Responses of broiler chicken to different oil levels within constant energy levels from 20 to 40 days of age under hot weather conditions

Youssef A. Attia\textsuperscript{a}, Mohammed A. Al-Harthi\textsuperscript{a} and Saber Sh. Hassan\textsuperscript{b}

\textsuperscript{a}Agriculture Department, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; \textsuperscript{b}Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Damanhour, Egypt

\textbf{ABSTRACT}

In total, 144 Arbour Acres broiler chickens were distributed among four treatment groups (six replicates per treatment; six chickens per replicate) during days 20–40 of age. The chickens were offered iso-caloric and iso-nitrogenous diets containing four dietary oil levels (DOL): 0 (oil non-supplemented diet, control), 2, 4, and 6% in a relatively low-energy diet (12.4 MJ ME/kg diet). During the experimental period, the chickens were reared under natural hot weather conditions (32.5 ± 4°C, 54 ± 7% relative humidity). Growth, feed conversion ratio (FCR), protein (PCR), metabolisable energy (MECR) ratio, and European production efficiency index (EPEI) were similar among groups fed up to 4% DOL but raising DOL to 6% impaired these traits. Besides, DOL at 6% decreased digestibility of dry matter, crude protein, and ash. Furthermore, a 6% DOL showed the lowest digestibility of dry matter, crude protein, and ash. Dressing percentage was the highest in 2%, and abdominal fat percentage showed the same trend in 6% DOL. The liver percentage increased significantly with fat/oil inclusion compared to the control. Meat dry matter and either extract increased considerably due to offering different DOLs, with maximum values at 6%. The inclusion of dietary oils in diets significantly increased serum malondialdehyde (MDA) but decreased serum total antioxidant capacity (TAC)/MDA ratio compared to the 0% DOL. In conclusion, under natural summer conditions, from 20 to 40 days of age, broilers’ best productive characteristics were achieved using 0–2% DOL, and the best immune response was obtained for 4–6% DOL.

\textbf{HIGHLIGHTS}

- Hot weather negatively influences the productivity of broilers.
- Fats/oils are essential for animal and human nutrition for several reasons; however, they are expensive compared to other energy sources.
- Improving the production index is essential to keep broilers farming profits under hot weather.

\section*{Introduction}

The closure of dry borders and ports and traffic restrictions in many countries to reduce the spread of COVID-19 have led to a reduction in feedstuffs for the poultry industry in some countries, with adverse effects on agricultural production, feed/food supply, and related economic activities (Hashem et al. 2020; Hussain et al. 2020; Poudel et al. 2020). One lesson learned from the COVID-19 outbreak is to depend on locally available human and animal nutrition resources to minimise imports (Al-Khalaifah et al. 2020; Hafez and Attia 2020; Siche 2020). After the outbreak of COVID-19 in early 2020, the cereal and oilseed markets collapsed (FAO http://www.fao.org/2019-ncov/q-and-a/impact-on-food-and-agriculture/en/) because of a drastic drop in feed sales during the lockdown (Hafez and Attia 2020).

In some areas of the world, temperatures can reach more than 40 °C, causing heat stress (HS) and substantial economic loss (Daghir 2008). Heat stress can be naturally induced due to natural heat waves and/or artificially generated by exposing animals to high temperatures (Attia and Hassan 2017). The severity of heat stress mainly depends on the temperature and/or duration of exposure to heat, resulting in different animal responses (Beckford et al. 2020). In previous studies,
HS increased energy expenditure and negatively impacted energy balance, causing thermal imbalance (Daghir 2008) and, consequently, homeostasis disturbance as well as physiological, performance, antioxidant imbalance, altered immune responses, and lower economic outputs (Al-Harthi et al. 2002; Al-Sultan and et al. 2019). Besides, HS adversely affects nutrient digestibility, inducing metabolic disturbance reflected in blood and transport mechanisms and, thus, altered deposition in the tissue (Sahin et al. 2009; Attia et al. 2017). Besides, HS increases the production of free radicals, hence inducing oxidative damages (Attia et al. 2009; Yang et al. 2010), adversely impacting immunological mechanisms (Mehaisen et al. 2019) and decreasing bursa, spleen, and thymus yield (Fouad et al. 2016).

In many developing countries, fats and oils are imported for human and animal food. Also, fats/oils are expensive energy sources that are mainly imported from developed countries. In some countries, some feedstuffs’ trading and pricing were adversely influenced by changes in the feed market during the first wave of COVID-19 (Poudel et al. 2020). Thus, using imported feed supplies, and costly ones, requires reconsideration to minimise their inclusion in poultry diets via reformulation to decrease fat/oil levels and/or energy levels (Hafez and Attia 2020).

An effective way for feeding broilers during HS is modifying the dietary nutrient profiles (Syafwan et al. 2011; Suganya et al. 2015) to enhance feed intake (Veldkamp et al. 2000). Feed, nutrient, and metabolisable energy (ME) intake decrease during high temperature (NRC, 1994; Daghir 2008; Attia et al. 2011). There is emerging evidence that including dietary fats/oils in broiler diets may encourage feed intake and reduce heat load while enhancing animals’ productivity (Aggoor et al. 2000; Ghazalah et al. 2008). The use of fats/oils has attracted great interest in broiler nutrition and the production of fast-growing animals under normal and HS conditions due to several beneficial effects (Attia et al. 2006, 2017, 2018; Pesti et al. 2015). The availability of ME during HS is vital to maintain production, as energy is critical to maintaining an energy balance essential for dispersing metabolic heat (Lin et al. 2005; Attia et al. 2006; Attia and Hassan 2017; Attia et al. 2018). In the literature, various results about dietary ME impacts on chickens subjected to HS can be found (Daghir 2008). For example, fat/oil supplementation in iso-caloric diets improved productive and economical traits of broilers raised under either normal or HS conditions (Veldkamp et al. 2000; Attia et al. 2018, 2020). On the contrary, fat/oil addition to broilers’ diets exposed to HS did not influence performance and economic traits (Sinurat and Balnave 1985). These contradictions lead to uncertainties about the recommended levels of fats/oils in broiler nutrition due to variations in fat sources, fatty acid profiles, dietary composition, ME, crude protein, environmental conditions, and age and size of birds during heat stress (Raju et al. 2004; Pesti et al. 2015; Alagawany et al. 2019). Besides, studies about the use of relatively low ME levels supplemented with elevated concentrations of fats/oils under a constant energy level, and the impacts on broilers’ productivity reared under hot conditions are scarce. Thus, this study’s objective was to test broiler chicken’s productive and physiological responses to different oil levels (0, 2, 4, and 6) within constant energy levels from 20 to 40 days of age under hot weather conditions.

### Materials and methods

#### Chickens, experimental design, and diets

This research was approved under protocol no 108-155-1441 by DSR, King Abdulaziz University, Saudi Arabia. In total, 144 Arbour Acres male broiler chickens with an initial body weight of 43.6 ± 2.7 g were randomly distributed in a straight run, completely randomised experimental design, with treatment groups during 20–40 days of age. The males were used herein to improve sample homogeneity and avoid gender differences within replicates and treatments. The chickens were fed iso-caloric and iso-nitrogenous diets containing four levels of vegetable oils, namely 0% (basal control diet), 2, 4, and 6% (Table 1). The change in diet profiles was done by including oil instead of corn and soybean meal with a gradual increase in the sand to compensate for the differences in energy between oil and corn and soybean meal. The differences in protein levels between the two ingredients were compensated by increasing soybean meal. This was done to keep iso-caloric and nitrogenous diets among different treatments. Under commercial broilers’ nutrition, broilers’ diets may contain up to 6% oil depending on the diet’s energy value, which usually is about 13 M.J./kg diet.

Each treatment consisted of six replicates and six male chickens per replicate. Each replicate was kept in battery brooders (35 × 25 × 30 cm length-× width-× height). From 20–40 days of age, chickens were exposed to natural hot weather conditions with an outdoor temperature of 34 ± 5°C and 52 ± 8% relative humidity. The indoor temperature during the stress period, which is usually observed, started at 10
am and ended by 5 pm; the duration is about 7 hours daily. The average temperature during this period was 31.5 ± 4°C, with 54 ± 7% relative humidity (RH). During this period, broilers showed signs of heat stress, such as increasing panting and lying down. From days 1–19 of age, the broilers were reared using standard husbandry practices, according to the broiler management guide. They fed a commercial mash diet containing 220 g/kg diet crude protein (CP), 12.8 MJ/kg diet, 10 g/kg Ca, and 5 g/kg available phosphorus (Table 1).

### Broiler rearing and management

During the preliminary experimental period (1–19 days of age), the broilers were reared under similar hygienic and management conditions. Mash from the corn-soybean meal based-diet was offered from 1 to 19 days of age (Table 1), following the Arbour Acres broiler breeder guidelines. Water and diets were provided ad libitum. Health care included vaccination with Hitchiner + IB at 8 days of age, Newcastle disease virus (NDV) via Lasota at 14, 20, and 30 days of age, Gumboro at 14 and 24 days of age, and avian influenza (AI) (H5N2) at 9 days of age. The light scheme was 23:1 hour light/dark cycle. The electric light was turned off one hour every 24 hours at the end of the day, around 8 p.m.

### Response measurements

As heat stress affected broilers after 3 weeks of age, causing substantial changes in metabolic profiles and loss in productive performance, we recorded data during 20 and 40 days of age, where the feed consumption was measured, and chickens were weighed (g) on a replicate basis as the experimental unit. Data for feed consumption and chemical analysis of feeds were then used for the calculation of feed (g), protein (g), and ME (kcal) intake as relative to body weight gain. Furthermore, the feed conversion ratio (FCR), protein (PCR), and energy (MECR) were estimated by dividing the consumption of feed, protein, and ME by the body weight gain. The European production efficiency index (EPEI) was reported as cited by Attia and Hassan (2017).

A digestibility trial was carried out during 40–47 days of age, using six individually caged broilers per treatment. After an adaptation period of 5 days, feed intake and excreta were collected daily at the same time for three successive days. The gut tract collection method (Total tract nutrient retention) was used for excreta collection, according to Moharrery (2011). Excreta were spiked with 1% boric acid solution to control nitrogen evaporation, dried in a forced ventilated oven at 60°C until constant weight, ground in a Wiley mill, mixed.

### Table 1. Ingredients and chemical composition of the experimental diets.

| Ingredients, (g/kg) | Preliminary diets 1–20 d of age | Dietary vegetable oils, % (20–40 d of age) |
|---------------------|----------------------------------|---------------------------------------------|
| Yellow corn         | 514                              | 701                                         |
| Soybean meal, 48% CP| 404                              | 263                                         |
| Di-calcium phosphate| 20                               | 15                                          |
| Limestone           | 12                               | 10                                          |
| NaCl                | 3                                | 3                                           |
| Vitamin + mineral premixa | 3                      | 2                                           |
| DL-Methionine,      | 2.5                              | 3.5                                         |
| L- Lysine           | 1.5                              | 2.5                                         |
| Soybean and sunflower oilb | 40                 | 0                                           |
| Sand                | 0                                | 0                                           |
| Total               | 1000                             | 1000                                        |

| Nutrient composition, calculatedc and determinedd |
|---------------------------------------------------|
| Metabolisable energy, MJ/kg dietc                | 12.8                                      |
| Crude proteind                                   | 220                                       |
| Calciumc                                         | 10.1                                      |
| Phosphorus, availablee                          | 5.11                                      |
| Methioninee                                      | 5.70                                      |
| Methionine + cysteinec                          | 9.21                                      |
| Lysine                                           | 12.8                                      |
| Ether extracted                                  | 65.9                                      |
| Crude fiberd                                    | 42.3                                      |
| Ash                                              | 35.9                                      |
| Dry matterd                                     | 906                                       |

| Nutrient composition, calculatedc and determinedd |
|---------------------------------------------------|
| Metabolisable energy, MJ/kg dietc                | 12.4                                      |
| Crude proteind                                   | 181                                       |
| Calciumc                                         | 8.27                                      |
| Phosphorus, availablee                          | 4.01                                      |
| Methioninee                                      | 5.25                                      |
| Methionine + cysteinec                          | 8.27                                      |
| Lysine                                           | 11.4                                      |
| Ether extractd                                   | 33.2                                      |
| Crude fiberd                                    | 42.1                                      |
| Ash                                              | 48.8                                      |
| Dry matterd                                     | 897                                       |

aVit + Min mixture provides per kg of the 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. Trace mineral (mg per kg of diet): Mn 80 Zn 60, Fe 35, Cu 8, Se 0.60.
bA mixture of soybean oil and sunflower oil at 50% of each.
cCalculated analyses.
d Determined analyses AOAC.
well-kept in screwed glass jars until analyses. The urine nitrogen was separated from faecal nitrogen, according to Davidson and Thomas (1969). Digestibility was calculated based on the amount of the intake and excreted of each nutrient (retained) divided by the amount consumed multiplied by 100.

According to the Islamic method, six broilers from each treatment were randomly selected to represent all treatment replicates and slaughtered at 40 days of age. Dressing percentage was calculated as the weight after slaughter (hot carcase), which is equal to empty carcase without blood, feather, intestinal, head, and legs, plus liver, empty gizzard, and heart divided by live body weight before slaughter and multiplying by 100. The abdominal fat, proventriculus, gizzard, intestines, pancreas, liver, heart, and lymphoid organs (spleen, bursa of Fabricius, and thymus) were separated, weighed, and expressed as relative to the weight of chickens before slaughtering.

Equal amounts of breast and thigh deboned meat were used for chemical analyses of meat samples ($n=6$ replicates per treatment). Meat samples (150 g) were collected from each slaughtered animal on day 40 of age, kept in plastic bags, and frozen at $-18^\circ$C until used for analyses. The dry matter, crude protein, ether extract, and ash content were determined in meat feeds, and faecal as well as crude fibre in feed and faecal according to AOAC (2004) using the methods 925.04, 990.3, 2003.06 and 942.05, 978.10, respectively. The water-holding capacity (WHC) and tenderness of the meat, and the colorimetric method was applied for the determination of the intensity of meat colour as the optical densities of the meat and drip (Husani et al. 1950). Meat acidity, pH values of the meat, and drip were determined according to Aitken et al. (1962).

Blood samples ($n=6$ per treatment) were gathered at 40 days of age in heparinised and un-heparinised tubes. The samples were centrifuged at 500 g for 15 min, and plasma and serum were collected and stored at $-18^\circ$C for determination of blood biochemical and haematological contents. The haematological traits consisted of counts of red blood cells (RBCs), as indicated by Hepler (1966). We further determined blood haemoglobin (Hgb) and packed cell volume (PCV) using the method of Eilers (1969). Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), and mean cell volume (MCV) were estimated as reported by (Eilers 1967). The counts of white blood cells (WBCs) and WBC portions were determined as noticed by Lucas and Jamroz (1961), and the activity of phagocytes (PA) and the index of phagocytes (PI) were obtained according to Leijh et al. (1986). The levels of plasma glucose (Trinder 1969, total serum protein (Weichselbaum 1946), serum albumin (Doumas 1971), serum globulin (Coles 1974), and the albumin-to-globulin ratio were estimated according to (Attia and Hassan 2017).

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated according to Reitman and Frankel (1957) technique. Serum urea and creatinine were established as described in Bartles et al. (1972) and Sampson et al. (1980), respectively. The urea-to-creatinine ratio was calculated, and alkaline phosphatase (ALP) enzymes were obtained using procedure Kind and King (1954). The plasma lipid profiles, such as cholesterol (Watson 1960), triglycerides (Randrup 1960), and very-low-density lipoprotein (vLDL), were assayed as triglycerides/5 (latexestonline.org/understanding/analytes/vldl/tab/sample/). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were gauged according to the procedures of Wieland and Seidel (1983) and Friedewald et al. (1972), respectively.

The TAC and MDA levels were estimated according to Koracevic et al. (2001) and Richard et al. (1992), respectively. The serum antibody body titres for avian influenza (AI) and Newcastle disease virus (NDV) were measured as suggested by Kai et al. (1988) and Takatsy (1956), respectively, and infectious bursal disease (IBD) was estimated according to the method of Cosgrove (1962).

**Statistical evaluation**

The number of replicates was estimated using the power analyses, and 0.07 difference in FCR of chickens at market age difference in FCR (1.93 vs. 2.0 kg feed/kg gain) was considered to be 0.07 based on published results by (30; 62; 63). The standard deviation of 0.04 kg feed/kg gain, the two-sided test, the P-value of 0.05, and the desired power of 80 was employed. The estimated number of replicates was 6 replicates, according to [https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html]. The normality of error distribution and data was tested using the Shapiro–Wilk test of normality (SAS Institute 2009). Also, the homogeneity of the variance (homoscedasticity) has been evaluated using the Levene's test (SAS, 2009). The assumptions of ANOVA were tested according to a random selection of the samples. One-way ANOVA, using SAS® (2009),
was run using the following statistical model:

\[ Y_{ij} = \mu + F_i + e_{ij}, \]  

where \( Y \) is the dependent variable, \( \mu \) is the overall mean, \( F_i \) is the effect of dietary oil levels, and \( e_{ij} \) is the random error. The experimental unit was the replicate. Prior to analysis of variance, data presented in percentages were transformed to log10 to normalise data distribution. Differences among means at \( P \leq .05 \) were examined using the Tukey’s procedure. The chi-square test was used for testing the survival rate.

**Results**

**Broiler growth**

Table 2 shows the effect of dietary oil level (DOL) on broiler performance during 20–40 days of age. The consumption of feed, protein, and energy did not differ among the treatments.

Growth of broilers on DOL up to 4% was similar, although raising DOL to 6% significantly lowered final body weight and body weight gain (BWG) compared to the other DOL levels. The conversion of feed, protein, and ME to gain was similar among groups fed a diet containing up to 4% DOL, whereas DOL inclusion up to 6% significantly impaired these traits and EPEI.

**Nutrient digestibility**

Table 3 represents the effect of DOL on nutrient digestibility. Feeding a diet with 6% DOL induced the lowest digestibility of dry matter and total tract retention of nitrogen (crude protein), and ash. Besides, other 2 and 4% DOLs significantly reduced the digestibility of ether extract and ash compared to the non-supplemented diet.

**Carcass traits and inner organs**

Table 4 indicates the effect of DOL on carcase traits and inner organs at 40 days of age. The dressing percentage was the highest for the 2% oil-supplemented diet. The diet supplemented with 6% oil resulted in the highest abdominal fat values; the gizzard percentage was lowest for 6% DOL. Liver percentage significantly increased similarly with increasing DOLs. There were no significant differences in the proventriculus, intestines, pancreas, and heart due to different DOLs.

**Chemical and physical meat characteristics**

Table 5 shows the effect of DOL on the chemical and physical characteristics of meat of 40-day-old broilers. Meat dry matter and ether extract increased

| Table 3. Nutrients digestibility of broilers exposed to natural high temperature and fed containing graded levels of oils within a constituent calorific level during 20-40 days of age. |
| --- |
| **Dietary oil levels** |
| Treatments | 0% | 2% | 4% | 6% | RMSE | \( p \) Value |
| Dry matter, % | 84.2<sup>a</sup> | 89.2<sup>b</sup> | 83.2<sup>a</sup> | 77.1<sup>b</sup> | 4.31 | .004 |
| Crude protein, % | 77.7<sup>a</sup> | 76.9<sup>a</sup> | 77.3<sup>a</sup> | 71.6<sup>b</sup> | 5.22 | .003 |
| Ether extract, % | 93.1<sup>a</sup> | 89.4<sup>b</sup> | 90.3<sup>b</sup> | 91.1<sup>a</sup> | 1.46 | .007 |
| Crude fibre, % | 36.4 | 37.5 | 37.6 | 33.4 | 3.19 | .421 |
| Ash, % | 49.8<sup>a</sup> | 42.8<sup>b</sup> | 41.7<sup>a</sup> | 35.9<sup>c</sup> | 3.35 | .001 |

<sup>abc</sup>Means within a row with different superscripts are significantly different (\( p < .05 \)).

RMSE: Root mean square error; \( p \) value: probability level.

| Table 2. Growth, feed intake, feed, protein, and energy conversion ratio, survival rate and European production efficiency index of broilers exposed to natural high temperature and fed graded levels of oils within a constituent calorific level during 20–40 days of age. |
| --- |
| **Dietary oil levels** |
| Treatments | 0% | 2% | 4% | 6% | RMSE | \( p \) Value |
| Feed intake, g | 2896 | 2915 | 3080 | 3000 | 221 | .549 |
| Protein intake, g | 524 | 528 | 557 | 543 | 39.9 | .551 |
| Energy intake, MJ | 37.6 | 37.8 | 40.0 | 38.9 | 2.87 | .549 |
| Growth of broiler chickens |
| Body weight, g, 20 d of age | 511 | 522 | 510 | 516 | 74.9 | .941 |
| Body weight, g, 40 d of age | 2009<sup>a</sup> | 2046<sup>a</sup> | 2094<sup>a</sup> | 1937<sup>b</sup> | 263 | .017 |
| Body weight gain, 20-40 d of age | 1498<sup>a</sup> | 1524<sup>a</sup> | 1584<sup>a</sup> | 1421<sup>b</sup> | 107 | .008 |
| Nutrient conversion ratio, period |
| Feed conversion ratio, kg/ kg | 1.93<sup>b</sup> | 1.91<sup>b</sup> | 1.94<sup>b</sup> | 2.11<sup>a</sup> | 0.038 | .001 |
| Protein conversion ratio, g/ g | 0.350<sup>b</sup> | 0.346<sup>b</sup> | 0.352<sup>b</sup> | 0.382<sup>a</sup> | 0.007 | .001 |
| Energy conversion ratio, MJ/g | 0.025<sup>b</sup> | 0.0248<sup>b</sup> | 0.0252<sup>b</sup> | 0.0274<sup>a</sup> | 0.0005 | .001 |
| Survival rate, % | 100 | 100 | 91.4 | 88.6 | 10.1 | .207 |
| European production efficiency Index | 369<sup>a</sup> | 380<sup>a</sup> | 355<sup>a</sup> | 264<sup>b</sup> | 40.2 | .002 |

<sup>abc</sup>Means within a row with different superscripts are significantly different (\( p < .05 \)).

RMSE: Root mean square error; \( p \) value: probability level, BWG: body weight gain.
significantly with increasing inclusion levels, with a maximum at 6% DOL. Meat crude protein and ash and physical characteristics of meat, such as meat colour measured as optical density, WHC, tenderness, and pH values, were similar among DOL groups.

**Biochemical constituents and indices of the liver and renal function**

Table 6 shows the biochemical constituents and the indices of the liver and renal function of 40-day-old chickens. Serum albumin and albumin/globulin ratio levels were significantly lower in the group fed 2% DOL than in the other groups. There were no

---

**Table 4.** Carcase traits and inner body organs of broilers exposed to natural high temperature and fed containing graded levels of oils within a constituent calorific level during 20–40 days of age.

| Treatments          | Dietary oil levels | RMSE | p Value |
|---------------------|--------------------|------|---------|
|                      | 0%                 | 2%   | 4%      | 6%     |       |
| Dressing, %          | 73.4<sup>b</sup>  | 74.6<sup>b</sup> | 71.4<sup>b</sup> | 72.6<sup>b</sup> | 2.68  | .010  |
| Abdominal fat, %     | 1.26<sup>a</sup>  | 0.978<sup>b</sup> | 1.33<sup>a</sup> | 2.20<sup>a</sup> | 0.614 | .016  |
| Proventriculus, %    | 0.322              | 0.402 | 0.483  | 0.380  | 0.173 | .465  |
| Gizzard, %           | 1.16<sup>a</sup>  | 1.08<sup>a</sup> | 1.07<sup>a</sup> | 0.939<sup>a</sup> | 0.127 | .053  |
| Intestinal, %        | 4.17               | 4.12  | 4.74   | 5.36   | 0.855 | .067  |
| Pancreas, %          | 0.234              | 0.199 | 0.285  | 0.217  | 0.005 | .213  |
| Liver, %             | 1.84<sup>a</sup>  | 2.18<sup>a</sup> | 2.18<sup>a</sup> | 2.21<sup>a</sup> | 0.148 | .001  |
| Heart, %             | 0.556              | 0.474 | 0.447  | 0.495  | 0.070 | .079  |

<sup>ab</sup>Means within a row with different superscripts are significantly different (p < .05).

RMSE: Root mean square error; p value: probability level.

---

**Table 5.** Chemical and physical meat quality traits of broilers exposed to natural high temperature and fed graded levels of oils within a constituent calorific level during 20–40 days of age.

| Treatments          | Dietary oil levels | RMSE | p Value |
|---------------------|--------------------|------|---------|
|                      | 0%                 | 2%   | 4%      | 6%     |       |
| Chemical composition of meat |                |      |         |        |       |
| Dry matter, %        | 25.6<sup>c</sup>  | 25.8<sup>b</sup> | 25.7<sup>b</sup> | 26.0<sup>a</sup> | 0.056 | .001  |
| Crude protein, %     | 19.3               | 19.4  | 19.2   | 19.2   | 0.050 | .193  |
| Ether extract, %     | 5.28<sup>a</sup>  | 5.42<sup>b</sup> | 5.50<sup>a</sup> | 5.76<sup>a</sup> | 0.057 | .001  |
| Ash, %               | 0.963              | 0.965 | 0.953  | 0.968  | 0.019 | .645  |
| Physical characteristics of meat |            |      |         |        |       |
| Meat colour, optical density, % | 0.183 | 0.174 | 0.181  | 0.181  | 0.018 | .907  |
| Water holding capacity, g/cm² | 16.8  | 16.6  | 16.5   | 16.4   | 0.303 | .580  |
| Tenderness, g/cm²    | 9.83               | 9.94  | 9.89   | 10.1   | 0.362 | .846  |
| pH                   | 6.01               | 6.08  | 6.03   | 6.07   | 0.108 | .803  |

<sup>abc</sup>Means within a row with different superscripts are significantly different (p < .05).

RMSE: Root mean square error; p value: probability level; pH: hydrogen power.

---

**Table 6.** Plasma biochemical and indices of liver and renal function of broilers exposed to natural high temperature and fed containing graded levels of oils within a constituent calorific level during 20–40 days of age.

| Treatments          | Dietary oil levels | RMSE | p Value |
|---------------------|--------------------|------|---------|
|                      | 0%                 | 2%   | 4%      | 6%     |       |
| Blood plasma protein |                    |      |         |        |       |
| Total protein, g/dl  | 6.26               | 6.30  | 6.46   | 6.32   | 0.153 | .223  |
| Albumin, g/dl        | 3.26<sup>a</sup>  | 2.96<sup>b</sup> | 3.26<sup>a</sup> | 3.36<sup>a</sup> | 0.155 | .008  |
| Globulin, g/dl       | 3.00               | 3.32  | 3.20   | 2.98   | 0.259 | .156  |
| Albumin/globulin ratio | 1.08<sup>a</sup>  | 0.904<sup>b</sup> | 1.02<sup>a</sup> | 1.15<sup>a</sup> | 0.131 | .049  |
| Blood plasma lipids  |                    |      |         |        |       |
| Triglycerides, mg/dl | 174<sup>b</sup>  | 176<sup>b</sup> | 184<sup>a</sup> | 185<sup>a</sup> | 2.49  | .001  |
| Total cholesterol, mg/dl | 208  | 202    | 202   | 202   | 5.88  | .332  |
| High density lipoprotein, mg/dl | 43.2<sup>a</sup> | 38.2<sup>b</sup> | 41.6<sup>a</sup> | 39.6<sup>b</sup> | 1.94  | .005  |
| Low density lipoprotein, mg/dl | 93.6  | 94.0   | 93.6  | 97.0  | 2.43  | .117  |
| HDL/LDL ratio        | 0.462<sup>a</sup> | 0.406<sup>b</sup> | 0.444<sup>a</sup> | 0.408<sup>b</sup> | 0.026 | .008  |
| Very low-density lipoprotein, mg/dl | 34.9<sup>a</sup> | 35.2<sup>a</sup> | 36.8<sup>a</sup> | 36.9<sup>a</sup> | 0.249 | .001  |
| Blood plasma glucose |                    |      |         |        |       |
| Blood plasma glucose, mg/dl | 192<sup>a</sup> | 188<sup>a</sup> | 165<sup>a</sup> | 156<sup>b</sup> | 21.2  | .001  |
| Liver function index |                    |      |         |        |       |
| AST, U/L             | 56.6<sup>a</sup>  | 53.6<sup>b</sup> | 56.0<sup>a</sup> | 55.8<sup>b</sup> | 1.26  | .009  |
| ALT, U/L             | 61.8               | 61.0  | 62.0   | 62.6   | 1.28  | .296  |
| AST/ALT ratio        | 0.916              | 0.878 | 0.903  | 0.892  | 0.034 | .364  |
| Alkaline phosphatase, U/L | 12.0  | 11.6   | 10.6  | 11.6   | 0.922 | .141  |
| Renal function index |                    |      |         |        |       |
| Urea, g/dl           | 22.2               | 24.0  | 23.2   | 22.2   | 1.74  | .323  |
| Creatinine, g/dl     | 12.0               | 12.6  | 12.2   | 12.2   | 1.29  | .902  |
| Urea/creatinine ratio | 1.86              | 1.93  | 1.91   | 1.83   | 0.153 | .702  |

<sup>ab</sup>Means within a row with different superscripts are significantly different (p < .05).

RMSE: Root mean square error; p value: probability level; HDL: High Density Lipoprotein; LDL: Low-density lipoprotein and VLDL: very Low-density lipoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AST/ALT: Aspartate aminotransferase to Alanine aminotransferase ratio.
significant differences in serum total protein and globulin among the different DOL groups.

Serum triglyceride and vLDL were significantly higher in groups fed 4 and 6% DOL than in the other groups, but the HDL to LDL ratio of 6% DOL was lower than that of the control. Besides, HDL was lower for 2 and 6% DOLs than in the control group. Serum total cholesterol, as well as LDL, were not significantly affected by DOL.

Plasma glucose levels decreased with increasing DOL at 6% DOL compared to the other DOLs groups. Most liver leakage enzymes and all renal function indices were not significantly affected by DOL, except for AST, which was considerably lower at 2%. We found no significant DOL effect on the renal function indices urea, creatinine, and urea-to-creatinine ratio.

**Haematological parameters**

Table 7 displays the impacts of DOL on RBCs parameters and different leukocytes proportional. There were no significant effects of DOL on RBC characteristics and most of the WBC parameters, except for a substantial decrease in WBCs of groups fed 2% DOL compared to the higher DOLs groups.

**Antioxidant status, lymphoid organs, phagocytosis, and antibody titre**

Table 8 represents the effect of DOL on antioxidants status, lymphoid organs, and phagocytosis, and antibody titre at 40 days of age. The inclusion of dietary oils in broiler diets significantly increased serum MDA compared to the control, whereas the TAC/MDA ratio was declined. There was no significant effect of DOL on TAC.

Spleen percentage was significantly lower in groups on 2% DOL than in the other groups. Besides, thymus percentage was markedly lower in groups fed 2% DOL than in those with 0 and 4% DOL. The phagocyte index was significantly higher at 4% DOL compared to the lower DOL groups.

Antibody titre against AI was increased in the 4 and 6% DOL groups compared to the other groups. Also, antibody titre against IBD tended to be numerically higher ($P < .07$) for 6% DOL compared to the other groups. We found no difference due to DOL in phagocyte index, antibody titre for NC, and heterophile-to-lymphocyte ratio.

**Discussion**

The changes in feed supply and chain during COVID-19 illustrate the needs for a new concept to reformulate broiler diets considering low-energy and low-cost diets to reduce feeding costs and minimise the demands for cereals, oilseeds, and fats/oils (Hafez and Attia 2020; Hashem et al. 2020; Hussain et al. 2020; Poudel et al. 2020; Siche 2020). A good example is the use of low-energy diets in broiler nutrition compared to diets supplemented with high DOL, especially under high ambient temperatures.

An indoor temperature of 31.5 ± 4°C during the experimental period induces heat stress in broilers, as evidenced by behavioural changes such as increased panting, lying down, and high body temperature (Daghir 2008; Attia et al. 2006). The effect of oil supplementation on broilers’ growth performance is
complex. An isocaloric diet containing 12.4 MJ/kg (ME), supplemented up to 4% DOL, resulted in similar growth performance and survival rate, and this related to the results digestibility. However, ether extract and ash digestibility of groups receiving 2 and 4% DOL were reduced. Besides, ash digestibility was further reduced by 6% DOL. Also, increasing DOL to 6% at unchanged ME levels reduced the performance index and dressing percentage of broilers compared to 2% DOL. The reduction in growth performance due to offering 6% DOL coincided with reducing digestibility DM, CP, ether extract diet or ash. The 6% DOL group’s lower performance may be related to increased fat deposition in the abdominal cavity compared to 2% DOL, and the liver and gizzard decreased. These changes indicate that the extra caloric effect provided by increasing DOL to 6% was directed to fat deposition in the abdominal cavity, liver, and meat, as meat lipid percentage also increased. These changes indicate that nutrient repatriating for fat deposition rather than muscle growth when DOL increased above energy requirements for muscle growth (Attia et al. 2018). Chickens offered diets containing different oils showed alterations in the carcass’s quality and composition (Baiao and Lara 2005; Attia et al. 2018). The group’s decreased performance fed 6% DOL may also be due to the low digestibility of nutrients due to reduced gizzard percentage. The gizzard is essential for the mechanical digestion of coarse parts of the diet (Daghir 2008; Pesti et al. 2015).

Our results suggest that broiler diets containing 12.4 MJ ME without or with supplementation of 2% DOL are adequate for broilers’ performance during hot weather conditions. The use of 2% oil could be recommended to meet broilers’ essential fatty acid needs under hot weather conditions (Attia et al. 2020). The recommended ME for broilers of 1–6 weeks is 13.4 MJ/kg diet (Attia et al. 2020); the level used herein was 7.5% lower (12.4 MJ/kg diet), saving about 2.8% of supplemented fat/oils in chicken diets. There is evidence that the DOL impacts broiler performance during hot weather conditions; a diet supplemented with fat/oil may enhance productive traits (NRC 1994; Raju et al. 2004; Baiao and Lara 2005; Attia et al. 2020). The primary energy sources for poultry nutrition are fats/oils and carbohydrates, although oils and fats are preferred. And, this may be due to the positive impact of fats/oils on digestibility, low heat increment, contents of fat-soluble vitamins, extra-calorific effect, and high-energy value; however, they are generally expensive (Pesti et al. 2015, Attia et al. 2020; Abd El-Hack et al. 2019).

In the literature, supplementation of oil/fat under high ambient temperatures is complex, and there was a lack of responses in some experiments (Sinurat and Balnave 1985; Attia et al. 2003). However, in others, the influence of oils/fats was dependent on the dose (Attia et al. 2018), the fat source (Attia et al. 2020), and the fatty acid profiles (Alagawany et al. 2019). Besides, reducing the ME level during high ambient temperatures promotes feed intake (Baghel and Pradhan 1990; Hoffmann et al. 1991), and some authors suggest that energy requirements are affected by weather temperature (Hurwitz et al. 1980; NRC 1994; Al-Harthi et al. 2002). The lower growth performance (growth rate, FCR, and survival) of broilers observed herein can be elucidated by the effect of heat stress on growth performance, such as feed

| Treatments                  | Dietary oil levels |
|-----------------------------|--------------------|
| 0% | 2% | 4% | 6% | RMSE | p Value |
| Total antioxidant capacity, mg/dl | 412 | 413 | 407 | 412 | 3.64 | .063 |
| MDA, μmol/l | 8.20b | 11.0b | 11.6b | 12.0b | 1.12 | .003 |
| TAC/MDA ratio | 50.3a | 37.6b | 35.4b | 35.0b | 4.03 | .001 |
| Spleen, % | 0.136a | 0.051b | 0.112a | 0.117a | 0.019 | .001 |
| Bursa of Fabricius, % | 0.085 | 0.051 | 0.075 | 0.114 | 0.044 | .128 |
| Thymus, % | 0.339ab | 0.127b | 0.410b | 0.226ab | 0.112 | .002 |
| Phagocytes | | | | |
| Phagocyte index | 1.32 | 1.30 | 1.46 | 1.30 | 0.141 | .255 |
| Phagocyte activity | 16.6b | 16.2b | 19.0b | 17.2b | 1.09 | .004 |
| Antibody titre, log2 | | | |
| Avian influenza | 3.25 | 3.26 | 4.00 | 4.25 | 0.395 | .043 |
| Infectious Bursa disease | 3.25 | 3.75 | 3.25 | 4.00 | 0.433 | .074 |
| Newcastle disease | 3.75 | 4.00 | 4.00 | 4.00 | 0.629 | .923 |
| Heterophil/lymphocyte ratio | 0.601 | 0.651 | 0.632 | 0.681 | 0.057 | .202 |

Means within a row with different superscripts are significantly different (p < 0.05); RMSE, Root mean square error; p value, probability level, TAC: Total antioxidants capacity; MDA: malondialdehyde.
intake, growth, and FCR. Performance of broilers can be affected by geographical area, region, season, type of diet, housing condition, type of house, slaughter age, nutritional level, hygiene and welfare, and epidemiology of diseases. The results recorded herein are in line with broilers' performance and survival in commercial enterprises in Saudi Arabia (Attia et al. 2018, 2020). Also, hot weather negatively affects animal performance and liveability. And, this coincided with lower feed intake, lower digestibility, impaired metabolic profiles, and acid-base balance (Daghir 2008; Al-Sultan et al. 2019; Beckford et al. 2020).

In our study, the group's dressing percentage fed 2% DOL was improved, which reflected increased growth performance. The present results show that up to 6% DOL's inclusion levels had no adverse effects on meat physical traits. High-quality meat has an increased customer acceptance, and meat with a greater juiciness/WHC is generally preferred (Attia et al. 2014, 2018).

An increase in DOL of up to 6% was associated with impaired lipid metabolism, as shown by the increased triglycerides of broilers on 4 and 6% DOL and the reduced HDL of the HDL to LDL ratio of 4 and 6% DOL. The present study revealed that lipid metabolites are the primary index of lipid metabolism; however, DOL is a valuable source of PUFA since it is mainly composed of sunflower and soybean oils. The increase in dietary fat intake can lead to cardiovascular issues, high blood pressure, and lipoedema. However, humans' dietary fat recommendations are sometimes contradictory and affected by dietary fat source, level, and fatty acid profile (Alagawany et al. 2019).

Interestingly, plasma glucose was gradually reduced with increasing DOLs, with a minimum level at 6%, indicating that oil supplementation can be used to control hyperglycaemia. In a previous study, fish oil and a PUFA-rich omega-3 source increased people's insulin sensitivity with metabolic disorders (Alagawany et al. 2019). A positive effect was noticed in AST, insulin sensitivity with metabolic disorders (Alagawany et al. 2019). Also, hot weather negatively affects animal performance and liveability. And, this coincided with lower feed intake, lower digestibility, impaired metabolic profiles, and acid-base balance (Daghir 2008; Al-Sultan et al. 2019; Beckford et al. 2020).

In our study, the group's dressing percentage fed 2% DOL was improved, which reflected increased growth performance. The present results show that up to 6% DOL's inclusion levels had no adverse effects on meat physical traits. High-quality meat has an increased customer acceptance, and meat with a greater juiciness/WHC is generally preferred (Attia et al. 2014, 2018).

An increase in DOL of up to 6% was associated with impaired lipid metabolism, as shown by the increased triglycerides of broilers on 4 and 6% DOL and the reduced HDL of the HDL to LDL ratio of 4 and 6% DOL. The present study revealed that lipid metabolites are the primary index of lipid metabolism; however, DOL is a valuable source of PUFA since it is mainly composed of sunflower and soybean oils. The increase in dietary fat intake can lead to cardiovascular issues, high blood pressure, and lipoedema. However, humans' dietary fat recommendations are sometimes contradictory and affected by dietary fat source, level, and fatty acid profile (Alagawany et al. 2019).

Interestingly, plasma glucose was gradually reduced with increasing DOLs, with a minimum level at 6%, indicating that oil supplementation can be used to control hyperglycaemia. In a previous study, fish oil and a PUFA-rich omega-3 source increased people's insulin sensitivity with metabolic disorders (Alagawany et al. 2019). A positive effect was noticed in AST, insulin sensitivity with metabolic disorders (Alagawany et al. 2019). Also, hot weather negatively affects animal performance and liveability. And, this coincided with lower feed intake, lower digestibility, impaired metabolic profiles, and acid-base balance (Daghir 2008; Al-Sultan et al. 2019; Beckford et al. 2020).

In our study, the group's dressing percentage fed 2% DOL was improved, which reflected increased growth performance. The present results show that up to 6% DOL's inclusion levels had no adverse effects on meat physical traits. High-quality meat has an increased customer acceptance, and meat with a greater juiciness/WHC is generally preferred (Attia et al. 2014, 2018).

An increase in DOL of up to 6% was associated with impaired lipid metabolism, as shown by the increased triglycerides of broilers on 4 and 6% DOL and the reduced HDL of the HDL to LDL ratio of 4 and 6% DOL. The present study revealed that lipid metabolites are the primary index of lipid metabolism; however, DOL is a valuable source of PUFA since it is mainly composed of sunflower and soybean oils. The increase in dietary fat intake can lead to cardiovascular issues, high blood pressure, and lipoedema. However, humans' dietary fat recommendations are sometimes contradictory and affected by dietary fat source, level, and fatty acid profile (Alagawany et al. 2019).

Interestingly, plasma glucose was gradually reduced with increasing DOLs, with a minimum level at 6%, indicating that oil supplementation can be used to control hyperglycaemia. In a previous study, fish oil and a PUFA-rich omega-3 source increased people's insulin sensitivity with metabolic disorders (Alagawany et al. 2019). A positive effect was noticed in AST, insulin sensitivity with metabolic disorders (Alagawany et al. 2019). Also, hot weather negatively affects animal performance and liveability. And, this coincided with lower feed intake, lower digestibility, impaired metabolic profiles, and acid-base balance (Daghir 2008; Al-Sultan et al. 2019; Beckford et al. 2020).

In our study, the group's dressing percentage fed 2% DOL was improved, which reflected increased growth performance. The present results show that up to 6% DOL's inclusion levels had no adverse effects on meat physical traits. High-quality meat has an increased customer acceptance, and meat with a greater juiciness/WHC is generally preferred (Attia et al. 2014, 2018).

An increase in DOL of up to 6% was associated with impaired lipid metabolism, as shown by the increased triglycerides of broilers on 4 and 6% DOL and the reduced HDL of the HDL to LDL ratio of 4 and 6% DOL. The present study revealed that lipid metabolites are the primary index of lipid metabolism; however, DOL is a valuable source of PUFA since it is mainly composed of sunflower and soybean oils. The increase in dietary fat intake can lead to cardiovascular issues, high blood pressure, and lipoedema. However, humans' dietary fat recommendations are sometimes contradictory and affected by dietary fat source, level, and fatty acid profile (Alagawany et al. 2019).

Interestingly, plasma glucose was gradually reduced with increasing DOLs, with a minimum level at 6%, indicating that oil supplementation can be used to control hyperglycaemia. In a previous study, fish oil and a PUFA-rich omega-3 source increased people's insulin sensitivity with metabolic disorders (Alagawany et al. 2019). A positive effect was noticed in AST, insulin sensitivity with metabolic disorders (Alagawany et al. 2019). Also, hot weather negatively affects animal performance and liveability. And, this coincided with lower feed intake, lower digestibility, impaired metabolic profiles, and acid-base balance (Daghir 2008; Al-Sultan et al. 2019; Beckford et al. 2020).

In our study, the group's dressing percentage fed 2% DOL was improved, which reflected increased growth performance. The present results show that up to 6% DOL's inclusion levels had no adverse effects on meat physical traits. High-quality meat has an increased customer acceptance, and meat with a greater juiciness/WHC is generally preferred (Attia et al. 2014, 2018).
The present results show that feeding broilers’ dietary oil levels under hot weather conditions depend on the production goal, as 2% can be suggested for growth performance and 4-6% for immune response. Thus, further research supports the recommended levels of dietary oils on broiler performance is essential. This should consider DOL, fatty acid profile, and ambient temperature. Due to the literature’s contradictory results (Sinurat and Balnave 1985; Lou et al. 2003).

However, our study is limited, mainly because of the lack of a control group reared under normal climatic conditions (Attia and Hassan 2017; Attia et al. 2018). Also, to the well-known demonstrated literature, group reared under normal environmental temperature had no additive value to the current experiment set-up due to primary goals in which the hot weather was investigated rather than heat stress per se. However, similar studies using carbohydrate-based-energy diets without oil supplementation compared to diets with 8–10% DOL, under heat stress, are lacking. The use of DOL may be suggested as a valuable nutritional technique to negate the harmful effects of hot weather; however, the most suitable level of oil/fat remains uncertain and depends on the production propose (Daghir 2008; Fouad et al. 2016).

**Conclusion**

Under natural high-temperature conditions from 20 to 40 days of age, broilers’ best production performance index was achieved at 0–2% DOL. However, feeding 6% DOL to broilers markedly decreased growth performance and impaired lipid metabolism, albeit with an improved immune status. This showed that broiler feeding under natural high temperatures is a factor of the achieved goal, such as improved growth performance or enhanced immune status. The results indicate that the effect of dietary oils on broilers depends on goals of production as growth performance needs less oils (−02%) than immune response, which showed greater responses to high oil supplemented diet (4–6%) %) and/or essential fatty acids requirements.

**Acknowledgments**

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, under grant no. “G-108-155-1441”. The author, therefore, acknowledges with thanks DSR for technical and financial support.

**Author contributions**

YAA, MAA, and SSH are equally contributed to all stages of the work and critically revised the manuscript and approved the final version.

**Disclosure statement**

The authors declare that the research was conducted lacked any commercial or financial correlations that could be construed as a potential conflict of interest.

**Funding**

The authors would like to express their gratitude to the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, under grant no. “G-108-155-1441”. The author, therefore, acknowledges with thanks DSR for technical and financial support.

**ORCID**

Youssef A. Attia http://orcid.org/0000-0001-6505-3240
Mohammed A. Al-Harthi http://orcid.org/0000-0002-0801-0399

**References**

Abaza M. 2002. Immune system and some physiological aspects in Japanese quail affected by antioxidants. Egypt Poult Sci J. 22:259–276.

Abd El-Hack ME, Alagawany M, Mahrose KM, Arif M, Saeed M, Arain MA, Soomro RN, Sialy FA, Fazlani SA, Fowler J. 2019. Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season. Anim Nutr. 5(1): 49–55.

Aggoor FAM, Attia YA, Qota EMA. 2000. A study on the energetic efficiency of different fat sources and levels in broiler chick vegetable diets. Mansoura Univ. J. Agric Sci. 25:801–820.

Aitken A, Case JC, Penny IF, Dvolys CA. 1962. Effect of drying temperature in the accelerated freezes drying of pork. J Feed Sci. 75:505–513.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Tiwari R, Yatoo MI, Prakash BP, Khurana SK, Kuldeep DK. 2019. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals. 9(8):573.

**Ethical approval**

The experimental work and ethical treatment of animals in this study were approved by DSR, King Abdulaziz University, under protocol no G-108-155-1441. The procedures suggested minimal stress, ensure rights and welfare by eliminating harm or suffering to animals.
Al-Harthi MA, El-Deek AA, El-Harbi BL. 2002. Interrelation
ships among triiodothyronine (T3), energy and sex on
nutritional and physiological responses of heat stressed
broilers. Egypt Poult Sci J. 22:349–385.
Al-Khalilfah H. 2020. Modulatory effect of dietary polysaturated
fatty acids on immunity, represented by phago-
cytic activity. Front Vet Sci. 7:569939.
Al-Khalilfah H, Al-Nasser A, Abdulmalek N, Al-Mansour H,
Ahmed A, Ragheb G. 2020. Impact of SARS-CoV-2 on the
poultry industry in Kuwait: a case study. Front Vet Sci. 7:
577178.
Al-Sultan SI, Abdel-Raheem SM, Abd-Allah SMS, Edris AM.
2019. Amelioration of chronic heat stress in broilers by diet-
ary supplementation of novel feed additive combinations.
SVR. 56(22-Suppl):269–279. doi.org/10.26873/SVR-766-2019
Association of Official Analytical Chemists, AOAC 2004.
Official Method of Analysis of the Association of Official
Analytical Chemists, Sindy Williams. 164th Ed. Arlington,
Virginia, USA: Association of Official Analytical Chemists, Inc.
Atapattu N, Silva LMSI. 2016. Effects of gradual feed dilution
with inert or less nutritive materials on growth perform-
ance, feed cost and meat organoleptic properties of
broiler chicken. Rev Bras Cienc Avic. 18(3):427–434.
doi.org/10.1590/1806-9061-2015-0136
Attia AI, Hassan II, EL-Zaiat AA, Abd EL-Maksoud AA. 2003.
Effect of dietary oil and ascorbic acid on the performance
of broiler chicks under Egyptian summer conditions.
Egypt J Nut Feeds. 6 (Special Issue):3–4.
Attia YA, Al-Harthi MA, Abo El-Maaty HAM. 2020. The effects
of different oil sources on performance, digestive
enzymes, carcass traits, biochemical, immunological, anti-
oxidant, and morphometric responses of broiler chicks.
Front Vet Sci. 7:181.
Attia YA, Al-Harthi MA, ElNaggar Asmaa S. 2018. Productive,
physiological and immunological responses of two broiler
strains fed different dietary regimens and exposed to heat
stress. Ita J Anim Sci. 17(3):686–697. doi.org/10.1080/
1828051X.2017.1416961
Attia YA, Al-Harthi MA, El-Shafey AS, Rehab YA, Kim WK.
2017. Enhancing tolerance of broiler chickens to heat
stress by supplementation with vitamin E, vitamin C and/
or probiotics. Ann Anim Sci. 17:1–15.
Attia YA, Böhmer BM, Roth-Maier DA. 2006. Responses of
broiler chicks raised under constant relatively high ambient
temperature to enzymes, amino acid supplementa-
tions, or diet density. Arch Geflügelk. 70:80–91.
Attia YA, El-Tahawy WS, Abd El-Hamid AE, Nizza A, El-Kelway
Mi, Al-Harthi MA, Bovera F. 2014. Effect of feed form, pel-
let diameter and enzymes supplementation on carcass
characteristics, meat quality, blood plasma constituents
and stress indicators of broilers. Arch Anim Breed. 57(1):
1–14.
Attia YA, Hassan RA, Qota EMA. 2009. Recovery from adverse
effects of heat stress on slow-growing chicks in the
tropics 1: Effect of ascorbic acid and different levels of
betaine. Trop Anim Health Prod. 41(5):807–818.
Attia YA, Hassan RA, Tag El-Din AE, Abou- Shehema BM.
2011. Effect of ascorbic acid or increasing metabolizable
energy level with or without supplementation of some
essential amino acids on productive and physiological
traits of slow-growing chicks exposed to chronic heat
stress. J Anim Phys Anim Nutr. 95(6):744–755.
Attia YA, Hassan SS. 2017. Broiler tolerance to heat stress at
various dietary protein/energy levels. Europ Poult Sci. 81:2017.
Baghel RPS, Pradhan K. 1990. Effect of season and age on the
utilization of metabolizable energy by broilers. Indian
J Anim Sci. 60:239–242.
Bagio NC, Lara JJC. 2005. Oil and fat in broiler nutrition. Rev
Bras Cienc Avic. 7(3):129–141.
Bartles H, Bohmer M, Heirli C. 1972. Serum creatinine deter-
mination without protein precipitation. [Article in
German]. Clin Chem Acta. 37:193–197.
Beckford RC, Elsestad LE, Proszkowiec-Weglarz M, Farley L,
Brady K, Angel R, Liu H-C, Porter TE. 2020. Effects of heat
stress on performance, blood chemistry and hypothalamic
and pituitary mRNA expression in broiler chickens. Poult
Sci. 99(12):6317–6325. doi.org/10.1016/j.psj.2020.09.052
Coles EH. 1974. Veterinary clinical pathology. Philadelphia,
London, Toronto: W.B. Saunders, Company p. 211–213.
Cosgrove AS. 1962. An apparently new disease of chickens.
Avian nephrosis. Avian Dis. 6(3):385–389.
Daghir N. 2008. Nutrient requirements of poultry at high
temperature. In: Daghir, NJ, editor. Poultry production in
hot climates 2nd ed., CAB.
Davidson J, Thomas OA. 1969. A scheme for the separation
and estimation of nitrogenous components of the excreta
from poultry. Br Poult Sci. 10(1):53–66. doi.org/10.1080/
00071666908415742
Doumas BT, Watson D, Biggs HG. 1971. Albumin standards
and the measurement of blood albumin with bromocresol
green. Clin Chem Acta. 31(1):87–96.
Ellers RI. 1967. Notification of final adoption of an inter-
national method and standard solution for hemoglobin-
ometry specifications for preparation of standard solution.
Am J Clin Pathol. 47(2):212–314.
FAO http://www.fao.org/2019-ncov/q-and-a/impact-on-food-
and-agriculture/en/. Accessed May 20, 2020.
Foudad AM, Chen W, Ruan D, Wang S, Xia WG, Zheng CT.
2016. Impact of heat stress on meat, egg quality, immunity
and fertility in poultry and nutritional factors that over-
come these effects: a review. Int J Poult Sci. 15(3):81–95.
doi.org/10.3923/ijps.2016.81.95.
Friedewald WT, Levy RI, Fredrickson DS. 1969. Estimation of
the concentration of low-density lipoprotein cholesterol in
plasma, without use of the preparative ultracentrifuge.
Clin Chem. 18(6):499–502.
Gao H, Geng T, Huang T, Zhao Q. 2017. Fish oil supplementa-
tion and insulin sensitivity: a systematic review and
meta-analysis. Lipids Health Dis. 16(1):131.
Ghazalah AA, Abd - Elsa MO, Ali AM. 2008. Influence of diet-
ary energy and poultry fat on the response of broiler
chicks to heat therm. Inter. Int J Poult Sci. 7(4):355–359.
Hafez MH, Attia YA. 2020. Challenges to the poultry industry:
current perspectives and strategic future after the COVID-
19 outbreak. Front Vet Sci. 7:516.
Hashem NM, González-Bulnes A, Rodriguez-Morales AJ. 2020.
Animal welfare and livestock supply chain sustainability
under the COVID-19 outbreak: an overview. Front Vet Sci.
7:582528.
Hepler OE. 1966. Manual of clinical laboratory methods.
Illinois, USA: Thomas, Sparing Field.
Hoffmann L, Schiemann R, Klein M. 1991. Energy metabolism of growing broilers in relation to environmental temperature. Archiv Anim Nutr. 41:167–181.

Hu R, He Y, Arowolo MA, Wu S, He J. 2019. Polyphenols as potential attenuators of heat stress in poultry production. Antioxidants. 8(3):67. doi.org/10.3390/antiox8030067

Hurwitz S, Weiselberg M, Eisner U, Bartov I, Riesenfeld G, Sharvit M, Niv A, Bornstein S. 1980. The energy requirements and performance of growing chickens and turkeys as affected by environmental temperature. Poult Sci. 59(10):2290–2299.

Husani SA, Deatherage FB, Kunlkle LE. 1950. Studies on meat.11: Observations on relation of biochemical factors to change in tenderness. Feed Tech. 4:366–369.

Hussain S, Hussain A, Ho J, Sparagano OA, Ubaid-Ur-Rehman. 2020. Economic and social impacts of COVID-19 on animal welfare and dairy husbandry in Central Punjab, Pakistan. Front Vet Sci. 7:589971.

Kai O, Nagase H, Ishikawa N, Suzuki M, Kakegawa T, Sato K. 1988. Effects propylthiouracil PTU on the immunological status of the chickens. Develop Comp Immun. 12(1):145–156.

Kind PRN, King EJ. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol. 7(4):322–326.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. 2001. Method for the measurement of antioxidant activity in human fluids. J Clin Path. 54(S):356–361.

Leijh PC, Van Furth R, Van Zwet TL. 1986. In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In: MD. Weir, L.A., Herzenberg, C., Blackwell, editors. Handbook of morphonuclear and mononuclear phagocytes. In: MD. Weir, L.A., Herzenberg, C., Blackwell, editors. Handbook of experimental immunology. Oxford: Blackwell Scientific Publications, 1986. p. 1–21.

Lin H, Zhang HF, Du R, Gu XH, Zhang ZY, Buyse J, Decuyper E. 2005. Thermoregulation responses of broiler chickens to humidity at different ambient temperatures. II. Four weeks of age. Poult Sci. 84(8):1173–1178.

Lou SM, Colian A, Shahroudi FE, Mahallati MN, Nermanshahi H. 2003. Effect of energy level and time of feed replacement from starter to finisher diets of broiler weighing less than two kg. J Sci Techn Agric. 7:153.

Lucas AM, Jamroz C. 1961. Atlas of Avian Hematology. Academic Press.

Sahraei M, Shariatmad F. 2007. Effect of different levels of commercial feed on egg production and performance of growing chickens and turkeys. Poultry Science. 6(3):280–282. doi.org/10.3923/ijps.2007.280.282

Mateos GG, Sell JL, Eastwood JA. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poult Sci. 61(1):94–100.

Mehaisen GM, Desoky AA, Sakr OG, Sallam W, Abass AO. 2019. Propolis alleviates the negative effects of heat stress on egg production, egg quality, physiological and immunological aspects of laying Japanese quail. PLoS One. 14(4):e0214839. doi.org/10.1371/journal.pone.0214839

Moharrery A. 2011. Comparison of performance and digestibility characteristics of broilers fed diets containing treated hulled barley or hullless barley. Czech J Anim Sci. 51(No. 3):122–131.

National Research Council NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Washington. DC., USA: National Academic Press.

Pesti GM, Bakalli RI, Driver JP, Atencio A, Foster EH. 2015. Lipids in poultry nutrition. PP. p. 9–1–9–17. in Poultry Nutrition and feeding (Text Book), 1st ed. Canada: Trafford Publishing.

Poudel PB, Poudel MR, Gautam A, Phuyal S, Tiwari CK, Bashyal N, Bashya S. 2020. COVID-19 and its global impact on food and agriculture. J Biol Todays’ World. 9:221–224.

Raju MVLN, Sunder GS, Chawak MM, Rao SVR, Sadagopan VR. 2004. Response of naked neck (Nana) and normal (nana) broiler chickens to dietary energy levels in a sub-tropical climate. Br Poult Sci. 45(2):186–193.

Randrup A. 1960. A specific and reasonably accurate method for routine determination of plasma triglyceride. Scand J Clin Lab Invest. 12:1–9.

Reitman S, Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 28(1):56–63.

Richard MJ, Portal B, Meo J, Coudray C, Hadjian A, Favier A. 1992. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin Chem. 38(5):704–709.

Sahin K, Sahin N, Kucuk O, Hayirli A, Prasad AS. 2009. Role of dietary zinc in heat-stressed poultry: a review. Poult Sci. 88(10):2176–2183. doi.org/10.3382/ps.2008-00560

Sampson EJ, Baird MA, Smith EM, Witte DL, Bayse DD. 1980. A coupled-enzyme equilibrium method for urea measuring in serum. Optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem. 26(7):816–826.

SAS Institute 2009. SAS® Users’ Guide: Statistics. Version 5th ed., Cary, NC, USA: SAS Institute Inc.

Siche R. 2020. What is the impact of COVID-19 disease on agriculture? Sci Agropecu. 11(1):3–6.

Sinurat AP, Balnave D. 1985. Effect of dietary amino acids and metabolisable energy on the performance of broilers kept at high temperatures. Br Poult Sci. 26(1):117–128.

Suganya T, Senthilkumar S, Deepa K, Amutha R. 2015. Nutritional management to alleviate heat stress in broilers. Int J Sci EnDOLi Tech. 4:661–666.

Syafwan S, Kwakkel RP, Verstegen MWA. 2011. Heat stress and supplementation of synthetic amino acids on performance and carcass parameters in commercial male turkeys. Poult Sci. 84(8):1173.

Takatsy GY. 1956. The use of spiral loops in serological and virological micromethods. Acta Microbiol Acad Sci. Hung. 5:218–221.

Veldkamp T, Ferket PR, Kwakkel RP, Nixey C, Noordhuizen JPTM. 2000. Interaction between ambient temperature and supplementation of synthetic amino acids on performance and carcass parameters in commercial male turkeys. Poult Sci. 79(10):1472–1477.

Volvoinskaia VP, Kelman BY. 1962. Modification of water holding capacity method of meat. F. D. Industry. Musco. 11:80.
Watson D. 1960. A simple method for determination of serum cholesterol. Clin Chem Acta. 5(5): 637–643.

Weichselbaum TE. 1946. An accurate and rapid methods for determination of proteins in small amount of blood, serum and plasma. Am J Clin Pathol. 16(3_ts): 40–48.

Yang L, Tan GY, Fu YQ, Feng JH, Zhang MH. 2010. Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ROS production and lipid peroxidation in broiler chickens. Comp Biochem Physiol C Toxicol Pharmacol. 151(2):204–208. doi.org/10.1016/j.cbpc.2009.10.010

Wieland H, Seidel D. 1983. A simple specific method for precipitation of low density lipoproteins. J Lipid Res. 24(7):904–909.