Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress

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ABSTRACT
The impact of dietary regimens on productive traits, meat quality, physiological adoption, immunological response and digestibility of nutrients was studied using Ross-308 and Cobb-500 strains. Chickens were reared under normal brooding conditions during most of the experimental period, but exposed to heat stress [(HS); 32°C and 55% RH] during 20–22 and 28–30 d of age. During 1–18 d of age, broilers were fed a standard-protein diet [(SPD); 22% crude protein (CP) with 12.97 MJ/kg], a high-protein diet [(HPD); 24% CP with 12.97 MJ/kg], or a high-protein with high-metabolisable energy (ME) diet [(HPMED); 24% CP with 13.60 MJ/kg]. During 19–35 d of age, the SPD group was fed 20% CP with 13.42 MJ/kg, the HPD group was fed 22% CP with 13.42 MJ/kg, and the HPMED group was fed 22% CP with 14.06 MJ/kg. Each group within each strain was replicated six times with five chicken males each. For the whole experimental period, Ross and Cobb chickens fed the HPMED showed higher BWG than chickens on the HPD. Broilers fed the HPMED had improved FCR compared to those on the SPD, independent of broiler strain. Further, independent of broiler strain, the HPMED regimen increased the dry matter, protein, and lipid contents of meat compared to the other regimens, and decreased cloacal temperature, respiration rate and Heterophile/lymphocyte (H/L ratio) compared to the HPD regimen. Growth and feed utilisation, percentage thymus, protein in meat were improved of Ross compared to Cobb, but abdominal fat, meat lipid and pH were decreased. On the other hand, Cobb on HPD had lower cloacal temperature and respiration rate than Ross strain, suggesting that production, physiology and immunological response of broiler chickens depend on feeding regimen that should be specific for each strain.

Introduction
Dietary protein is an important factor in the production and reproduction of poultry, particularly in relation to the feeding costs and benefits of poultry farming. In the literature, a significant relationship between dietary protein levels and the genetic make-up required for the optimum production of chickens have been reported (Razuki and Al-Rawi 2007; Attia et al. 2010a, 2010b, 2016). Dietary protein and thus amino acid requirements differ based on the age of the chicken, the goal of production, dietary metabolisable energy (ME), environmental conditions, sex, age and feed intake (Pedroso et al. 2003; Corrêa et al. 2006; Razuki and Al-Rawi 2007; Attia et al. 2010a, 2010b, 2016). Under thermoneutral conditions, average daily gain, feed intake and utilisation were similar among different CP concentrations (18.5, 20.5 and 22.5%) during 14–49 d of age. The breast and drumstick meat were significantly greater in broilers on the 22.5% CP than those on the 20.5 and 18.5% CP (Laudadio et al. 2012).

Hot climate is a worldwide problem that causes poor performance and loss of economic benefits (Daghir 2008; Attia and Hassan 2017), particularly after the third week of age (Attia et al. 2011). Moreover, under heat stress conditions, growth was found to be adversely affected by increasing dietary protein concentration (Cahaner et al. 1995). In addition, respiratory system and cardiovascular development are negatively affected by high ambient temperature, which causes low performance (Yahav 2000; Steiner et al. 2008). Losses in productivity of broilers caused by continuous heat exposure could be explained by the decrease in nutrients digestibility (Bonnet et al. 1997; Souza et al. 2017).
Heat production from protein turnover increased with increasing dietary protein/amino acid, and became even worse in hot climates (Musharaf and Latshaw 1999; Attia and Hassan 2017). The dissipation of heat produced in hot climates increased maintenance energy requirements (Attia et al. 2006; Lin et al. 2016) that may be caused by peripheral vasodilation and reduced blood flow in the gut and thus decreased intestinal function (Yamauchi 2002; Attia and Hassan 2017).

Heat stress increased the need for heat production from protein turnover and reduced blood flow in the gut and thus decreased intestinal function (Yamauchi 2002; Attia and Hassan 2017). Therefore, the problem may be complicated because of the decrease in feed intake (NRC 1994; Daghir 2008). Thus, elevating ME by oil/fat addition could be an efficient way to improve the performance of animals in hot regions, due to increase digestibility of nutrient, fat-soluble vitamins and extra caloric effect of fats/oils (Dale and Fuller 1979; Attia et al. 2011).

Dietary modification of poultry feeds may offer a possible solution to overcome poor performance of chickens in hot zones (Razu and Al-Rawi 2007; Daghir 2008; Attia et al. 2011; Suganya et al. 2015; Attia and Hassan 2017). Dietary CP and metabolisable energy (ME) are the main factors affecting the cost of dietary composition for poultry (Kamran et al. 2010; Attia and Hassan 2017). In literature, findings in relation to the impact of dietary CP on the performance of broilers under normal and heat stress conditions are contradictory. The use of low-CP diets for broiler chickens caused low performance compared to standard protein with sufficient amino acids (Berres et al. 2010). On the other hand, Widiyaratne and Drew (2011) and Laudadio et al. (2012) found that low-protein diets under thermoneutral conditions did not influence the performance of broilers. However, in hot climates, low-protein feed increases growth performance (Thim et al. 1997) and decreases excreta N, thus eliminating environmental pollution from N (Sterling et al. 2005; Kamran et al. 2010; Attia and Hassan 2017). However, protein/amino acid requirements may not be elevated during HS due to the decrease in growth and thus protein needs for production (Attia et al. 2006, 2011; Attia and Hassan 2017). Breakdown and synthesis of protein are adversely influenced by heat stress (Lin et al. 2006) and was found to result in low-protein deposition (Temim et al. 2000). Heat stress decreased consumption of feeds and digestibility of dry matter and protein, and thus growth, but did not affect FCR (Souza et al. 2016). Broilers exposed to continuous heat stress increased ME intake (20.3%) and heat production (35.5%), and decreased ME retention (20.9%), ME efficiency (32.4%), nitrogen intake, nitrogen retention (50.4%) and nitrogen efficiency (33.1%) compared to controls. This indicates that a continuous HS had a stronger adverse effect than cyclical ones, which has a less effect on performance and digestibility of nutrients (Souza et al. 2016).

Information regarding the influence of the elevation of dietary of ME on enhancing chickens’ tolerance of high ambient temperatures is lacking in the literature, particularly in relation to elevating dietary protein concentration. Nonetheless, the influence of HS in the utilisation of ME still requires further verification, as different authors have found an elevation (Keshavarz and Fuller 1980), a decline (Yamazaki and Zi-Yi 1982; Souza et al. 2016; Attia and Hassan 2017) and no influence (Geraert et al. 1992; Faria Filho et al. 2007). Additionally, HS influences meat yield, carcase composition, and ME, independently of feed intake (Cahaner et al. 1995; Geraert et al. 1996; Attia and Hassan 2017). Protein metabolism has also been found to be affected by a decline in nitrogen consumption and nitrogen retention (Temim et al. 1999; Souza et al. 2016). Moreover, a reduction in the deposition of protein in muscle in chickens exposed to HS has been reported (Temim et al. 2000; Attia and Hassan 2017).

Blood metabolites offer a good indication of the influences of dietary factors on metabolic, immunological and antioxidant conditions (Attia and Hassan 2017). Plasma hormones, liver and renal functions, protein and antioxidant status are affected by HS and dietary factors (Yahav 2000; Attia et al. 2006, 2009, 2011; Attia and Hassan 2017). Hence, this study aims to investigate tolerance of Ross-308 and Cobb-500 strains exposed to heat stress and fed standard and high-protein diet without or with high energy diet on productive traits, meat quality, physiological response and digestibility of nutrients from 1 to 35 d of age.

Materials and methods

Experimental design, chicks and feeding regimen

A total of 180, one-day-old commercial male broiler strains from Ross-308 (90 chicks) and Cobb-500 strains (90 chicks) were randomly distributed, keeping equal initial body weight (44.1 ± 2.7) in factorial design, totaling six treatment groups. Each treatment group within each strain contained six replicates with five chicken males per replicate (2 strains × 3 dietary treatments × 6 replicates × 5 male chicks = 180 chicks).

During 1–18 d of age, broilers were fed a standard-protein diet ([SPD]; 22% CP with 12.97 MJ/kg based on NRC (1994)], a high-protein diet ([HPD];
24% CP with 12.97 MJ/kg) or a high-protein with high-metabolisable energy diet [(HPMED); 24% CP with 13.60 MJ/kg]. During 19–35 d of age, the SPD group was fed a diet containing 20% CP with 13.39 MJ/kg, the HPD group was fed a diet containing 22% CP with 13.39 MJ/kg, and the HPMED group was fed a diet containing 22% CP with 14.02 MJ/kg. Chickens were reared under normal brooding conditions (34, 32, 30, 28, 25 °C during the first, second, third, fourth and fifth week of age, respectively) except for when they exposed to heat stress. Chickens were exposed to HS (32 °C and 55% RH) for 6 h a day from 10 am to 4 pm, during 20–22 and 28–30 d of age, and returned to thermoneutral conditions thereafter. The reason for this regimen is that continuous heat stress does not normally occur in natural environments, as HS usually occurs in waves from 3 to 5 d, followed by a broken wave, and occurs for other waves depending on the environmental situation (Mashaly et al. 2004; Attia and Hassan 2017). Chicks were raised in battery brooders. Each replicate was kept in a cage (30 × 35 × 45). Vegetable diets (Table 1) were formulated using NRC (1994) tabulated values of feedstuffs. Broilers were offered free access to water and mash feed, and illuminated with 24 h of light during one to three days of age and a 23:1 light-dark cycle afterward. Broilers were vaccinated with Hatchiner’s B1 + IB at 8 d of age, avian Influenza h5n2 and Gambaro at 11, 13 and 26 d of age, and Lasota at 35 d of age. The Care and handling of the chickens were approved by the department committee that recommended animal rights, welfare and minimal stress that is subjected to, the Government Law No 9 in 24-8-2010, about the regulations of Research ethics on living creatures.

### Data collection

Chickens were weighed (g) at 1, 18 and 35 d of age. Body weight gain, feed intake and FCR were recorded at 18 and 35 d of age using a replicate as the experimental unit. The FCR was calculated from feed intake and BWG. Cloacal temperature was measured just after exposure to HS by using a digital thermometer with an accuracy of 0.1 °C, using one chick per replicate. The respiration rate just after exposure to HS was estimated by counting the breaths/min by watching movement of the abdomen for a minute.

| Table 1. The composition, calculated and chemical analysis of the experimental diets fed during days 1–18 (starter phase) and days 19–35 (grower-finisher phase). |
| Ingredient | 22% (SPD) | 24% (HDP) | 24% (HPMED) | 20% (SPD) | 22% (HDP) | 22% (HPMED) |
| Yellow corn | 552.80 | 491.75 | 458 | 564 | 528.5 | 520 |
| Soybean meal, 48% | 330 | 385 | 390 | 260 | 310 | 310 |
| Corn gluten meal | 30 | 30.00 | 30.00 | 50.00 | 50.00 | 50.00 |
| Dicalcium phosphate | 18.30 | 18.00 | 18.00 | 17.50 | 16.80 | 16.80 |
| Limestone | 13.50 | 13.00 | 13.00 | 11.00 | 11.00 | 11.00 |
| NaCl | 4.00 | 4.00 | 4.00 | 3.00 | 3.00 | 3.00 |
| Premix x | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Methionine | 2.10 | 1.60 | 1.60 | 2.20 | 1.70 | 1.70 |
| Lysine | 2.30 | 0.65 | 0.65 | 3.20 | 1.60 | 1.60 |
| Mixture of vegetable oils | 44.00 | 53.00 | 81.75 | 61 | 62.50 | 81.90 |
| Sand | 0.00 | 0.00 | 0.00 | 25.10 | 11.90 | 1.00 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

Vit + Min mixture provides (per kg diet): vitamin A (retinyl acetate) 24 mg, vitamin E (dl-α-tocopheryl acetate) 20 mg, menadione 2.3 mg, Vitamin D3 (cholecalciferol) 0.05 mg, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, choline chloride 600 mg, vitamin B12 10 µg, vitamin B6 3 mg, thiamine 3 mg, folic acid 1 mg, d-biotin 0.50 mg, Mn 80 mg Zn 60 mg, Fe 35 mg, Cu 8 mg, Se 0.60 mg; bthe calculated values; cthe determined values.
The European broiler index (EBI) was calculated according to the following formula (http://poultry-performanceplus.com/information-database/broilers/285-european-broiler-index-or-european-production-efficiency-factor):

$$\text{EBI} = \frac{\text{Average grams gained day} \times \% \text{ survival rate}}{\text{Feed conversion} \times 10}$$

Digestibility of nutrients was determined using the total gut collection method during 35–40 d of age, as 2 d of preliminary experiments and 3 d of collection, using five replicates of three males per replicate, as reported by Attia and Hassan (2017). Concentrations of nutrients in the excreta and those in the feed were estimated according to AOAC (2007). They were expressed on a dry matter basis. The daily amount retained (g/d) divided by the daily amount intake (g/d) was used for the calculation of percent digestibility.

At 35 d, chicken males (n = 6 representing all treatment replicates per strain) were taken randomly from each treatment, weighed after fasting overnight, slaughtered, and had feathers picked, and dressed carcass weight without head, legs and viscera were weighed. The inner organs, bursa of fibrous, thymus, spleen and abdominal fat were isolated and individually weighed and expressed as a percentage of live body weight.

Meat samples (n = 6 representing all treatment replicates per strain) were collected from the slaughtered chickens per treatment. The meat samples were a mixture of 50% breast meat +50% thigh meat. The samples were weighed, dried and ground to pass through a sieve (1 mm²). The meat samples were subjected to chemical composition according to AOAC (2007). A part of the fresh meat samples was used for determination of the physical characteristics of meat as reported by Attia and Hassan (2017) included meat colour, pH, water holding capacity (WHC) and tenderness.

At 35 d of age, samples (n = 6 showing all treatment replicates per strain per treatment) of the ileum of the slaughtered chickens were collected for histological investigation. The samples were collected immediately and fixed in a 10% formalin saline buffer. For light microscopy, the segments were opened and rinsed with a phosphate buffer (0.1M, pH 7.4) and fixed in Bouin’s solution for 24 h. Then, samples were dehydrated in a graded series of ethanol, diaphanised in xylene and paraffin embedded. Cross sections of the ileum were made at 5 μm and they were stained with haematoxylin and eosin. A morphometric estimation of the ileum villi length was done using binocular microscope equipped with a clear Nikon camera coupled with an image-analysing system from Optika according to Aptekmann et al. (2001).

Blood samples were collected at 35 d of age from six chicken males of each strain per treatment demonstrating all treatment replicates. The samples were collected without or with heparin to determine the haematological, phagocyte index and activity and biochemical constituents of blood and determinations were as cited by Attia and Hassan (2017) and Hepler (1966).

Blood plasma was obtained by centrifugation of blood at 1500 × g for 15 min and was stored at −18 °C for analyses of the alkaline phosphatase (ALP), malondialdehyde (MDA), total antioxidant capacity, plasma tri-iodothyronine (T3), glucose (g/dL), total protein (g/dL), albumin (g/dL) and total plasma cholesterol using commercial diagnostic kits. The kits were produced by Diamond diagnostics (23 EL-Montazah St. Heliopolis, Cairo, Egypt, http://www.diamonddiagnostics.com).

Statistical evaluation

Data were statistically evaluated using the PROC ANOVA of SAS® 9.3, and the difference among means was tested using the student Newman–Keuls test (SAS Institute 2007; Cary, NC). The replicate was the experimental unit. The experimental model was as follows: $y_{ijk} = \mu + t_j + h_k + (th)_{jk} + \epsilon_{ijk}$, where $\mu$ = the general mean, $t_j$ = the effect of dietary regimen, $h_k$ = the effect of strain broilers $(th)_{jk}$ = the interaction effect, and $\epsilon_{ijk}$ = the experimental error. All the percentages were converted as log10 to normalise data distribution.

Results

Productive performance

Feeding regimen and/or genotype had a significant influence on BWG during all experimental periods (Table 2). Broilers on the HPMED had a significantly higher BWG than those fed SPD during days 1–18 of age. Broilers fed the HPMED and SPD had a significantly higher BWG than those fed the HPD during days 19–35 of age in the heat stress period, and broilers on the HPMED had a significantly higher BWG than those fed the SPD and the HPD for the whole period (1–35 d of age). During 19–35 d of age, regardless of kind of diet, the Ross chickens had a significantly higher BWG than the Cobb strain. A significant interaction between broiler genotype and dietary regimen was found in BWG during the experimental periods (Table 3). During 1–18 d of age, Cobb chickens on
Table 2. Effect of dietary regimen and/or broiler strain on body weight gain, feed intake and feed conversion ratio of broiler chickens during days 1–35 of age.

| Treatments | Body weight gain, g/chick | Feed intake, g/chick | Feed conversion, g feed/g gain | Survival rate, % | European broiler index, % |
|------------|---------------------------|----------------------|--------------------------------|------------------|--------------------------|
|            | 1–18 Days | Days | 19–35 Days | 1–35 Days | Days | 19–35 Days | 1–35 Days | 1–18 Days | Days | 19–35 Days | 1–35 Days | 1–18 Days | Days | 19–35 Days | 1–35 Days |
| SPD        | 457<sup>b</sup> | 1170<sup>a</sup> | 1627<sup>b</sup> | 657 | 2437<sup>a</sup> | 3094<sup>b</sup> | 1.44 | 2.08<sup>a</sup> | 1.90<sup>a</sup> | 97.2 | 238<sup>b</sup> |
| HPD        | 482<sup>c</sup> | 1133<sup>b</sup> | 1615<sup>b</sup> | 672 | 2278<sup>b</sup> | 2950<sup>a</sup> | 1.39 | 2.01<sup>a</sup> | 1.83<sup>ab</sup> | 97.2 | 246<sup>b</sup> |
| HPMED      | 490<sup>c</sup> | 1212<sup>a</sup> | 1702<sup>b</sup> | 684 | 2360<sup>b</sup> | 3044<sup>a</sup> | 1.40 | 1.95<sup>b</sup> | 1.78<sup>b</sup> | 100 | 273<sup>c</sup> |
| p Value    | .001 | .003 | .007 | NS | .001 | NS | .010 | NS | .001 | .010 | NS | .010 |
| SEM        | 11.200 | 18.900 | 23.200 | 25.400 | 22.300 | 28.700 | 0.034 | 0.021 | 0.031 | 1.170 | 4.900 |

<sup>a,b,c</sup>Means within a column under similar criteria not sharing a common superscript are significantly different (p < .05).

SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; DR: dietary regimen; S: strain; NS: not significant; SEM: standard error of mean.

Table 3. The observed significant interaction effects between strain of broiler and dietary regimen for body weight gain, European broiler index, cloacal temperature, respiration rate and digestibility of protein, lipids and crude fibre.

| Criteria | Ross | Cobb |
|----------|------|------|
|          | SPD  | HPD  | HPMED | SPD  | HPD  | HPMED | p Value | SEM |
| Body weight gain |      |      |       |      |      |       |         |     |
| Days 1–18 | 460<sup>b</sup>,<sup>c</sup> | 451<sup>c</sup> | 481<sup>b</sup>,<sup>c</sup> | 454<sup>b</sup>,<sup>c</sup> | 513<sup>a</sup> | 499<sup>b</sup>,<sup>c</sup> | .010 | 15.900 |
| Days 19–35 | 1184<sup>a</sup> | 1176<sup>b</sup> | 1215<sup>b</sup> | 1156<sup>a</sup> | 1090<sup>b</sup> | 1209<sup>a</sup> | .001 | 26.800 |
| Days 1–35 | 1644<sup>b</sup> | 1627<sup>c</sup> | 1696<sup>ab</sup> | 1610<sup>b</sup>,<sup>c</sup> | 1603<sup>c</sup> | 1708<sup>b</sup> | .010 | 32.900 |
| European broiler index, % | 238<sup>b</sup> | 264<sup>b</sup> | 277<sup>a</sup> | 237<sup>b</sup> | 227<sup>a</sup> | 270<sup>a</sup> | .010 | 8.400 |
| Cloacal temperature and respiration rate |      |      |       |      |      |       |         |     |
| Cloacal temperature, °C | 42.100 | 42.400 | 41.900 | 41.700 | 40.000 | 41.700 | .010 | 0.113 |
| Respiration rate, breath/min | 52.400 | 58.600 | 51.000 | 52.100 | 51.200 | 50.400 | .010 | 1.520 |
| Digestibility of nutrients |      |      |       |      |      |       |         |     |
| Protein, % | 79.000<sup>a</sup> | 70.100<sup>d</sup> | 76.000<sup>b</sup> | 77.400<sup>d</sup> | 72.900<sup>a</sup> | 74.600<sup>b</sup>,<sup>c</sup> | .010 | 0.786 |
| Lipids, % | 86.500<sup>b</sup>,<sup>c</sup> | 85.500<sup>c</sup> | 88.700<sup>a</sup>,<sup>b</sup> | 82.100<sup>d</sup> | 89.300<sup>a</sup>,<sup>b</sup> | 90.200<sup>a</sup> | .010 | 1.030 |
| Dietary fibre, % | 18.200<sup>b</sup> | 14.200<sup>c</sup> | 22.000<sup>a</sup> | 15.200<sup>d</sup> | 14.400<sup>c</sup> | 14.000<sup>c</sup> | .010 | 0.654 |

<sup>a,b,c</sup>Means within a row not sharing a common superscript are significantly different (p < .05).

SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; SEM: standard error of mean.

the HPD had a significantly higher BWG than Cobb and Ross chickens fed the SPD and Ross chickens fed HPD. During the period (19–35 d of age) of exposure to HS, Cobb chicks fed the HPD had a significantly lower BWG than that of the other groups. During 1–35 d of age, Ross and Cobb chickens fed the HPMED had a significantly higher BWG than those fed the HPD. In addition, Cobb chickens receiving the HPMED had a significantly higher BWG than the same genotype and Ross fed the SPD. Feed intake during 19–35 and 1–35 d of age was significantly affected by dietary regimen (Table 2). Chickens fed the HPD consumed a significantly lower amount of feed than those fed the SPD during 19–35 d of age, and those on the other regimens during 1–35 d of age.

Genotype had a significant impact on feed intake during most of the experimental periods (Table 2). The Ross strain consumed a significantly lower feed than the Cobb strain during 1–18 and 1–35 d of age, but differences during 19–35 d of age were not significant. There was no significant interaction between broiler genotype and dietary regimen on feed intake.

Dietary regimen had no significant effect on FCR during the first experimental period (Table 2). However, feeding with the HPMED significantly improved FCR compared to the SPD and HPD during 19–35 d of age, and compared to SPD during 1–35 d of age. Genetic difference was observed in FCR, with the Ross strain utilising feeds significantly more efficiently than the Cobb strain during the experimental periods (Table 2). The interaction between strains of broilers and dietary regimen was not significant for FCR (Table 2). Survival rate was not significantly affected by the dietary regimen and/or genetic makeup.

The European broiler index showed a significant effect of dietary regimen, strain of broilers and interaction dietary regimen × broiler strain. The results
indicate that the HPD and HPMED regimens increased the EBI of Ross broilers compared to SPD. In addition, the HPMED regimen raised the EBI of the Cobb strain compared to the SPD and HPD regimens. Furthermore, Ross chickens on the HPD regimen yielded a higher EBI than the Cobb strain on the same feeding regimen (Table 3).

**Digestibility of nutrients**

Dietary regimen had a significant influence on the CP, lipids and crude fibre digestibility (Table 4). Feeding with the SPD resulted in a significantly higher protein digestibility than the HPD regimen, but lower digestibility of lipid. Feeding with the HPMED significantly increased digestibility of lipid compared to the SPD, and digestibility of crude fibre compared to the HPD.

Genotype had no significant effect on most nutrients' digestibility, except crude fibre digestibility, which was significantly lower in Cobb chickens than the Ross strain (Table 4). Ross chickens fed the SPD and HPMED had significantly greater protein digestibility than the same strain on the HPD regimen (Table 3). The Cobb on the HPD showed lower digestibility of protein than the same strain on the SPD, but feeding HPMED restored protein digestibility to the SPD. Cobb fed the HPD had a significantly greater protein digestibility than the Ross on the same regimen.

Lipid digestibility was increased due to increasing CP, with Ross strain; lipid digestibility was significantly higher on the HPMED than those on the HPD (Table 3). Within Cobb chickens, feeding the high-protein diet increased lipid digestibility compared to those fed the SPD. In addition, the difference between HPD groups was significant, and was higher for the Cobb than the Ross.

Ross chickens fed the HPMED had significantly greater crude fibre digestibility than chickens on other regimens (Table 3). In addition, the Ross strain on the SPD showed improved crude fibre digestibility compared to the rest of the groups. Cobb chickens fed the HPD had the lowest crude fibre digestibility.

**Cloacal temperature and respiration rate**

Dietary regimen and/or genotype only had a significant impact on cloacal temperature and respiration rate (Tables 3 and 4). Cloacal temperature and respiration rate were higher values of Ross strain fed HPD than the rest of the experimental groups (Table 3).

**Carcass and organ traits**

Dietary regimen showed a significant impact only on proventriculus, ileum villi length, abdominal fat and heart percentage (Table 5). Feeding with the HPMED regimen was associated with a significantly higher proventriculus than the SPD regimen. Ileum villi length was greater with the SPD regimen than the HPMED regimen. Feeding with the HPMED regimen resulted in significantly greater abdominal fat and heart percentage than the HPD.

Genotype displayed a significant influence only on abdominal fat with Cobb chicks had a significantly higher abdominal fat percentage than Ross (Table 5). There was no significant interaction between strains of broilers and dietary regimen on carcase and organs parameters.

**Table 4.** Effect of dietary regimen and/or broiler strain on digestibility nutrient at day 35 of age, cloacal temperature and respiration rate.

| Treatments | Dry matter | Protein | Lipids | Crude fibre | Ash retention | Physiological adoption |
|------------|------------|---------|--------|-------------|---------------|------------------------|
|            | %          |         |        |             |               | CT, °C                 | RR, Br/Min             |
| Dietary regimen |          |         |        |             |               |                        |                        |
| SPD        | 73.500     | 78.200 b| 84.300 b| 16.700 a    | 21.500        | 41.900 b              | 52.300 b              |
| HPD        | 75.800 b   | 71.500 b| 87.400 a| 13.100 b    | 23.200        | 42.200 a              | 54.900 a              |
| HPMED      | 74.200 a,b | 75.300 a,b| 89.500 a| 18.200 a    | 20.800        | 41.800 b              | 50.700 b              |
| p Value    | NS         | .010    | .010   | .010        | NS            | .001                  | .001                  |
| SEM        | 1.970      | 0.321   | 0.687  | 0.447       | 0.930         | 0.080                 | 1.07                  |
| Strain     |            |         |        |             |               |                        |                        |
| Ross       | 73.400 a   | 75.100  | 86.800 | 18.100 a    | 21.100        | 41.800 b              | 51.400 b              |
| Cobb       | 72.100     | 75.000  | 87.300 | 13.900 b    | 22.500        | 42.100 a              | 53.800 a              |
| p Value    | NS         | NS      | NS     | .010        | NS            | .010                  | .010                  |
| SEM        | 1.470      | 0.212   | 0.571  | 0.158       | 0.758         | 0.070                 | 0.870                 |
| DR × S interaction | NS | .010 | .001 | .010 | NS | .010 | .010 |

Means within a column under similar criteria not sharing a common superscript are significantly different (p < .05).

SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; CT: cloacal temperature; RR: respiration rate; SEM: standard error of mean; DR: dietary regimen; S: strain.
Meat quality

Dietary regimen had a significant impact on the chemical composition of meat, except ash. Feeding with the HPMED yielded significant higher dry matter, crude protein and lipids than feeding with the SPD and HPD. Feeding with the HPD yielded significantly more meat lipids than feeding with the SPD (Table 6). The broiler strain showed a significant influence on meat protein, lipids and pH. The Ross strain displayed higher meat protein than the Cobb genotype, but lower meat lipids and pH. Dietary regimen or strain of broilers did not affect the colour, tenderness or WHC of meat. In addition, the interaction between genetic make-up and dietary regimen in relation to meat quality traits was not significant.

Blood plasma biochemical constituents

Plasma protein, albumin, globulin and globulin to albumin ratio, alkaline phosphatase, cholesterol, T₃, TAC and MDA were not significantly affected by dietary regimen (Table 7). However, plasma glucose was higher (p < .05) of group fed the HPD than of the other groups. Genotype only had a significant impact on T₃.

Blood haematological constituents and immunological response

Dietary CP regimen and/or strain of broilers did not significantly affect RBC’s, Hgb and PCV%, MCV, MCH and MCHC. (Data was not presented). For example, the values ranged from 1.72 to 1.75 x 10¹² for RBCs, 9.43–9.60 g/L for Hgb and 28.8–29.2% for PCV. The values for MCV, MCH and MCHC ranged 166.9–167.4, 54.6–54.9 and 32.7–32.9, respectively. Lymphoid organs were not affected by dietary regimen and strain of broilers except for thymus % which was greater of Ross than Cobb. In addition, most of immune indices were not affected by dietary regimen, except for phagocyte activity, heterophile and H/L ratio (Table 8). Feeding with the HPMED regimen resulted in lower heterophile levels than the SPD and HPD regimens, but higher phagocytes activity. Feeding with the HPD regimen had a significantly higher H/L ratio than the other groups. In addition, broilers fed

| Table 5. Effect of dietary regimen and/or broiler strain on carcase and organs criteria of broiler chickens at day 35 of age. |
|--------------------------------------------------|
| Treatments | Dressing (%) | Proventriculus (%) | Pancreas (%) | Gizzard (%) | Intestinal (%) | Ileum villi length, μm | Liver (%) | AF (%) | Heart (%) |
| Dietary regimen | | | | | | | | | |
| SPD | 66.100b | 0.2610b | 0.251 | 2.330 | 4.110 | 1828b | 2.690 | 1.230b | 0.339b |
| HPD | 65.400b | 0.301b | 0.240 | 2.410 | 3.950 | 1731b | 2.870 | 1.110b | 0.295b |
| HPMED | 65.800 | 0.358b | 0.295 | 2.370 | 4.520 | 1433b | 2.960 | 1.520b | 0.355b |
| p Value | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| SEM | 1.370 | 0.023 | 0.033 | 0.078 | 0.236 | 117.100 | 0.156 | 0.136 | 0.018 |
| Strain | | | | | | | | | |
| Ross | 65.300 | 0.322 | 0.241 | 2.460 | 4.090 | 1558 | 2.760 | 1.120b | 0.349 |
| Cobb | 66.200 | 0.291 | 0.283 | 2.280 | 4.290 | 1694 | 2.920 | 1.460b | 0.311 |
| p Value | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| SEM | 1.120 | 0.018 | 0.027 | 0.064 | 0.193 | 95.800 | 0.127 | 0.111 | 0.015 |
| DR × S interaction | NS | NS | NS | NS | NS | NS | NS | NS |

a,bMeans within a column under similar criteria not sharing a common superscript are significantly different (p < .05). AF: abdominal fat; SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; DR: dietary regimen; S: strain; NS: not significant; SEM: standard error of mean.

| Table 6. Effect of dietary regimen and/or broiler strain on chemical composition and physical characteristics at day 35 of age. |
|--------------------------------------------------|
| Treatments | Chemical composition, % | Physical characteristics |
|--------------------------------------------------|
| Treatments | Dry matter | Protein | Lipids | Ash | pH | Colour | Tenderness, cm²/g | WHC, cm²/g |
|--------------------------------------------------|
| Dietary regimen | | | | | | | | |
| SPD | 25.600b | 19.100b | 4.690c | 1.210 | 6.700 | 0.273 | 9.030 | 16.700 |
| HPD | 25.700b | 19.200b | 5.470b | 1.130 | 6.620 | 0.279 | 9.270 | 17.000 |
| HPMED | 26.300a | 19.700a | 6.130a | 1.070 | 6.670 | 0.281 | 9.020 | 16.800 |
| p Value | NS | NS | NS | NS | NS | NS | NS | NS |
| SEM | 0.044 | 0.056 | 0.041 | 0.011 | 0.072 | 0.006 | 0.156 | 0.256 |
| Strain | | | | | | | | |
| Ross | 25.700 | 19.600a | 5.340b | 1.120 | 6.530b | 0.275 | 8.990 | 17.000 |
| Cobb | 26.100 | 19.000b | 5.380b | 1.160 | 6.790b | 0.277 | 9.220 | 16.800 |
| p Value | NS | NS | NS | NS | NS | NS | NS | NS |
| SEM | 0.035 | 0.046 | 0.034 | 0.009 | 0.059 | 0.005 | 0.127 | 0.209 |
| DR × S interaction | NS | NS | NS | NS | NS | NS | NS | NS |

a,bMeans within a column under similar criteria not sharing a common superscript are significantly different (p < .05).

DM: dry matter; pH: hydrogen power; WHC: water holding capacity; SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; DR: dietary regimen; S: strain; NS: not significant; SEM: standard error of mean.
with the SPD regimen displayed a significantly lower H/L ratio than those on the HPD, but similar phago-
cyte activity and heterophile levels. Blood haemato-
logical constituents were not significantly influenced
by the interaction between strains of broilers and diet-
ary regimen (Table 8).

**Discussion**

The results indicate that feeding the HPMED to Ross
and Cobb chickens resulted in similar BWG to the
other regimens during 1–18 d of age. The results indi-
cate that SPD (22.66% CP with 12.97 MJ/kg ME) was
adequate for Ross strain, but feeding HPD (24.21% CP
with 12.97 MJ/kg ME) was beneficial for Cobb from
BWG point of view. In contrast, dietary feeding regi-
men had no effect on feed intake and FCR during
1–18 days of age. This was supported by ileum villi
length. There is a relationship between the intestinal
villi and digestibility of feeds in the lumen of the small
intestine (Yamauchi 2002). According to Laudadio et al. (2012) feeding dietary protein levels (20.5%) under thermoneutral conditions significantly improved the growth of broilers compared to levels of 22.5 and 18.5%, whereas BWG and feed intake and FCR
remained unaffected by dietary treatment during
14–49 d of age. On the other hand, low-protein diets
can sustain productive performance similar to high-
protein diets (Widyaratne and Drew 2011). In this
regard, ileum villi did not alter by feeding with the
low-CP diet. Instead, reducing the dietary protein to
20.5% resulted in a higher villus height and villus height to crypt depth ratio in the duodenum and ileum (Laudadio et al. 2012), which is similar to the present findings that the SPD increased ileum villi length compared to the HPMED regimen and this may be due to higher nutrient availability of HPMED.

During the HS period (19–35 d of age), the HPD
regimen decreased the growth of only the Cobb
strain compared to the SPD and HPMED regimens, in
contrast to the observation during 1–18 d of age. Thus, for the whole period, the HPMED (22.1% %CP
with 14.06 MJ/kg ME) regimen showed greater BWG
in Ross and Cobb chickens than the HPD regimen,
and surpasses the growth of only Cobb SPD group.
The results indicate that Ross and Cobb chickens
responded similarly to the HPMED regimen, with
more favourable growth than those on the HPD regi-
men. The increased growth of broilers on HPMED
regimen observed herein was supported by increas-
proteins and lipid digestibility in Ross strain com-
pared to HPD. In literature, performance of different
strains of broilers was found to be affected by feed-
ing regimen (Corrêa et al. 2006; Razuki and Al-Rawi
2007; Attia et al. 2010b, 2016). Moreover, the effect
of the dietary CP of broilers was found to be incon-
sistent and differed from one study to another; for
example, utilisation of low-CP diets for broiler chick-
ens caused low performance compared to standard
protein with sufficient amino acids (Berres et al.
2010). On the other hand, Laudadio et al. (2012) and
Widyaratne and Drew (2011) found that low-protein
diets under thermoneutral conditions did not influ-
ence the performance of broilers. Alternatively, in hot
climates, low dietary protein increased growth per-
performance (Thim et al. 1997). This may be due to pro-
tein/amino acid requirements not being elevated
during HS as a result of the decrease in growth and
thus protein needs for production (Attia et al. 2006,
2011; Attia and Hassan 2017). Additionally, low pro-
tein showed a beneficial effect on the environment
by decreasing N excretion (Sterling et al. 2005;
Kamran et al. 2010; Attia and Hassan 2017).

In the literature, evidence indicates an adverse
effect of HS on protein metabolism, such as low syn-
thesis and breakdown of protein (Lin et al. 2006), and
a decrease in protein deposition (Temim et al. 2000),
feed intake, and digestibility of dry matter and protein
and thus weight gain. However, HS has been found to
not affect FCR (Souza et al. 2016).

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**Table 7. Effect of dietary regimen and/or broiler strain on blood biochemical of broiler chicken at day 35 of age.**

| Treatments | Total protein, g/dL | Albumin, g/dL | Globulin, g/dL | Globulin:albumin ratio | Glucose, µg/dL | Alkaline Phosphate, U/L | Cholesterol, mg/dL | T3, ng/mL | TAC, µmol/L | MDA, µmol/L |
|------------|-------------------|---------------|----------------|------------------------|----------------|------------------------|-------------------|------------|-------------|-------------|
| SPD        | 5.140             | 2.920         | 2.220          | 0.818                  | 183<sup>a</sup> | 11.500                 | 199               | 2.880      | 506         | 1.320       |
| HPD        | 5.130             | 3.010         | 2.120          | 0.758                  | 197<sup>a</sup> | 12.390                 | 203               | 2.840      | 520         | 1.380       |
| HPMED      | 5.060             | 2.910         | 2.140          | 0.802                  | 187<sup>b</sup> | 12.920                 | 201               | 2.850      | 510         | 1.350       |
| p Value    | NS                | NS            | NS             | NS                     | NS             | NS                     | NS                | NS         | NS          | NS          |
| SEM        | 0.092             | 0.101         | 0.070          | 0.040                  | 1.420          | 0.560                  | 1.570             | 0.070      | 8.660       | 0.070       |
| Ross       | 5.080             | 2.890         | 2.180          | 0.820                  | 191            | 12.130                 | 201               | 2.810      | 510         | 1.380       |
| Cobb       | 5.150             | 3.000         | 2.140          | 0.770                  | 193            | 12.340                 | 203               | 2.910<sup>a</sup> | 515         | 1.320       |
| p Value    | NS                | NS            | NS             | NS                     | NS             | NS                     | NS                | NS         | NS          | NS          |
| SEM        | 0.080             | 0.070         | 0.040          | 0.062                  | 1.150          | 0.450                  | 1.280             | 0.057      | 7.030       | 0.060       |
| DR x S interaction | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

<sup>a,b</sup>Means within a column not sharing a common superscript are significantly different at .05.

SPD: standard-protein diet; HPD: high-protein diet; HPMED: high-protein and metabolisable energy diet; T3: tri-iodothyronine; TAC: total antioxidant capacity; MDA: malondialdehyde; NS: not significant; SEM: standard error of mean.
From a feed utilisation standpoint, the HPMED improved FCR compared to the SPD and HPD regimens during the heat stress period (19–35 d of age) and improved FCR compared to the SPD during the whole period. In addition, the HPMED improved the EBI of both strains of broilers. This indicates that the HPMED regimen is beneficial for the FCR of broilers during HS and beneficial for FCR and EBI for the whole periods. It seems that growth and FCR have different requirements of protein and ME, with FCR needing higher levels of CP with ME than BWG.

The results indicate that feeding with the HPD during the HS period decreased feed intake by 6.5% compared to the SPD, and during the whole period by 4.7 and 3.1% compared to the SPD and HPMED, respectively; this coincided with a reduction in BWG in the group on the HPD. The decrease in feed intake of the group on the HPD may demonstrate an attempt of the broilers to decrease heat production due to the high heat increment of the HPD (Attia and Hassan 2017). The improved growth and FCR of broilers on the HPMED may be due to increasing the oil contents of diet by 2% to raise ME levels. Oil/fat had a lower heat increment than protein, resulting in an increase in energy availability behind the calculated value, due to extra caloric effect and fat-soluble vitamins, and thus improved FCR (Dale and Fuller 1979; Daghir 2008).

In the literature, evidence has suggested that increasing diet density by fat addition enhances the growth performance of broilers exposed to HS (Attia et al. 2006, 2011; Attia and Hassan 2017), and enhances the performance index and FCR compared to the control group (Swatson et al. 2002). In contrast, the effect of ME on broiler performance exposed to HS is inconclusive, as findings have included an elevation (Keshavarz and Fuller 1980), a decline (Yamazaki and Zi-Yi 1982; Souza et al. 2016; Attia and Hassan 2017), and no influence (Geraert et al. 1992; Faria Filho et al. 2007). These can be attributed to the severity of heat stress, the strain of broilers, the feeding regimen and environmental conditions.

The positive effect of the HPMED regimen on the growth performance of broilers, phagocyte activity and decrease stress index (H:L ratio). Further, the decrease in cloacal temperature and respiration rate showed an improvement in the physiological status of the broilers. This was associated with increased lipid digestibility, abdominal fat deposition, and dry matter, and protein and lipids in the meat indicate improved protein and energy availability for production and immunity. Attia and Hassan (2017) and Attia et al. (2006, 2011) and reported similar results. The increase
in PA in broilers on the HPMED regimen indicates an improved immunity. The impact positive of increasing nutritional concentrations on immunity has also been evaluated (Attia et al. 2011). Chicks fed on a HPD had higher antibody titre at 10 and 15 d after inoculation with sheep red blood cells than those fed on 19% CP (Praharaj et al. 1998). The adverse influence of the HPD on the productive traits of broilers, particularly during the HS period, was associated with increased blood glucose, cloacal temperature, respiration rate, and H/L ratio and a decrease in the internal innate immunity, e.g. PA. In addition, a decrease in the digestibility of crude protein and crude fibre were found. These results indicate that the HPD regimen during heat stress had a harmful effect on broiler performance, which was mediated by changes in physiology condition (increased cloacal temperature and respiration rate), stress index (H/L ratio) and immunity (PA). This could be due to the increased heat increment of protein, the reduction in energy availability for maintenance and heat dissipation, and a decrease in nutrient digestibility (Bonnet et al. 1997; Souza et al. 2016; Attia and Hassan 2017) that may be caused by peripheral vasodilation and reduced blood flow in the digestive system. These results are in agreement with those observed by Cahalan et al. (1995) and Attia and Hassan (2017). Heat production from protein turnover increased together with increasing dietary protein/amino acids concentrations and became even worse under HS (Musharaf and Latshaw 1999; Attia and Hassan 2017). Dissipation of heat produced during HS increased maintenance energy requirements (Attia et al. 2006; Lin et al. 2006; Rosa et al. 2007; Attia and Hassan 2017). The problem may be complicated because of the decrease in feed intake (Daghir 2008; NRC 1994).

The Ross strain showed superior growth and feed utilisation and improved percentage thymus (T-cells) and protein in meat, deposited less abdominal fat and lipid in meat, and had a lower pH of meat than the Cobb chickens. On the other hand, Cobb on HPD showed better tolerance to HS than Ross due to low cloacal temperature and respiration ratio than Ross strain. These results indicate the production, physiology and immunology of broiler chickens depend on feeding regimen. The difference in the genetic potential of broiler strains could be related to genetic makeup variations (Smith et al. 1998; Smith and Pesti 1998; Rosa et al. 2007; Benyi et al. 2009; Abdullah et al. 2010; Yakubu et al. 2010; Attia et al. 2016). In contrast, Corzo et al. (2004) reported that BWG and FCR were similar among females of three different chicken strain crosses.

Conclusions

In conclusion, Ross and Cobb chickens fed the HPMED showed higher BWG than chickens on the HPD, and broilers fed the HPMED had improved FCR compared to those on the SPD, independent of broiler strain. Further, independent of broiler strain, the HPMED regimen increased the dry matter, protein, and lipid contents of meat compared to the SPD and HPD regimens, and decreased cloacal temperature, respiration rate and H/L ratio compared to the HPD regimen.

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Disclosure statement

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