group similarly had a markedly higher level than CH 40 %. Corticosterone levels were not altered by low dietary energy intake after 5 weeks of feed restriction, and ecotype-specific differences in the levels were absent.

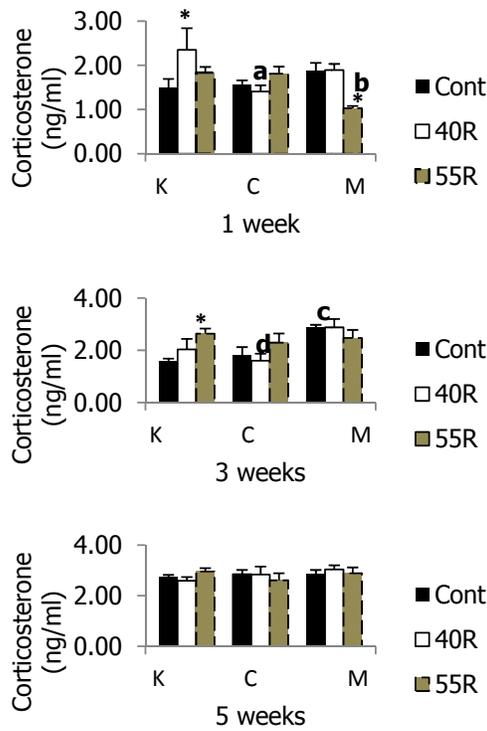
**Uric Acid:** Serum uric acid concentration results are presented in Figure 4. After a week of dietary energy restriction, there were significant elevations ( $p < 0.05$ ) in serum uric acid levels but no inter-ecotype differences were observed in both restricted groups in all ecotypes. Serum uric acid levels for MM (both groups) and KU (55 % restriction group) were markedly elevated ( $p < 0.05$ ) after 3 weeks of dietary energy restriction. Moreover, the KU 55 % restriction group had significantly higher amounts ( $p < 0.05$ ) than both CH and MM at the same restriction level. Similarly, after 7 weeks of dietary energy restriction marked levels ( $p < 0.05$ ) of serum uric acid were evident in both restriction groups for all the ecotypes. For the controls, CH had significantly higher ( $p < 0.05$ ) levels than both KU and MM; and similarly for the 55 % restriction group, CH had significantly higher ( $p < 0.05$ ) levels than KU.



**Figure 1: Liver HSP70 (A) and iNOS (B) gene expression after 3 weeks of dietary energy restriction; \*significantly different ( $p < 0.05$ ) from the control; Values are presented as Mean  $\pm$  SE. Cont: control, 40R: 40% dietary energy restriction, 55R: 55% dietary energy restriction, KU: Kuchi, CH: Ching'wekwe, MM: Morogoro medium**

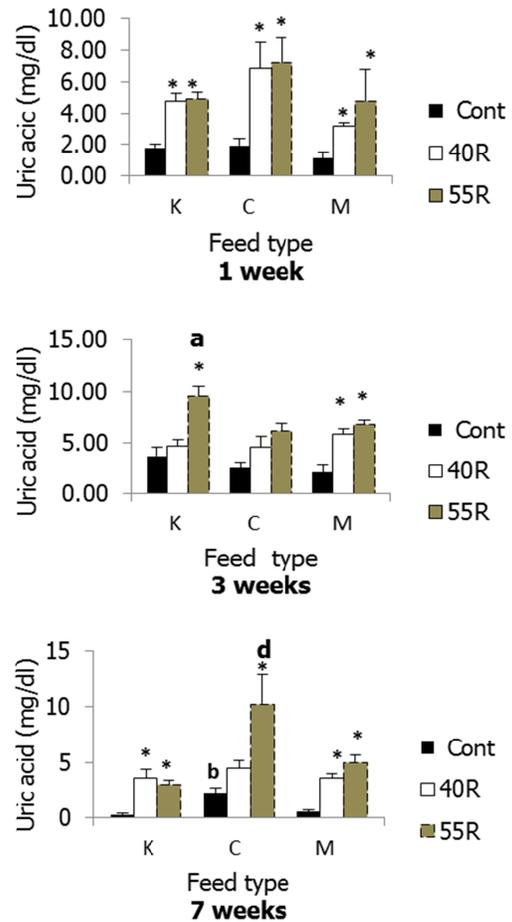


**Figure 2: Liver HSP70 (A) and iNOS (B) gene expression after 7 weeks of dietary energy restriction; \*significantly different ( $p < 0.05$ ) from the control; b: significantly higher than in CH. Values are presented as Mean  $\pm$  SE. Cont: control, 40R: 40% dietary energy restriction, 55R: 55% dietary energy restriction, KU: Kuchi, CH: Ching'wekwe, MM: Morogoro medium**



**Figure 3: Serum corticosterone levels after 1, 3 and 5 weeks of dietary energy restriction; \*: significantly different from the control; a: significantly lower ( $p < 0.05$ ) than K40R; b: significantly lower ( $p < 0.05$ ) than K55R and C55R; c: significantly higher ( $p < 0.05$ ) than cont in K and C; d: significantly lower ( $p < 0.05$ ) than M40R; K: Kuchi; C: Ching'wekwe; M: Morogoro medium; 40R: 40% dietary energy restriction; 55R: 55% dietary energy restriction; Cont: control**

**Glucose and triglycerides:** Results for serum glucose and triglycerides concentrations are shown in Table 2. After a week of feed restriction, serum triglycerides levels in both 40 and 55% restricted groups markedly reduced ( $p < 0.05$ ) in all the ecotypes, with no ecotype specific differences. A similar trend was maintained by both restriction groups for all ecotypes after 3 weeks, though at 40% restriction level CH had significantly higher ( $p < 0.05$ ) levels than MM. After 7 weeks, the serum levels of triglycerides in KU for both restriction levels were not altered but levels in CH and MM markedly declined ( $p < 0.05$ ) for the 55% restricted groups. The CH control group had significantly higher amounts than both KU and MM controls.



**Figure 4: Serum uric acid levels after 1, 3 and 7 weeks of dietary energy restriction; \*: significantly different from the control; a: significantly higher ( $p < 0.05$ ) than in C and M; b: significantly higher ( $p < 0.05$ ) than in K and M; c: significantly higher ( $p < 0.05$ ) than in K; d: significantly higher ( $p < 0.05$ ) than in K; K: Kuchi; C: Ching'wekwe; M: Morogoro medium; 40R: 40% dietary energy restriction; 55R: 55% dietary energy restriction; Cont: control**

Serum glucose levels were notably reduced for the MM 55% restriction group after a week of dietary energy restriction, and for the 40% restricted group, MM had significantly lower levels than KU. After 3 weeks, serum glucose levels were not altered by low energy diets in all groups except the CH 40% restricted group which had a surge. The KU 55% restricted group recorded markedly higher ( $p < 0.05$ ) levels of serum glucose, which were higher than that of MM at the same restriction level after 7 weeks of dietary energy restrictions.

**Table 2: Comparative effects of low energy diets on serum glucose (mg/dl) and triglycerides (mg/dl) between the chicken ecotypes after 1, 3 and 7 weeks**

Feed type	Week 1		Week 3		Week 7	
	Glucose	Triglyceride	Glucose	Triglyceride	Glucose	Triglyceride
K Cnt	180.2±31.5	204.4±60.9	84.3±30.4	155.9±27.2	130.6±17.3	84.9±22.8 <sup>b</sup>
K 40	189.2±14.1 <sup>b</sup>	117.5±38.6 <sup>ni</sup>	67.5±23.6 <sup>b</sup>	90.8±27.6 <sup>ni</sup>	135.5±15.2	90.9±46.4
K 55	175.0±26.6	98.2±21.0 <sup>ni</sup>	58.1±43.0	88.6±25.1 <sup>ni</sup>	161.6±17.9 <sup>ni</sup> <sup>b</sup>	80.0±19.3
C Cnt	150.6±33.1	150.6±33.1	77.5±25.0	208.6±74.4	147.7±23.5	180.7±60.4 <sup>bc</sup>
C 40	164.7±17.6	164.7±17.6	115.8±18.8 <sup>ni</sup> <sup>bc</sup>	157.6±61.8 <sup>b</sup>	129.1±41.5	133.7±39.2
C 55	168.2±22.8	168.2±22.8	84.0±20.2	90.9±56.4 <sup>ni</sup>	152.2±28.8	76.6±18.3 <sup>ni</sup>
M Cont	174.8±25.1	174.8±25.1	95.0±24.5	151.2±26.8	132.8±17.4	124.2±21.3 <sup>c</sup>
M 40	160.9±23.5 <sup>b</sup>	160.9±23.5 <sup>b</sup>	70.2±17.2 <sup>c</sup>	83.4±30.6 <sup>ni</sup> <sup>b</sup>	143.6±10.3	103.3±47.8
M 55	144.7±28.0 <sup>ni</sup>	144.7±28.0 <sup>ni</sup>	85.7±20.6	90.2±45.8 <sup>ni</sup>	123.0±26.0 <sup>b</sup>	70.8±15.4 <sup>ni</sup>

<sup>ni</sup>; means in a column for each ecotype are significantly different from control ( $p < 0.05$ ); <sup>b</sup> and <sup>c</sup>: means bearing same letter within a column between ecotypes are significantly different, and those with a pair are significantly different from those bearing either letter of the pair ( $p < 0.05$ ). K: kuchi; C: ching'wekwe; M: Morogoro medium; 40: 40% restriction; 55: 55% restriction; Cont: control

**Table 3: Comparative effects of low energy diets on Hb (g/dL) and Hct (%) between the chicken ecotypes after 5 and 7 weeks**

	Treatments								
	K Cont	K 40	K 55	C Cont	C 40	C 55	M Cont	M 40	M 55
<b>Week 5</b>									
Hb	9.2±0.9	9.4±0.9	9.4±0.7	9.6±1.0	8.0±2.4	8.6±0.8 <sup>ni</sup>	9.4±2.0	8.5±1.0	9.8±0.9
Hct	24.8±2.9	25.8±2.2	25.9±1.6	24.8±2.2	22.5±4.4	21.3±4.0 <sup>ni</sup>	24.7±4.0	23.1±2.5	25.5±3.1
<b>Week 7</b>									
Hb	11.3±1.6	10.7±2.5	11.3±2.2	11.9±1.5	9.9±0.9 <sup>ni</sup>	10.1±2.0 <sup>ni</sup>	11.6±1.1	11.4±1.5	11.1±1.2
Hct	25.8±3.2	24.7±3.9	24.3±3.9	26.7±3.3	21.4±2.9 <sup>ni</sup>	22.3±3.4 <sup>ni</sup>	25.8±1.9	26.1±4.2	24.8±2.3

<sup>ni</sup>; means in a row for each ecotype that are significantly different from the control ( $p < 0.05$ ). K: kuchi; C: ching'wekwe; M: Morogoro medium; 40: 40% restriction; 55: 55% restriction; Cont: control; Hb: hemoglobin; Hct: hematocrit; RBC: red blood cells; wk: week

**Hb and Hct:** Results for Hb and Hct levels are shown in Table 3. The Hb and Hct levels were not significantly altered by low energy diets during the first four weeks of the study in all ecotypes. Nonetheless, after 5 and 7 weeks of the study, Hb and Hct levels for CH markedly declined ( $p < 0.05$ ) for both restricted groups.

**DISCUSSION**

Dietary energy is important for basal metabolism, maintenance, growth and tissue accretion in chickens, and therefore, reduction in dietary energy intake results in reduced growth and tissue accretion (Veldkamp *et al.*, 2005). In the current study, liver hsp70 and iNOS up-regulation at 3 weeks of dietary energy restriction may be linked to cytoprotection under escalated stressful conditions. In consistent with this, Al-Aqil and Zulkifli (2009) showed that 60 % feed restricted female broiler

chicks had higher hsp70 density than those of the *ad libitum* fed group. Delezie *et al.* (2007) also reported increased hsp70 gene expression levels in broiler chickens after feed deprivation. The current findings indicated significantly higher liver hsp70 expression levels for KU 55 % group than CH and MM, suggesting that it was the most affected ecotype with low dietary energy at the time (after 3 weeks of study). It appears the chickens were affected similarly at 40% energy restriction level and the impact of low dietary energy stress was minimal.

The up-regulated liver hsp70 and iNOS expressions for KU and CH 55 % restriction groups even at 7 weeks of study suggests that the stress effect remained high for these groups. The MM ecotype appeared to be better tolerant and/or was able to quickly adapt to low dietary energy stress by 7 weeks of the study and this is reflected in the low liver hsp70 and iNOS expression levels, unlike KU and CH

chicken ecotypes, which had considerable up-regulations. This finding is in agreement with work on the same chickens which showed that MM performed better than KU and CH at lowest energy levels used in the study (1319 Kcal/kg ME) with respect to growth rate, mean percent weight gain, feed efficient utilization, behavioral and mortality indicators (Khondowe *et al.*, 2017). These results therefore, may imply that liver inflammation due to low energy diets was evident in KU after 3 and 7 weeks but for CH ecotype inflammation was only evident by the 7<sup>th</sup> week of the study. Consistent with some of the findings of the current study, Kang *et al.* (2011) reported increased liver iNOS gene expression after stress caused by feed restriction (75 % of voluntary) and high stocking density in White Leghorn laying hens. Liver iNOS expression may function as an adaptive response to minimise inflammatory injury (Taylor *et al.*, 1998). Generally iNOS is absent in normal liver but is markedly increased in response to inflammation and some oxidative stresses (Clemens, 1999).

Significant elevations of serum corticosterone levels were noted for the KU ecotype restriction groups after 1 and 3 weeks. This is consistent with previous research whereby feed restriction caused a significant elevation in plasma corticosterone concentration in broiler chickens (Al-Aqil and Zulkifli, 2009; Prieto and Campo, 2011). In the current study, the trends in corticosterone levels coupled with liver hsp70 and iNOS gene expression levels, suggest that KU was the most stressed local chicken ecotype by low dietary energy levels. Although the body weight differences were still not very pronounced at this age, the larger mean body weight for KU may have partly contributed to the differences due to escalated metabolic needs for basal metabolism and maintenance.

At 5 weeks of dietary energy restriction serum corticosterone levels were not altered by low energy diets in all ecotypes. This may entail that although differences may exist on how these chickens respond to low dietary energy stress, the period of time in which adaptation may take place might be similar. Surprisingly for CH and MM ecotypes, the levels in serum

corticosterone were not significantly different from the controls even earlier at 1 and 3 weeks of the study. It may be that elevations could have been evident earlier such that by the time of blood sampling after a week, levels would have dropped already to prevent chronic elevations. Chronic stressors such as feed restriction cause corticosterone use to be up-regulated earlier than expected, but in cases of extended chronic stress, down-regulation may ensue, thereby avoiding the adverse effects of chronically elevated levels (Walker *et al.*, 2005). Feed restriction initially causes a physiological stress response, although chickens quickly habituate and the response is minimized (Prieto and Campo, 2011). Other studies have also shown that repeated feed restriction or deprivation can lead to habituation of the corticosterone responses in poultry (Zulkifli *et al.*, 2006). In the current study, while all the three ecotypes seem to be well adapted to their environments, the better tolerance and adaptation exhibited by the MM ecotype might be due to natural genetic selection overtime.

Serum glucose levels for the restricted groups were generally maintained at similar levels with controls except for MM 55 % restricted group at 1 week (lowered), CH 40 % restricted group (elevated) at 3 weeks, and KU 55 % restricted group (elevated) at the end of the study. Plasma levels of glucose are typically very stable in birds, even during fasting or starvation (de Jong *et al.*, 2002). However, the continuous stimulation of the adrenal cortex leads to intermittent increase in the level of corticosterone, which is responsible for the formation of glucose molecules from reserves of carbohydrates, lipids and proteins (Ognik and Sembratowicz, 2012). In the current study, it seems that the chickens' physiological response progressed at different rates having been affected differently as shown by inconsistent changes in levels of serum glucose. On the other hand serum triglyceride levels were consistently significantly reduced in both restricted groups after 1 and 3 weeks for all the ecotypes and at both times there were no between-ecotype differences. After 7 weeks of the study, triglyceride levels did not significantly differ from the control except for CH and MM 55

% restricted groups. Therefore it shows that CH and MM 55 % restricted groups had lowered triglyceride levels throughout the study period. This is consistent with studies by Zhan *et al.* (2007), who reported decreased serum triglycerides levels in feed restricted broilers on day 21 of feed restriction. The major fuels of muscle include glucose and fatty acids; and fatty acids in muscle are derived from circulating triglycerides and endogenously stored intramuscular triglycerides (Zhan *et al.*, 2007). In the current study, therefore, as low energy levels persisted for the chickens, there was high demand of these metabolites to fuel muscular function. The study chickens' response to lower energy levels seems to be similar in all ecotypes with respect to stimulating triglyceride uptake from the blood though it seems CH and MM triggered a continuous response, thus better coping up than KU.

The results of the current study have shown markedly higher ( $p < 0.05$ ) serum levels of uric acid in the restricted groups for the entire period of study in all chicken ecotypes. The elevations are consistent with Chen *et al.* (2012) who reported that energy restriction significantly increased serum uric acid in 30 % energy restricted broilers. The ecotype-specific differences in the levels of uric acid were seen in the later stages of the study, with KU 55 % and CH 55 % restricted groups recording higher levels after 3 and 7 weeks, respectively. Despite these differences in levels, it seems the consistence and rate of the physiological response to low energy levels with respect to release of uric acid among the ecotypes is similar. Avoidance of oxidative stress relies on antioxidants and antioxidative enzymes; and uric acid is an important antioxidant and primary end-product of nitrogen metabolism in birds (Hartman *et al.*, 2006). It has the ability to inactivate an oxidant via an electron transfer before the oxidant can react with the targeted biological molecule, and can inactivate strong oxidants like nitrite and hydroxyl generated radicals (Simic and Jovanovic, 1989).

The low energy diets did not induce changes in the levels of Hb and Hct for the entire study period except for the CH ecotype, which showed significantly lower Hb and Hct

after 5 and 7 weeks for both restricted groups. Findings by other researchers (Boostani *et al.*, 2010; Tamzil *et al.*, 2014) reported reductions in Hb and Hct after feed restriction in broiler chickens. In the current study, reductions in these parameters for the CH ecotype may have led to a decrease in oxygen carrying capacity and the acid base balance could be compromised as evaluated Hct is advantageous in adaptation to stress through maintenance of high oxygen carrying capacity. However, Junqueira *et al.* (2003) reported that Hb and Hct values were not affected by feed restriction of broilers from 22 to 42 days of age. This is consistent with responses of KU and MM ecotypes in the current study. Therefore, ecotype-specific differences in Hb and Hct responses to low dietary energy may have been influenced by apparent differences in the genotypes of the chickens.

**Conclusion:** Low energy diets induced stress in all the chicken ecotypes studied and ecotype-specific responses and tolerance were manifested in the Liver HSP70 and iNOS up-regulations. Adaptation patterns through changes in blood corticosterone and uric acid in some cases also show inter-ecotype differences. The MM ecotype showed to be better tolerance at lowest energy levels used in this study whilst KU appeared the least tolerant. These findings have important implications for future research that would enhance selection for low dietary energy tolerance among the local chicken stocks.

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