EFFECT OF DIETARY FAT AND ENVIRONMENTAL TEMPERATURE ON GROWTH PERFORMANCE IN BROILER CHICKENS

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Abstract

This study investigated the effects of dietary fat level and environmental temperature on growth performance, nutrient utilisation, and survival in Ross 308 broiler chickens. Modern broilers have high growth potential, but their performance can be influenced by nutritional composition and environmental temperature. Dietary fat is commonly used as an energy-dense ingredient and may reduce metabolic heat production because of its lower heat increment (HI) compared with protein and carbohydrate. Therefore, this experiment evaluated whether a high-fat diet could improve broiler performance under an elevated temperature regimen. A 2 × 2 factorial design was used, with two dietary treatments, low-fat and high-fat, and two environmental temperature regimens, normal and elevated. Birds were fed experimental pelleted diets from day 10 to day 34. Both diets were formulated to contain similar targets of apparent metabolisable energy (AME) and similar crude protein levels; however, the measured AME content was numerically different between the low-fat and high-fat diets. During the experimental period, the normal-temperature regimen declined from approximately 25°C on day 10 to 21°C on day 34, whereas the elevated-temperature regimen declined from approximately 30°C on day 10 to 23°C on day 34. Thus, the elevated-temperature group remained higher than the normal-temperature group throughout the experimental period, including a 2°C difference at day 34, with an additional cyclic daytime increase during weekdays. Growth performance was assessed through body weight, average daily gain (ADG), daily feed intake, and feed conversion ratio. Apparent metabolisable energy, ileal nutrient digestibility, and survival were also measured.

Overall, elevated temperature significantly increased ADG and daily feed intake, but did not affect feed conversion ratio. Dietary fat level did not significantly influence overall ADG, daily feed intake, or feed conversion ratio, although the high-fat diet improved feed conversion ratio during Week 4. A significant Diet × Temperature interaction was observed for overall daily feed intake, but not for ADG or feed conversion ratio. Digestibility results showed that diet had a stronger effect than temperature. The low-fat diet produced a higher AME coefficient and starch digestibility, while the high-fat diet increased protein digestibility and digestible fat content. Survival ranged from 72.22% to 91.67% across the four treatment combinations, with no statistically detectable effects of diet, temperature. The results suggest that the elevated temperature regimen used in this study was moderate and should not be treated as an

experimentally-based severe heat stress model. High dietary fat did not improve overall growth performance, although it may support feed efficiency during later growth stages.

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List of Abbreviations:

ADF	Acid Detergent Fibre
ADG	Average Daily Gain
AME	Apparent Metabolisable Energy
AOAC	Association of Official Analytical Chemists
BSF	Black Soldier Fly
BW	Body Weight
BWG	Body Weight Gain
BWE	Body Weight at End of Week
BWS	Body Weight at Start of Week
CIAD	Coefficient of Ileal Apparent Digestibility
DCP	Dicalcium Phosphate
DFI	Daily Feed Intake
DM	Dry Matter
ET	Elevated Temperature
FCR	Feed Conversion Ratio
FI	Feed Intake
GE	Gross Energy
GH	Growth Hormone
GLM	General Linear Model
GPx	Glutathione Peroxidase
GWAS	Genome-Wide Association Studies
H/L ratio	Heterophil-to-Lymphocyte Ratio
HF	High Fat
HPA	Hypothalamic–Pituitary–Adrenal

HI	Heat Increment
HS	Heat Stress
IDF	Ileal Digestibility of Fat
IDP	Ileal Digestibility of Protein
IDS	Ileal Digestibility of Starch
IGF-1	Insulin-like Growth Factor-1
LCT	Lower Critical Temperature
LF	Low Fat
LSMeans	Least Squares Means
ME	Metabolisable Energy
MDA	Malondialdehyde
MUFA	Monounsaturated Fatty Acids
NDF	Neutral Detergent Fibre
NE	Net Energy
NGS	Next-Generation Sequencing
NT	Normal Temperature
PUFA	Polyunsaturated Fatty Acids
PV	Peroxide Value
QTL	Quantitative Trait Loci
RFI	Residual Feed Intake
RO Water	Reverse Osmosis Water
SAS	Statistical Analysis System
SD	Standard Deviation
SE	Standard Error
SFA	Saturated Fatty Acids

SOD	Superoxide Dismutase
T3	Triiodothyronine
T4	Thyroxine
TiO_2	Titanium Dioxide
TNZ	Thermoneutral Zone
UCT	Upper Critical Temperature
UFA	Unsaturated Fatty Acids
WB	Weighed Back

1. GENERAL INTRODUCTION

Broiler chicken production is an efficient way to supply high-quality animal protein, but achieving consistent performance depends strongly on nutrition and the rearing environment (Dal Bosco et al., 2021). Modern broiler strains have been intensively selected for rapid growth and improved feed efficiency; however, these genetic gains can be limited when birds face environmental challenges such as sub-optimal temperature. Temperature affects birds' ability to maintain normal body function and allocate nutrients toward growth rather than thermoregulation. As a result, variation in ambient temperature can change feed intake patterns and overall growth performance, making environmental control a key part of efficient broiler production.

Exposure to high environmental temperatures affects broiler chicken feed intake, nutrient use, and energy partitioning, which may diminish productive efficiency (Wanjala et al., 2023). Therefore, the nutritional strategies that support sufficient energy and nutrient provision under different thermal conditions could be useful in broiler production.

Dietary energy density and the source of dietary energy are important considerations in broiler nutrition. The high energy density and relatively low heat increment of fats and oils make them a common addition to broiler diets. Dietary fat level, therefore, can also affect energy utilisation and energy efficiency, but this will vary with the composition of the complete feed and the rearing environment. Feed intake and nutrient utilisation could also be affected by environmental temperature. It is therefore essential to learn how the level of dietary fat affects environmental temperature to determine feeding strategy for specific thermal conditions. (Wanjala et al., 2023).

The present thesis investigates the broiler chicken's reaction to two extreme dietary formulations and two environmental temperature regimens in a 2 × 2 factorial design. The birds were kept under either normal- or elevated-temperature conditions and received a low-fat or high-fat diet throughout the experimental phase, days 10 to 34. The two diets were designed to the same target apparent metabolisable energy (AME; 3150 kcal/kg) and similar crude protein concentrations but varied in inclusion of soybean-oil (1.62 g/kg vs 6.01 g/kg) and other ingredients and nutrient fractions. Therefore, responses to the dietary treatments should be interpreted as effects of the contrasting diet formulations rather than being attributed exclusively to dietary fat. Growth performance was measured by body weight (BW), feed intake, ADG and FCR, which provided

evidence on the influence of diet composition and temperature alone and in combination on broiler productivity (Katu et al., 2025).

2. LITERATURE REVIEW

2.1. Introduction to the Literature Review

Climate variability and nutritional constraints are increasingly limiting broiler growth performance and production efficiency in commercial poultry systems (Kyriazakis et al., 2024). This literature review addresses the interactive effects of dietary fat and environmental temperature on broiler chickens in terms of physiological responses, feed efficiency, and production results. With the current strains of broilers like Ross 308 having high metabolism and growing at a rapid rate, a broiler is especially susceptible to heat stress, which can greatly affect productivity and welfare.

Broiler chickens have played a significant role in the supply of meat in the world, as they provide about 41% of total meat-protein supply (*Food Balance Sheets 2010–2022. Global, Regional and Country Trends*, 2024). Optimisation of growth performance is based on high body weight gain and efficient feed conversion ratio, as well as preserving the quality of meat and the welfare of birds. Heat stress in broiler chickens can be caused by high environmental temperature, especially when ambient conditions are beyond their thermoneutral range. Heat stress is a significant environmental issue as it decreases productivity due to decreased feed consumption, disturbed nutrient metabolism, compromised immune system, and lower carcass quality (Nawaz et al., 2021). As feed intake declines under heat stress, energy-dense diets are commonly used to help maintain nutrient intake, although their effects may vary under hot conditions, making the Diet × Temperature interaction an important research focus.

Nutrient strategies which could be beneficial for broiler performance in warmer environments have been gaining more recognition with the rising environmental temperature. Increased inclusion of dietary fat has been suggested, as fat is energy dense and has a lower heat increment than protein and carbohydrate, which may lead to a decreased production of metabolic heat while maintaining the dietary energy supply (Loyiso Ndlebe et al., 2023). However, the reported benefits of higher-fat diets are not consistent across studies and may depend on factors such as bird age, severity and duration of temperature exposure, dietary energy concentration, fat source, and the overall ingredient composition of the diet. The aim of the present study is to investigate growth performance, apparent metabolisable energy, ileal digestibility of nutrients and survival rates in response to two dietary formulations and two environmental temperature regimens to address this uncertainty.

2.2. Broiler Chicken Physiology and Growth Performance

2.2.1 Growth phases of broiler chickens

The growth pattern of broiler chickens is extremely fast in nature, as the chicken undergoes three distinct production stages, namely: starter, grower, and finisher, with their nutritional and physiological requirements varying. In the starter stage (0-14 days), chicks undergo fast growth of organ and muscle tissues and thus demand high-protein diets (22-24%) to aid the growth of cells and initial skeletal development. The grower phase (15-28 days) is the process of intensifying muscle accretion and energy storage, where balanced levels of energy-to-protein ratios are required to convert nutrients efficiently. Lastly, the finisher phase (29-42 days) focuses on yield and fat deposition of the carcass, with reduced protein (~19-20) and increased energy consumption to achieve maximum market weight and feed conversion ratio (Maharjan et al., 2021).

Early studies by Jaap (1970) outlined the self-accelerating growth stage in broilers, which was characterized by extreme rises in body weight throughout the early growth and development, and a curve of diminishing returns as growth approaches its genetic potential. This growth pattern indicates physiological and genetic limits, as the proportion of energy allocated to muscle tissue decreases and the energy allocated to maintenance and fat storage increases.

2.2.2 Genetic selection and rapid growth in modern strains

The growth rate and feed efficiency of commercial broiler strains have improved significantly in the past few decades due to genetic selection. However, performance varies among genetic lines and is influenced by management, nutrition, and environmental conditions. Most commercial varieties, such as in the modern Ross 308, have been bred for fast growth and feed conversion efficiency. In the study conducted by Marcu et al. (2013) there was also significant variation in growth performance and economic efficiency between Ross 308 and Cobb 500 broilers, which suggested that genetic type can have an effect on the production performance. The results presented here should therefore not be extrapolated to generalise for all modern broilers, but only to the particular strain and production environment.

Genomic tools in the present era have increased the accuracy of selection. Next-generation sequencing (NGS) and genome-wide association studies (GWAS) have revealed a great number of quantitative trait loci (QTLs) that correlate with economically valuable phenotypes such as body weight, residual feed intake (RFI), and feed efficiency (Gilyazova et al., 2025). Other genes that

were linked with muscle development and energy systems, including SLIT2, PPARGC1A (PGC-1α), MYOCD, and ADGRA3 (GPR125), were associated with variation in growth potential and feed-utilisation efficiency.

The results show that genomic and transcriptomic studies have enhanced the knowledge on molecular pathways that regulate the growth of skeletal muscle and lipid metabolism in broilers. Genomic and marker-assisted selection have accelerated improvement in broilers, especially for growth rate, feed efficiency, and overall robustness. An example is the Ross 308 strain, which is not only optimised in terms of growth rate but also metabolic efficiency and has the ability to adapt to various production climates. Nonetheless, increased physiological needs on metabolism and cardiovascular performance have also been a consequence of these genetic advancements, posing new management and welfare challenges.

2.2.3 Physiological determinants of growth

Metabolic factors, hormonal factors, and nutritional factors interact to control the growth of broilers. Muscle growth involves the interaction of growth hormone (GH), insulin-like growth factor-1 (IGF-1) as well as thyroid hormones, which promote the accretion of proteins and the maintenance of lipolysis balance (Maharjan et al., 2021). Maharjan et al. (2021) further highlighted that the rapid accumulation of muscle in the contemporary strains in most cases is followed by the reduction of the oxidative capacity, and such a situation may result in metabolic overload when the dietary protein and energy are not controlled accordingly.

2.2.4 Environmental and welfare considerations

Genetic selection has maximized growth but has increased exposure to environmental conditions as well. The amount of feed intake, the use of energy, and the stress physiology can be greatly influenced by temperature changes, humidity, and stocking density (birds/m^2 or kg liveweight/m^2). According to Riber and Wurtz (2024), increased growth rates are associated with welfare issues like skeletal defects, cardiovascular pressure, as well as decreased mobility, especially when there is thermal stress or in crowded living conditions. This suggests that the genetic potential of modern broilers can only be realised fully when management and environmental conditions are carefully controlled to protect welfare and maintain physiological stability.

2.3. Dietary Energy and Fat in Broiler Nutrition

Dietary energy in broilers can be understood as a stepwise partitioning process, beginning with gross energy and progressing through digestible energy and AME to net energy. At each stage, part of the energy is lost through excreta, urinary and gaseous losses, and heat increment. Net energy therefore represents the fraction ultimately available for maintenance and growth. As shown in Figure 2.1, lower HI improves the efficiency of energy utilisation by allowing a greater proportion of AME to be retained as net energy.

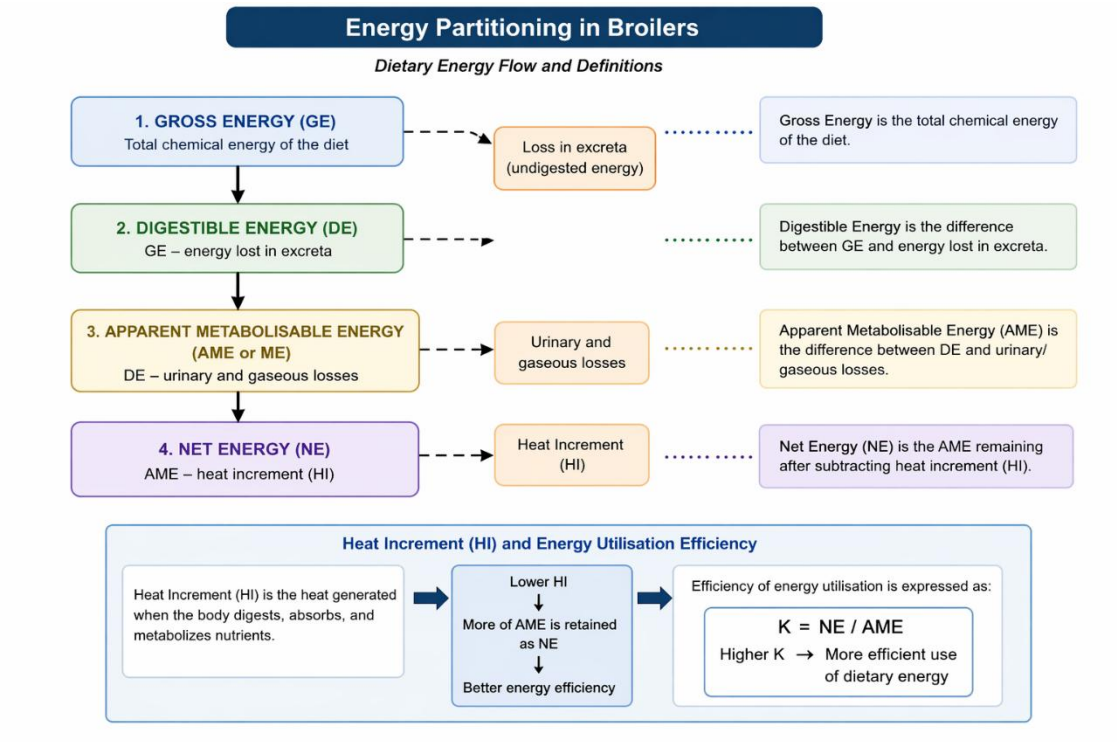

Figure 2.1 Energy partitioning of dietary energy in broilers (OpenAI, 2025)

Note: This figure was generated using ChatGPT (OpenAI, 2025)

2.3.1 Role of dietary fat in poultry

Dietary fat forms a significant part of the broiler diet since it gives greater gross energy than carbohydrates or proteins and is usually added to enhance the energy density of the diet and to enhance palatability. In addition to being an energy source, fat can also have extra-caloric effects, i.e., greater performance responses than that of the same energy density. These effects have been linked to better digestion and absorption, reduced passage of digesta, and increased conversion of metabolisable energy into net energy since it has a low HI (Brue and Latshaw, 1985). Therefore,

dietary fat has the potential to enhance feed efficiency and growth performance. Fat also plays a role in the quality of pellets, which can enhance voluntary feeding and growth that is more uniform. In addition, dietary fats are important for cell membrane structure, hormone production, and lipid metabolism. In broilers, liver is a significant location of lipid metabolism and thus lipid synthesis and deposition can be affected by dietary fat manipulation (Fébel et al., 2008).

2.3.2 High-fat vs. Low-fat diets

Dietary fat level can influence broiler growth performance, feed efficiency, carcass yield, and fat deposition. Generally, higher fat diets will help in feed efficiency, due to the increased energy density of the fat and its lower HI compared to protein or carbohydrate. However, other factors can also increase deposition of fat in the abdomen when the energy consumed is in excess of what the bird needs (Fouad and El-Senousey, 2014). With increased fat inclusion, apparent fat digestibility may also be increased by stimulation of bile secretion and micelle formation, but this depends on the chemical nature of the fat and the age of the birds. Lower fat diets, on the other hand, might minimise the deposition of fat in the carcass but could also result in less dietary energy per unit quantity. Therefore, comparisons between high-fat and low-fat diets are important because they reflect the trade-off between energy utilisation, growth response, and body fat deposition.

2.3.3 Types and sources of fat

a. Animal vs. Vegetable fats

Animal fats like tallow, lard and poultry fat are typically rich in saturated fatty acids (SFA), while vegetable fats such as soybean oil, sunflower oil and linseed oil are typically rich in unsaturated fatty acids (UFA), which include monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). This difference is important since unsaturated fats are generally better digested, especially in younger broilers, while the saturated fats are likely to be more efficiently incorporated into the micelles and thus to have higher apparent digestibility. However, UFA-rich oils are more readily absorbed and oxidized for energy, whereas highly unsaturated oils could be more susceptible to lipid oxidation (Fébel et al., 2008; Fouad & El-Senousey, 2014).,

Fébel et al. (2008) compared lard with vegetable oils (sunflower, soybean, linseed) and evaluated the effects of these oils on tissue fatty-acid composition and antioxidant status. Growth performance did not differ among treatments, but diets richer in PUFA, such as those containing linseed oil, increased the incorporation of omega-3 fatty acids into muscle. The authors also found

higher plasma radical-scavenging capacity for the birds fed the PUFA rich diets, suggesting differences in antioxidant properties of fat sources.

Waldroup et al. (1995) reported that blended animal–vegetable fats with acceptable oxidative quality and moderate free fatty acid levels can be used without compromising growth performance or feed conversion ratio (FCR). Oil rancidity is commonly assessed using indicators of lipid oxidation such as peroxide value (PV), which reflects primary oxidation products (hydroperoxides). Yaseen et al. (2021) showed that higher PV in thermally oxidised sunflower oil reduced feed intake, increased FCR (poorer feed efficiency), impaired gut morphology, and increased serum cholesterol, whereas lower PV oil (e.g., ~20 mEq/kg) had smaller negative effects. Overall, these findings highlight that dietary fats/oils should be fresh and of good oxidative quality to support broiler health and efficient nutrient utilisation.

b. Saturated vs. Unsaturated fatty acids

The level of unsaturation of fatty acids has a major influence on lipid metabolism, carcass structure and oxidative stability. Unsaturated fatty acids, particularly the polyunsaturated fatty acids (PUFAs), increase the fatty acid oxidation rate and decrease the deposition of abdominal fat, as well as augment the vulnerability of tissues to lipid peroxidation. Hence, to prevent oxidative instability and maintain meat quality, high-PUFA diets should be fortified with antioxidants (e.g., vitamin E, selenium) (Fébel et al., 2008; Long et al., 2020). Long et al. (2020) also noted that supplementation with PUFA oils improved the quality of meat, muscle fatty acid composition, and antioxidant capacity, and increased nutritional value in consumers. Research by Selim et al. (2021) has determined that development of rice bran oil supplementation led to better growth performance, antioxidant status, and liver morphology, and reduced liver fat status, which suggests that certain vegetable oils have the potential to support metabolism, as well as prevent or reduce oxidative effects.

2.4. Environmental Temperature and Broiler Performance

As broilers age, the ambient temperature is recommended to be lowered due to the different metabolic heat output and thermoregulatory ability they have. Therefore, thermal comfort should be referred to the age of the birds instead of represented by a single range throughout the production cycle.

Table 2.1 Typical recommended ambient temperatures for broilers at different ages

Age/Period	Recommended temperature
Day 1	32–34°C
Week 2	29–31°C
Week 3	26–28°C
Week 4	23–25°C
Weeks 5–6 (Day 35–42)	21–23°C

2.4.1 Temperature ranges and comfort zones

The thermoneutral zone (TNZ) of broiler chickens is age-dependent and varies depending on the age of the birds; there is no single thermoneutral zone for all production stages. (Apalowo et al., 2024). The lower critical temperature (LCT) occurs below 18 °C, where birds must increase metabolic heat production, while the upper critical temperature (UCT) exceeds 30 °C, beyond which evaporative cooling (panting) becomes energetically costly.

The temperature should be maintained through proper ventilation, insulation, humidity, and stocking density. Humidity should be above 70 %, which prevents heat loss through evaporation and worsens heat stress (Apalowo et al., 2024). Adequate airflow and tunnel ventilation can minimise ambient heat load, while light-coloured roofing and reflective insulation can minimise solar heat absorption.

Thermal comfort in open-sided poultry houses typical of the tropics requires management interventions, including mist-spraying, evaporative cooling pads, and reduced stocking density (less than 30 kg/m²) (Bilal et al., 2021). On the other hand, cold weather can cause respiratory stress due to over-ventilation hence, dynamic adjustment is necessary to maintain oxygen and temperature balance.

New technologies such as infrared thermography are currently being applied to check the thermal comfort of broilers. Hernandez-Sanchez et al. (2024) conducted a systematic review that validated

temperature measurements in the comb, eye, and shank areas as reliable non-invasive thermal stress indicators to intervene early enough before performance falls.

2.4.2 Thermal stress in poultry

Thermal stress is one of the most important environmental factors adversely affecting broiler production worldwide, and this includes both heat stress and cold stress. Birds are homeothermic, maintaining a stable core temperature through physiological and behavioural regulation. Modern broilers, however, have a rapid metabolic rate and a dense plumage with limited sweating capacity, making them extremely prone to heat accumulation. Heat stress (HS) occurs when the ambient temperature surpasses the bird's ability to dissipate heat, causing hyperventilation, decreased feed consumption, and decreased productivity (Bilal et al., 2021).

Heat stress

Heat stress is defined as the environmental temperature exceeding upper critical limits of the thermoneutral zone (TNZ) which is 25–30 °C for broilers (Apalowo et al., 2024). Physiological responses include panting, increased levels of corticosterone, respiratory alkalosis, and oxidative stress all of which negatively affect nutrient metabolism and immune competence (Apalowo et al., 2024).

During heat stress, feed consumption may drop by up to 60 %, resulting in depressed weight gain and FCR (Bilal et al., 2021). Oxidative stress leads to lipid peroxidation, while chronic hyperthermia suppresses IgM (immunoglobulin M) and IgG (immunoglobulin G) antibody titers, compromising humoral immunity (Pawar 2016, cited in Bilal et al.). Mirzaie et al. (2018) demonstrated that supplementing heat-exposed broilers with *Spirulina platensis* improved antioxidant enzyme activity (SOD, GPx) and reduced serum corticosterone, suggesting nutritional modulation can mitigate heat-induced oxidative damage.

Cold stress

Although less common in tropical climates, cold stress can also cause severe metabolic strain. It triggers sympathetic activation, elevating oxygen consumption and cardiac output, often leading to ascites and pulmonary hypertension (Bilal et al., 2021). As stated by Bilal et al. (2021), cold exposure increases red-blood-cell counts and hematocrit, but depresses feed efficiency and induces

oxidative stress. Hypothermia in chicks reduces thermogenic efficiency and may increase mortality through immunosuppression and respiratory stress.

2.4.3 Effects of elevated temperatures

a. Feed intake and growth

One of the first behavioural responses to heat stress is reduced feed intake, which lowers nutrient and energy consumption. Kpomasse et al. (2021) reported that broilers exposed to sustained tropical temperatures (≥32 °C) showed 36% lower weight gain and 21% poorer feed efficiency, confirming the direct impact of high ambient temperatures on metabolic balance. Reduced feed intake is compounded by increased maintenance energy requirements for panting and thermoregulation.

b. Hormonal and metabolic responses

At the endocrine level, heat stress activates the hypothalamic–pituitary–adrenal (HPA) axis, releasing corticosterone, which suppresses thyroid hormones such as triiodothyronine (T3) and thyroxine (T4), and reduces protein synthesis and growth hormone secretion (Bilal et al., 2021). In addition, chronic HS causes a disruption in insulin sensitivity and glucose utilisation and also results in fat deposition and changed carbohydrate metabolism (Kpomasse et al., 2021).

Katafuchi et al. (2024) reported that repeated exposure to the high ambient temperature had led to a reduction in the biochemical quality of muscle protein, as measured by the markedly reduced levels of muscle imidazole dipeptides, which are important molecules in the pH buffering and antioxidative defense of muscle tissue. This molecular evidence confirms the correlation between oxidative stress, meat quality deterioration, and growth suppression.

c. Immune and antioxidant responses

Thermal stress compromises both innate and adaptive immunity. Mirzaie et al. (2018) reported that the heat-stressed broilers had higher levels of corticosterone and lipid peroxidation (MDA) and lower levels of SOD and GPx activities. However, supplementing Spirulina (1%) improved antioxidant capacity and increased antibody titers to sheep red-blood-cell antigens.

Similarly, Kim et al. (2025) emphasised that functional nutrients like selenium, vitamin E, betaine, and phytogenic extracts may reduce stress indicators, including the heterophil-to-lymphocyte

(H/L) ratio, corticosterone concentration, and increase cellular immunity and antioxidant gene expression in HS-stressed poultry. These findings suggest a metabolic adaptation approach to nutritional interventions for supporting thermotolerance.

d. Gut morphology and nutrient absorption

Heat stress damages intestinal morphology and reduces digestibility. Kedsirin Sakwiwatkul et al. (2025) found that feeding yam bean pulp (4–8 %) improved villus height and crypt ratio in heat-exposed broilers, enhancing nutrient absorption. However, these changes did not fully restore growth, showing that digestive improvements alone cannot offset the systemic metabolic costs of heat stress.

e. Economic and welfare implications

According to Apalowo et al. (2024), HS results in an estimated global cost of more than USD 2 billion per year, primarily due to lost feed efficiency, loss of life, and meat quality loss. The high temperature coupled with poor ventilation also poses a risk for the disease and can lower welfare. Kpomasse et al. (2021) also emphasised that chronic heat exposure increases oxidative stress and decreases growth and immunity, making broilers more vulnerable to infections and metabolic disorders in tropical areas.

2.4.4 Management and mitigation strategies

Poultry managers use a mixture of environmental management and nutritional management to relieve the impact of heat stress. On the environmental front, the ventilation rates, the reduction of the stocking density, and evaporative cooling systems are suggested as the means of ensuring the best TNZ conditions (Bilal et al., 2021). Behaviourally, access to cool water, and changing the feeding schedule, which is to provide feed in cool conditions, can be used to reduce the load of heat.

Metabolically, antioxidant supplementation, incorporation of functional nutrients, and fat-enriched diets with reduced HI lower the production of heat generation and oxidative stress. The approach of continuous thermal monitoring through the integration of infrared thermography, as proposed by Hernandez-Sanchez et al. (2024), provides precision-based early detection and adaptive cooling control.

Environmental temperature has significant impacts on the physiology, metabolism, and welfare of broilers. Stress from heat causes decreased feed consumption, poor growth, hormonal disturbances, oxidative stress, and immunosuppression, while cold stress increases metabolic oxygen consumption. It is critical to keep broilers in a thermoneutral environment (21-28 °C) using better housing, ventilation and adjusting food to maximize productivity. Recent research observes that environmental control combined with nutritional fortification such as antioxidants, functional nutrients and energy-efficient diets are effective in improving thermotolerance and maintaining performance during climatic stress.

2.5. Interaction Between Diet and Temperature in Broiler Performance

2.5.1 Synergistic effects: Diet as a buffer against thermal stress

The high metabolic rate and low heat dissipation capacity of broilers make them very susceptible to heat stress as a result of environmental factors. In the high temperatures, birds decrease their food consumption leading to inadequate nutrient and energy uptake, which affects growth and immunity. In this case, dietary fat becomes one of the vital nutrition tactics. Dietary fat decreases the thermogenic load of heat-stressed broilers due to its lower heat increase when compared with proteins and carbohydrates (Choi et al., 2023).

Diets rich in fat, especially with added contents of vegetable oils or insect lipid-rich meals, have been promising in maintaining energy supply under thermal stress. Mangan and Siwek (2023) emphasised that fats contain more calories per gram and generate less heat in the body of broilers subjected to chronic heat exposure, which makes them the best option. However, dietary fat level and dietary energy concentration are two different attributes; fat inclusion may not always imply increased metabolisable energy concentration of the diet if other ingredients are changed during the diet formulation. For instance, the low fat and high fat diets of the present study were both designed to a common AME target with differing fat inclusions.

Also, unsaturated fats help increase the fluidity of cell membranes, which enhance physiological durability to oxidative and thermal injuries (Choi et al., 2023). Such an effect on nutritional buffering is essential in hot climates, where birds must endure periodic or prolonged temperature spikes that disrupt protein and lipid metabolism.

2.5.2 The thermogenic effect of feed components

Macronutrients differ in heat increment (thermogenic effect) during digestion and metabolism: protein is highest, followed by carbohydrate, while fat is lowest. A lower HI will result in a higher proportion of dietary metabolisable energy (ME) being saved as net energy (NE) for maintenance and growth. This efficiency is sometimes represented as K = NE/ME, where a higher value of K represents more efficient utilisation of energy with lower heat production. Therefore, partially substituting dietary energy from protein/carbohydrate with fat can reduce metabolic heat load and may be advantageous under warm conditions and heat stress (Onagbesan et al., 2023).

Choi et al. (2023) also indicate that broilers under heat stress fed high-energy, low-protein diets showed positive growth performance, reduced mortality, and improved meat quality as compared to broilers fed on conventional diets. Such macronutrient composition change is not only able to maintain muscle accretion but also lowers systemic oxidative stress and metabolic heat generation.

Besides, vitamins C and E, selenium, betaine, and polyphenols are examples of beneficial nutrients with thermoprotective effects in conjunction with fat supplementation. These compounds have antioxidant and osmoprotectant activities, and increase the stability and water balance of the thermally stressed tissue (Mangan and Siwek, 2023).

2.5.3 Evidence from factorial design studies

Several studies have used Diet × Temperature experimental designs to determine the effect of specific nutrient changes on performance under thermal stress.

Onagbesan et al. (2023) reviewed nutrition and nutrition management for heat stress mitigation in poultry and concluded that dietary supplementation with fats and oils can be beneficial for the performance as they contain high amount of energy and relatively low heat increment. The review reported that studies where dietary metabolisable energy was raised to around 3,300 kcal/kg with up to 5% fat inclusion resulted in better performance and nutrient utilisation in heat stressed broilers. It also found positive effects from up to 8% of the vegetable-oil supplementation. The findings are obtained under different dietary formulations and experimental conditions, however, and should not be considered as evidence for a single optimal level of dietary fat inclusion.

Likewise, Choi et al. (2023) pointed out tests showing that feeding diets with higher energy concentration (3,200–3,400 kcal/kg) could mitigate the growth depressant effects of heat stress,

despite a reduced feed intake. These energy levels were elevated by diets formulated with higher metabolisable energy concentrations (approximately 3,200–3,400 kcal/kg) than the control diets. These diets were often paired with bioactive compounds like polyphenols and exogenous enzymes to optimize nutrient uptake and mitigate oxidative damage.

An innovative approach from Seyedalmoosavi et al. (2022) examined black soldier fly (BSF) larvae meal as a lipid-rich, sustainable fat substitute in broiler diets. Black soldier fly larvae are rich in medium-chain fatty acids, which not only reduce dietary thermogenesis but also exhibit antimicrobial and anti-inflammatory properties beneficial under heat stress. Although direct trials under thermal conditions were limited, the review suggests that BSF may enhance thermotolerance and nutrient utilisation efficiency in broilers compared to conventional animal protein or soy-based diets.

2.5.4 Considerations for welfare and husbandry

Diet and temperature interaction also have profound implications on broiler welfare. Wilcox et al. (2023) note that nutritional manipulation (like heat-reducing diets) are to be incorporated into the behavioural and housing interventions to help enhance the comfort of birds and reduce stress-induced behaviours, including wing spreading, panting, and aggression. Moreover, Mangan and Siwek (2023) also accentuate that dietary changes should be related to stocking density, lighting schedules, and feeding times.

Lastly, more precise feeding and environmental sensors can be used to adjust the diet plans dynamically in accordance with the thermal index of the environment and the reactions of birds. These combined strategies are the future of poultry nutrition that can withstand climate changes.

Interaction between the dietary composition and the environmental temperature plays the crucial role that helps optimize the performance of broilers during heat stress. Fats and insect-derived lipids in particular provide an energy source which is thermally efficient and has the ability to supplement any low feed intake in the period of thermal challenges. Substitution of protein with fat in the right proportions decreases metabolic heat generation and enhances the ability of organisms to withstand oxidative stress. Combined with functional additives and housing adaptations, dietary modulation provides a practical and effective tool for enhancing broiler growth, welfare, and meat quality in hot climates.

2.6. Objectives and Hypotheses

Objective: To determine growth performance, apparent metabolisable energy (AME), ileal nutrient digestibility, and survival of Ross 308 broiler chickens in response to two dietary treatments (low-fat and high-fat) and two environmental temperatures (normal and elevated) regimes from day 10 to day 34, and to investigate Diet × Temperature interactions.

Research questions:

1. Does elevated temperature change body weight gain, feed intake, and FCR compared with normal temperature?
2. Does increasing dietary fat level improve feed efficiency and/or increase fat deposition risk under these conditions?
3. Is there a Diet × Temperature interaction, i.e., does dietary fat level alter the performance response to elevated temperature?

Hypotheses:

H1: Broilers exposed to the elevated-temperature regimen will have lower average daily gain (ADG) than broilers exposed to the normal-temperature regimen.

H2: High-fat diets will improve feed efficiency (lower FCR) compared with low-fat diets.
H3: The effect of dietary fat on performance will differ between normal and elevated temperature (interaction).

3. MATERIAL AND METHODS

3.1 Introduction

This study examined the effects of dietary fat level and environmental temperature on the growth performance of Ross 308 broiler chickens. The study aimed to determine whether feeding a high-fat or a low-fat diet affects growth response under either a normal or an elevated temperature regimen and whether there is an interaction between these two factors. Accordingly, the experiment was structured to determine the effects of dietary fat level and environmental temperature on growth performance indicators, particularly body weight gain, feed intake, and feed conversion ratio, in Ross 308 broiler chickens, as well as any interaction between these factors.

The overall trial took place between day 1 & 34 of age. Birds were brooded and temperature-conditioned on days 1 to 9 then the factorial treatments were applied from day 10 to day 34. All experimental procedures described in this thesis were approved by the Massey University Animal Ethics Committee (MUAEC 08/11) and complied with the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching.

3.2 Study Location and Experimental Facilities

The experiment was conducted in an environmentally controlled poultry research facility at Massey University, Palmerston North, New Zealand, using four temperature-controlled rooms to implement the environmental treatments. Two rooms were assigned to the normal temperature regimen and two rooms were assigned to the elevated temperature regimen. During the factorial period (day 10–34), the normal treatment was programmed to decline from 25°C at day 10 to 21°C at day 34, whereas the elevated treatment declined from 30°C at day 10 to 23°C at day 34, with an additional cyclic daytime increase of approximately 1°C between 09:00 and 16:00 on weekdays. Birds were kept in cages in each room and fed and watered ad libitum during the experimental period. Ventilation and air flow were controlled by fan-assisted room control for stable environmental conditions and for bird comfort.

Standard biosecurity practices were followed throughout the study to reduce disease risk and variation among replicates. These involved limiting access to the rooms, regular cleaning and disinfecting the experimental space and equipment, and hygiene measures at entry points. Husbandry procedures were uniform throughout all rooms so that the effect of the experimental

factors (temperature and diet) would be the major factor in any differences in performance. Table 3.1 provides an overview of the experimental rooms, system and key equipment utilized in the feeding, watering, ventilation and temperature control.

Table 3.1 Overview of facilities and equipment used in the experiment

Component	**Description / Use in the study**
Experimental rooms	4 environmentally controlled rooms (2 normal temperature; 2 elevated temperature)
Housing	Cages used as replicate units; cages distributed within each room
Feed delivery	Feeders/troughs suitable for cage feeding; feed offered ad libitum
Water system	Nipple drinkers; water provided ad libitum
Temperature control	Room-level temperature programming and monitoring (thermometers/data logging as per facility practice)
Ventilation	Fan-assisted airflow and ventilation control to support stable thermal conditions
Measurement equipment	Weighing scale for bird body weight; weighing equipment for feed allocation/remaining feed

3.3 Experimental Birds, Pre-trial Management, and Treatment Allocation

A total of 288 one-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery and inspected on arrival for health status. During the brooding and pre-trial conditioning phase (Day 1–9), birds were housed in floor pens, fed a commercial starter diet, and provided feed and water ad libitum. To prepare for the later environmental treatments, chicks were divided into two thermal-conditioning groups: the elevated-temperature group was reduced from 30°C to 28°C, while the normal-temperature group declined from 30°C to 26°C by Day 9. Birds were monitored daily for general condition, feed and water access, and mortality as shown in Table 3.2.

Table 3.2 Timeline of major management procedures and measurements (Day 1–34)

Day(s)	Phase / Activity	Key actions and records
1–9	Brooding + early temperature conditioning	Floor pens; commercial diet; water ad libitum; temperature conditioning (normal vs elevated)
10	Start of factorial trial	Birds weighed; allocated to treatments; placed into 2 normal and 2 elevated temperature rooms
10–34	Factorial experimental period	Temperature and diet treatments applied; daily husbandry; mortality recorded
14, 21, 28, 34	Scheduled measurements	Body weight and feed intake recorded at each time point
34	End of trial	Final body weight and feed intake recorded; trial terminated

On Day 10, 144 birds were selected, weighed, and allocated to the experimental trial. The study followed a 2 × 2 factorial design with two temperature regimens (normal and elevated) and two diet fat levels (low fat and high fat) as shown in Table 3.3. Birds were assigned to two normal-temperature rooms and two elevated-temperature rooms, with cages within each room randomly allocated to one of the two diets. Each treatment combination had 12 replicate cages, with three birds per cage, giving 48 cages in total. The cage was treated as the experimental unit because diet was applied and feed intake measured at cage level, while body weight was recorded individually and averaged per cage for analysis. Mortality was recorded daily and later accounted for using survival-adjusted measures such as chicken-days.

Table 3.3 Treatment structure and replication (2 × 2 factorial design)

Temperature regimen	Diet fat level	Replicates (cages)	Birds per replicate
Normal	Low fat	12	3
Normal	High fat	12	3
Elevated	Low fat	12	3
Elevated	High fat	12	3

Table 3.4 Husbandry checklist and frequency during the factorial period (Day 10–34)

Activity	Frequency	Notes
Check bird health/behaviour	Daily	Observe panting, lethargy, lameness, and general activity
Check water system (nipple drinkers)	Daily	Ensure availability and correct flow
Check feeders / refill feed	Daily (as needed)	Feed offered ad libitum
Remove and record mortalities	Daily	Mortality recorded daily
Basic cleaning/sanitation	Routine	Maintain consistent hygiene across rooms

3.4 Environmental Temperature Management

3.4.1 Target temperature programme

During the brooding/conditioning phase (Day 1–9), chicks in both groups were initially maintained at approximately 30°C on Day 1. Temperature was then reduced gradually, reaching 26°C in the normal-temperature group and 28°C in the elevated-temperature group by Day 9.

During the experimental period (Day 10–34), the normal-temperature regimen was programmed to decline from 25°C on Day 10 to 21°C on Day 34. The elevated-temperature regimen was programmed to decline from 30°C on Day 10 to 23°C on Day 34. In addition, elevated-temperature

rooms were subjected to a cyclic daytime increase of approximately 1°C between 09:00 and 16:00 on weekdays to simulate daytime heat load. On weekends, elevated rooms were maintained at 25°C.

Table 3.5 Target temperature schedule for normal and elevated treatments (Day 1–34)

Phase	**Day range**	**Normal temperature (target)**	**Elevated temperature (target)**	**Notes on how the programmewas applied**
Brooding / conditioning	1–9	~30°C → 26°C	~30°C → 28°C	Both started ~30°C Day 1; reduced to different end-points by Day 9
Factorial period (maximum temperature targets)	10–34	Max ~25°C (Day 10) → 21°C (Day 34)	Max ~30°C (Day 10) → 23°C (Day 34)	Elevated regimen included weekday cyclic heating (09:00–16:00) and weekends held at 25°C
Cyclic challenge window (Elevated only)	10–34	—	+~1°C increase during 09:00–16:00 (weekdays)	Used to create a daytime heat-load pattern

3.4.2 Actual temperature recording and monitoring

Throughout the study, birds had free access to feed and water (ad libitum). To verify that the two temperature regimens were maintained and remained meaningfully separated, environmental temperature was recorded routinely throughout the trial using a structured temperature log. Temperature readings were taken repeatedly across the day (including the intended heat-challenge window) and across the night period. This approach served two functions: (1) to document the achieved thermal conditions experienced by birds, and (2) to confirm that the elevated regimen produced a higher daily thermal profile than the normal regimen, particularly during the daytime

challenge period. A summary of the recorded temperatures by treatment and period is presented in Table 3.6.

Temperature monitoring was conducted at a position intended to reflect the birds' immediate environment (i.e., at approximately bird height within each room), rather than at ceiling level, because microclimate at bird level is the most relevant for performance and welfare outcomes. For the normal temperature regimen, temperature adjustments followed the breeding company recommendations (Ross Broiler Manual; Aviagen, 2009).

Table 3.6 Summary of recorded temperatures by treatment and period

Temperature treatment	Days	Mean ± SD (°C)	Min (°C)	Max (°C)
Elevated	1–9	28.67 ± 1.00	28.0	30.0
Elevated	10–34	25.62 ± 1.18	23.0	30.0
Normal	1–9	27.89 ± 1.36	26.0	30.0
Normal	10–34	22.60 ± 1.78	21.0	26.0

3.5 Diet Composition

3.5.1 Diets, feeding phase, and physical form

Two pelleted experimental diets were used to assess the effect of dietary fat level under the two environmental temperature regimens. The diets were formulated as low-fat and high-fat rations and were fed from Day 10 to Day 34, with cages within each room randomly assigned to one of the two diets. Feed and water were provided ad libitum throughout the experimental period, with water supplied via nipple drinkers to maintain consistent hydration. Because the diets differed in fat inclusion, feed was stored carefully in closed containers away from heat and moisture to minimize rancidity and oxidation, which could affect palatability and nutrient utilisation. Both diets were formulated to maintain comparable nutrient targets, with AME held constant at 3150 kcal/kg and crude protein at 21%. Crude fat, however, differed between the diets, being 4.3% in the low-fat diet and 8.08% in the high-fat diet.

3.5.2 Ingredient composition of experimental diets

The two diets were formulated using maize and soybean meal as major ingredients, with fat level primarily manipulated by altering soybean oil inclusion. The high-fat diet increased soybean oil from 1.62% (low fat) to 6.01% (high fat), while maintaining total formulation at 100%. Minor adjustments in ingredients (e.g., maize starch, wheat bran, mineral sources, and amino acid supplements) were made to balance the diet structure while meeting intended nutrient specifications. Ingredient inclusion rates for the two experimental diets are shown in Table 3.7.

Table 3.7 Ingredients (%) of the low-fat and high-fat experimental diets (pelleted)

Ingredient	**Low fat (%)**	**High fat (%)**
Maize	47.55	51.76
Maize starch	15	0
Soybean meal 48%	24.1	32.18
Wheat bran	0	5.9
Maize gluten meal	2.79	0
Meat and bone meal	6.92	0
Soybean oil	1.62	6.01
Dicalcium Phosphate	0	1.78
Limestone	0.26	0.66
DL-methionine	0.34	0.33
Lysine	0.37	0.22
L-threonine	0.14	0.1
Salt	0.18	0.24
Sodium bicarbonate	0.2	0.29
Vitamin premix	0.08	0.08
Mineral premix	0.15	0.15
Titanium dioxide	0.3	0.3
Total	100	100

3.5.3 Calculated nutrient composition

Although both diets were formulated to the same target AME (3150 kcal/kg) and total protein concentration (210 g/kg), they were not controlled solely for dietary fat. The high fat formulation

also had a higher calculated crude fibre content (31 g/kg versus 22.5 g/kg) and lower starch content (338 g/kg versus 442 g/kg), and a significantly different ingredient makeup. Therefore, any dietary treatment effects observed should be interpreted as a result of the total composition of the dietary treatments rather than solely as a result of the difference in dietary fat. The calculated nutrient composition of the two diets is presented in Table 3.8.

Table 3.8 Calculated nutrient composition of the low-fat and high-fat diets

Nutrient (calculated)	**Low fat**	**High fat**
AME (kcal/kg)	3150	3150
Starch (g/kg)	442	338
Total protein (g/kg)	210	210
Digestible protein (g/kg)	166	171.7
Digestible methionine (g/kg)	5.9	5.9
Digestible methionine + cysteine (g/kg)	8.4	8.4
Digestible lysine (g/kg)	11	11
Digestible threonine (g/kg)	7.3	7.3
Digestible arginine (g/kg)	11.4	12.5
Crude fat (g/kg)	43	80.8
Crude fibre (g/kg)	22.5	31
Calcium (g/kg)	9	9
Available phosphorus (g/kg)	4.5	4.5
Na (g/kg)	1.9	1.9
Chloride (g/kg)	1.9	1.9

3.6 Growth Data Recording and Measurements

3.6.1 Body weight measurement schedule

Body weight was measured repeatedly to quantify growth responses to the factorial treatments of environmental temperature (normal vs elevated) and dietary fat level (low vs high). Body weight

was measured at the beginning of the experimental period (day 10) to provide initial body weight and for random assignment of the birds to the four treatment combinations. BW was subsequently recorded at regular intervals on day 14, 21, 28, and 34 to create growth curves and to determine weight gain for specified periods.

All weight measurements were made in the same way to minimize the stress of handling and variability in measurements. Handling of the birds was calm and the weighing was done within a short time window on each weighing day. Birds were not fasted prior to weighing as this can affect gut fill and may add stressor variation. For each cage, individual bird weights were recorded and averaged to obtain mean body weight per cage, because the cage was treated as the experimental unit for performance outcomes.

Table 3.9 Measurement schedule and variables recorded

Day of age	Study phase	Main measurements recorded
1–9	Brooding/conditioning	Daily husbandry checks; temperature programme applied by group
10	Start of factorial period	Baseline BW; allocation to treatments; start of experimental diets
10–34	Factorial period	Mortality recorded daily ; feed offered/remaining tracked by replicate; environmental temperature monitored
14, 21, 28, 34	Repeated performance measures	BW and feed intake recorded at each interval

3.6.2 Feed intake measurement and correction (Evaporation/Weight loss)

Feed intake (FI) was recorded at the replicate level (cage) because birds within a cage shared the same feeder and diet assignment. Feed was provided ad libitum, and FI over each period was determined using a feed disappearance approach:

FI (uncorrected) = Feed offered − Feed remaining (weighed back).

Because pelleted feed could undergo weight loss over time due to drying or moisture evaporation, particularly under warm conditions, a correction factor was applied to reduce bias in feed intake estimates. To account for this, a representative feed sample was weighed (“weight before

evaporation"), then reweighed after set time points (e.g., day 4 and day 11), and the average weight loss was calculated. This average loss was then added to the measured feed remaining (weighed back) to estimate the "true" remaining feed mass at the time of collection:

Corrected feed remaining = Measured feed remaining + Average weight loss
Corrected FI = Feed offered − Corrected feed remaining

Corrected feed intake was calculated for each cage as feed offered minus corrected feed remaining. Total feed intake per cage and average daily feed intake per cage were then determined for each time period and across the overall experimental phase. Where mortality occurred, intake was adjusted using chicken-days so that feed intake reflected the actual number of birds present during the measurement period.

Table 3.10 Feed intake variables recorded and correction steps (replicate/cage level)

Variable (as recorded)	**What it represents**	**Used for**
Total Fed (g)	Total feed offered to the cage during the period	FI calculation
Total WB (g)	Feed remaining ("weighed back") at end of period	FI calculation
Weight before evaporation	Reference sample weight at start	Evaporation estimate
Weight after evaporation (day 4; day 11)	Sample weight after storage time	Evaporation estimate
Avg weight loss (g)	Mean loss from the reference sample	Correction factor
Total WB + Evaporation	Corrected remaining feed	Corrected FI
FI (g)	Corrected feed intake (g)	Performance indices
FI/Chicken (g)	FI standardised by chicken-days	Fair comparison across mortality

Table 3.11 Example of feed intake correction (one cage; worked example)

Step	Value (g)	Calculation
Feed offered	9000	Total Fed
Feed remaining (weighed back)	5520	Total WB
Average evaporation loss	20	Avg weight loss
Corrected remaining feed	5540	5520 + 20
Corrected FI	**3460**	9000 − 5540

3.7 Digestibility:

3.7.1 Apparent metabolizable energy (AME)

For AME measurements, excreta were collected from each cage between days 31 and 34. Collected excreta were weighed, mixed and subsampled and each sub sample freeze dried and ground to pass through a 0.5 mm sieve. Excreta samples were analysed for dry matter, ash, titanium dioxide and gross energy.

3.7.2 Ileal collection and analysis:

Birds were euthanized by intravenous injection of pentobarbitone (1 ml/2kg body weight) and Ileal digesta was collected. The ileum which is defined as the portion of the intestines extending from the Meckel's diverticulum to a point ~40mm proximal to the ileocecal junction, was first divided into two halves and digesta was then flushed from the lower half of the ileum using reverse osmosis water (RO water). The ileal digesta collected from birds of the same cage were pooled together. The digesta were then freeze-dried and ground to pass through a 0.5 mm sieve. Two samples from each cage in the same Diet × Temperature treatment group were then pooled together, to create six ileal composite samples per treatment group. The available experimental records did not specify the rationale for pairing two cages or the criterion used to select individual cage pairs. After pooling, each two-cage composite sample was used as one analytical unit for ileal digestibility analysis (n = 6 for each Diet × Temperature treatment combination). The mixed ileal samples

were sent to Massey University Nutrition Laboratory for the analysis of dry matter, ash, nitrogen, fat, starch and titanium dioxide.

3.8 Mortality / Survival:

Survival rate was evaluated to determine whether dietary fat level and environmental temperature influenced bird survival during the experimental period. The study followed a 2 × 2 factorial design with two dietary treatments, low fat (LF) and high fat (HF), and two temperature regimens, normal (N) and elevated (E), giving four treatment combinations: LF–N, LF–E, HF–N, and HF–E. Birds were allocated to treatment groups as described for the main growth performance trial.

Mortality was monitored daily throughout the experiment. Dead birds were noted by treatment group and promptly removed from the cages. Mortality percentage was calculated as the number of birds that died during the experimental period divided by the number of birds at risk at the beginning of the relevant period, multiplied by 100. Survival percentage was calculated as the number of birds alive at the end of the experimental period divided by the number of birds present at the beginning of the experimental period, multiplied by 100."

3.9 Chemical Analysis

Chemical analyses were conducted to determine the nutrient composition of the experimental diets. The determination of dry matter (DM) was carried out using standard AOAC procedures (methods 930.15 and 925.10), and gross energy (GE) was measured using bomb calorimetry. The fat content of diet and ileal samples was analysed using the Soxtec extraction method (AOAC method 991.36), while nitrogen content was analysed by total combustion method using LECO analyser (AOAC method 991.36) and crude protein was calculated accordingly. The starch content of diet and ileal samples was measured using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase, following the approach described by McCleary et al. (1997). For diet, excreta and ileal samples, ash content was determined using AOAC method 942.05, with samples ashed at 550°C.

Fibre fractions were determined for diet samples, including neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin, using the method described by Robertson and Van Soest (1981) and the Tecator Fibreter System (AOAC method 2002.04). In addition, titanium dioxide (TiO_2) was analysed in diet and ileal samples using sulphuric acid digestion followed by colourimetric determination.

Calculations:

AME coefficients were calculated using the following formula*:*

$$\text{AME \%} = \frac{(\text{GE in Diet}/\text{Titanium in diet}) - (\text{GE in excreta}/\text{Titanium in excreata})}{(\text{Ge in Diet}/\text{Titanium in Diet})}$$

AME content in Diet (MJ/Kg DM) = AME % x GE in diet (MJ/Kg DM)

Ileal apparent nutrient digestibility coefficients were calculated using the following formula*:*

$$\text{CIAD of the nutrient} = \frac{(\text{Nutrient in Diet}/\text{Titanium in diet}) - (\text{Nutrient in digesta}/\text{Titanium in digesta})}{(\text{Nutrient in Diet}/\text{Titanium in Diet})}$$

Ileal digestible nutrient content in Diet (g/Kg DM) = Nutrient CIAD x Nutrient in diet (g/Kg DM)

Nutrient intake (g/day) = Feed intake (kg DM/day) x Ileal digestible nutrient content in Diet (g/Kg DM)

3.10 Statistical Analysis

All data were analysed using SAS software (version 9.4). For growth performance, AME, and ileal digestibility variables, the cage was treated as the experimental unit (three birds per cage). Data were analysed using the General Linear Models (GLM) procedure for a 2 × 2 factorial arrangements, with dietary fat level, environmental temperature, and their interaction included in the model. Least square means (LSMeans) were obtained, and differences were considered significant at $P < 0.05$.

SAS software was used for the survival data analysis. The statistical model comprised of the main effects of diet, temperature and their interaction (Diet × Temperature). Because survival is proportional data, results were analysed on the logit scale, and back-transformed survival percentages were presented for interpretation. Differences were considered statistically significant at $P < 0.05$.

Growth performance parameters were body weight, ADG, daily feed intake and feed conversion ratio. Apparent metabolisable energy variables were AME coefficient (%) and AME content (MJ/kg DM). Ileal digestibility variables consisted of the digestibility coefficient of protein, fat and starch, and digestible contents of protein, fat and starch on dry matter basis.

Mortality data was taken throughout the experimental period and summarised according to the different treatments as mortality and survival percentages. Survival data were analysed as a binomial variable (alive or dead) after logit transformation using the PROC GENMOD in SAS.

4. RESULTS

4.1 Overview of the Chapter

This study presents the results of the experiment investigating the effects of dietary fat level (Low Fat vs High Fat) and environmental temperature regimen (Normal vs Elevated), and their interaction, on broiler growth performance across the experimental period. Results are shown for each week (Week 1, Week 2, Week 3, Week 4) and for all weeks (Week 1 – 4). The performance outcome is expressed in terms of body weight at the beginning of the week (BWS), body weight at the end of the week (BWE), ADG, daily feed intake (DFI), and feed conversion ratio (FCR).

For each period, tables report least-squares means for Diet, Temperature, and the four treatment combinations (LF–NT, LF–ET, HF–NT, HF–ET), along with standard errors (SE) and the P-values for Diet, Temperature, and the Diet × Temperature interaction.

4.2 Diet Composition and Chemical Profile (Context for Performance Results)

Before presenting growth performance outcomes, the nutrient profile of the experimental diets is summarised in Table 4.1. Both diets were formulated to provide the same metabolisable energy level, while differing primarily in fat inclusion. This formulation difference was reflected in the analysed composition, with the high-fat diet showing a higher fat concentration and a lower starch concentration than the low-fat diet. Titanium dioxide (TiO_2) concentration was similar between diets, indicating consistent marker inclusion.

Table 4.1 Analysed chemical composition of experimental diets (Low fat vs High fat)

Parameter	**Low fat**	**High fat**
DM (g/kg)	894.0	897.0
Ash (g/kg DM)	54.0	63.0
Crude Protein (g/kg DM)	228.0	220.0
Fat (g/kg DM)	46.0	102.0
Starch (g/kg DM)	392.0	308.0
NDF (g/kg DM)	112.0	130.0
ADF (g/kg DM)	27.0	37.0
Lignin (g/kg DM)	9.0	9.0
TiO_2 (g/kg DM)	3.0	3.0
GE (MJ/kg DM)	18.7	19.6

DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre;
TiO_2 = titanium dioxide; GE = gross energy

4.3 Week 1 Growth Performance Results

Week 1 growth performance responses to dietary fat level and environmental temperature are summarised in Table 4.2. At the start of Week 1, body weight at the start of the week (BWS) was similar between dietary treatments, with birds receiving the low-fat diet (353.71 g) and high-fat diet (354.29 g) showing no difference (P = 0.7385). Likewise, BWS did not differ significantly between temperature regimens (Normal: 355.75 g vs Elevated: 352.25 g; P = 0.0500). When the four treatment combinations were considered, BWS ranged from 351.92g in HF–ET to 356.67g HF–NT, with no evidence of a Diet × Temperature interaction (P = 0.4754), indicating comparable starting weights across the factorial groups.

By the end of Week 1, body weight at the end of the week (BWE) remained statistically similar across treatments, although the main effect of Diet approached significance (P = 0.0525). Birds on

the low-fat diet tended to finish the week heavier (617.58 g) than those on the high-fat diet (608.79 g). Temperature did not significantly affect BWE ($P = 0.1452$), with mean end weights of 609.92 g under normal temperature and 616.46 g under elevated temperature. Across treatment combinations, the highest BWE was observed in LF–ET (621.75 g), while HF–NT recorded the lowest (606.42 g); however, the interaction effect was not significant ($P = 0.6866$).

For growth rate, ADG in Week 1 differed significantly for both main effects. Birds fed the low-fat diet achieved higher ADG (37.79 g/d) than those fed the high-fat diet (36.42 g/d), resulting in a significant Diet effect ($P = 0.0284$). Similarly, birds reared under elevated temperature showed higher ADG (37.83 g/d) than those under normal temperature (36.38 g/d), producing a significant Temperature effect ($P = 0.0205$). In contrast, the Diet × Temperature interaction for ADG was not significant ($P = 0.9455$), suggesting that the diet effect on ADG was consistent across temperature regimens.

Daily feed intake (DFI) did not differ significantly due to Diet ($P = 0.1922$) or Temperature ($P = 0.5579$). Mean DFI was 54.58 g/d for low-fat birds and 53.23 g/d for high-fat birds, while normal and elevated temperature regimens produced similar intakes (53.60 vs 54.21 g/d). At the interaction level, DFI ranged from 52.12 g/d in HF–NT to 55.08 g/d in LF–NT, with no significant Diet × Temperature effect ($P = 0.1263$).

In terms of efficiency, FCR was not affected by Diet ($P = 0.2258$), but was significantly influenced by Temperature ($P = 0.0356$). Birds under elevated temperature achieved a lower (improved) FCR (1.38) compared with those under normal temperature (1.45). The Diet × Temperature interaction for FCR was close to significance ($P = 0.0512$) but did not reach the 0.05 threshold; descriptively, LF–ET showed the lowest FCR (1.32), whereas LF–NT showed the highest (1.46).

Table 4.2 Growth performance of broiler chickens during Week 1 of the experimental period, showing least-square means ± SE and P-values for diet, temperature, and Diet × Temperature interaction

Effect / Treatment	BWS (g)	BWE (g)	ADG (g/d)	DFI (g/d)	FCR
Diet					
Low fat	353.71	617.58	37.79	54.58	1.39
High fat	354.29	608.79	36.42	53.23	1.43
SE	1.23	3.12	0.43	0.73	0.02
P (Diet)	0.7385	0.0525	0.0284	0.1922	0.2258
Temperature					
Normal	355.75	609.92	36.38	53.60	1.45
Elevated	352.25	616.46	37.83	54.21	1.38
SE	1.23	3.12	0.43	0.73	0.02
P (Temp)	0.0500	0.1452	0.0205	0.5579	0.0356
Diet×Temp					
Low×Normal	354.83	613.42	37.08	55.08	1.46
Low×Elevated	352.58	621.75	38.50	54.09	1.32
High×Normal	356.67	606.42	35.67	52.12	1.44
High×Elevated	351.92	611.17	37.17	54.33	1.43
SE	1.74	4.41	0.61	1.03	0.03
P (Diet×Temp)	0.4754	0.6866	0.9455	0.1263	0.0512

BWS = body weight at start of week; BWE = body weight at end of week

ADG = average daily gain; DFI = daily feed intake; FCR = feed conversion ratio; LF = low fat; HF = high fat; NT = normal temperature; ET = elevated temperature; SE = standard error.

4.4 Week 2 Growth Performance Results

Week 2 growth performance response to dietary fat level and environmental temperature is summarised in Table 4.3. At the beginning of week 2, body weight at the beginning of the week (BWS) was numerically greater in the low-fat group (617.58g) than the high-fat group (608.79g) but the difference was not significant (P = 0.0525). Body weight at start of week was also not different in those groups of temperature regimens (Normal: 609.92g; Elevated: 616.46g; P = 0.1452). The range of BWS was 606.42g in HF–NT to 621.75g in LF–ET, where no significant interaction between Diet and Temperature was found (P = 0.6866) and suggested that the starting weights for Week 2 were similar.

Body weight at the end (BWE) of Week 2 was not significantly affected by Diet and Temperature main effects (Diet: P = 0.0884; Temperature: P = 0.1776). Birds fed the low-fat diet had a numerically greater BWE (1264.21g) than did the high-fat diet (1236.13g), and birds under elevated temperature had a numerically greater BWE (1261.21g) than did those fed at normal temperature (1239.13g). However, the interaction effect of BWE was not significant (P = 0.8291).

For growth rate, ADG was not significantly different because of Diet (P = 0.2572) or Temperature (P = 0.2815). ADG was on average 92.38 g/d under low fat and 89.50 g/d under high fat and normal and elevated temperature regimens were found to have comparable gain (89.88 vs 92.00 g/d). Daily feed intake (DFI) also did not differ by Diet (P = 0.4399) or Temperature (P = 0.7469). Although the Diet × Temperature interaction for DFI was not significant (P = 0.2360), the means of the interaction pointed to the lowest DFI under HF–NT (114.92 g/d) and higher DFI under LF–NT (120.00 g/d). Feed conversion ratio (FCR) was not significantly influenced by Diet (P = 0.2207), Temperature (P = 0.1464) or the interaction between them (P = 0.1177).

Table 4.3 Growth performance of broiler chickens during Week 2 of the experimental period, showing least-square means ± SE and P-values for diet, temperature, and Diet × Temperature interaction

Effect / Treatment	BWS (g)	BWE (g)	ADG (g/d)	DFI (g/d)	FCR
Diet					
Low fat	617.58	1264.21	92.38	101.08	1.09
High fat	608.79	1236.13	89.77	100.91	1.12
SE	3.12	11.40	1.38	1.81	0.0104
P (Diet)	0.0525	0.0884	0.2572	0.4399	0.2207
Temperature					
Normal	609.92	1239.13	89.88	117.46	1.3074
Elevated	616.46	1261.21	92.00	118.29	1.2857
SE	3.12	11.40	1.38	1.81	0.0104
P (Temp)	0.1452	0.1776	0.2815	0.7469	0.1464
Diet×Temp					
Low×Normal	613.42	1254.92	91.64	120.00	1.31
Low×Elevated	621.75	1273.50	93.08	117.75	1.2648
High×Normal	606.42	1223.33	88.08	114.92	1.3048
High×Elevated	611.17	1248.92	90.92	118.83	1.3065
SE	4.41	16.12	1.95	2.57	0.0147
P (Diet×Temp)	0.6866	0.8291	0.7180	0.2360	0.1177

4.5 Week 3 Growth Performance Results

Week 3 growth performance responses to dietary fat level and environmental temperature summarised in Table 4.4. At the beginning of Week 3, body weight at the start of the week (BWS) was numerically greater in birds fed a low-fat diet (1264.21 g) than in birds fed a high-fat diet (1236.13 g), but not significantly ($P = 0.0884$). BWS also did not significantly differ between the two temperature regimens (Normal: 1239.13 g; Elevated: 1261.21 g; $P = 0.1776$). Consistent with this, the Diet × Temperature interaction for BWS was not significant ($P = 0.8291$), which indicated similar starting weights among the four treatment combinations.

By the end of Week 3, body weight at the end of the week (BWE) is significantly affected by Diet ($P = 0.0197$). Birds on the low-fat diet recorded a higher BWE (2030.13 g) than the birds on the high-fat diet (1951.88 g). Temperature did not have a significant effect on BWE ($P = 0.1831$) with end weights of 1969.13 g (Normal) and 2012.88 g (Elevated). The Diet × Temperature interaction did not significantly ($P = 0.6269$) affect BWE although the maximum BWE was in LF–ET (2044.08 g) and the minimum in HF–NT (1922.08 g).

For growth and intake, ADG was numerically higher in the low-fat group (109.38 g/d) than in the high-fat group (102.17 g/d), but not statistically significant ($P = 0.0747$). Daily feed intake was also numerically greater for low (160.79 g/d) than high (152.17 g/d) fat but not significant ($P = 0.0983$), and unaffected by Temperature ($P = 0.2561$). Feed efficiency ratio did not differ because of Diet ($P = 0.4175$) and Temperature ($P = 0.9514$). Overall, Week 3 did show a clear effect of diet on end of week body weight, whereas the observed differences in ADG and DFI were numerical only and did not meet the predefined significance threshold of $P < 0.05$.

Table 4.4 Growth performance of broiler chickens during Week 3 of the experimental period, showing least-square means ± SE and P-values for diet, temperature, and Diet × Temperature interaction

Effect / Treatment	BWS (g)	BWE (g)	ADG (g/d)	DFI (g/d)	FCR
Diet					
Low fat	1264.21	2030.13	109.38	160.79	1.4726
High fat	1236.13	1951.88	102.17	152.17	1.4977
SE	11.40	22.87	2.79	3.61	0.0216
P (Diet)	0.0884	0.0197	0.0747	0.0983	0.4175
Temperature					
Normal	1239.13	1969.13	104.25	153.54	1.4842
Elevated	1261.21	2012.88	107.29	159.42	1.4860
SE	11.40	22.87	2.79	3.61	0.0216
P (Temp)	0.1776	0.1831	0.4453	0.2561	0.9514
Diet×Temp					
Low×Normal	1254.92	2016.17	108.75	162.42	1.4982
Low×Elevated	1273.50	2044.08	110.00	159.17	1.4469
High×Normal	1223.33	1922.08	99.75	144.67	1.4702
High×Elevated	1248.92	1981.67	104.58	159.67	1.5252
SE	16.12	32.34	3.95	5.11	0.0306
P (Diet×Temp)	0.8291	0.6269	0.6523	0.0808	0.0903

4.6 Week 4 Growth Performance Results

Week 4 growth performance responses to dietary fat level and environmental temperature are summarised in Table 4.5. At the start of Week 4, body weight at the start of the week differed significantly between diets (P = 0.0197), with birds on the low-fat diet starting Week 4 heavier (2030.13 g) than those on the high-fat diet (1951.88 g). In contrast, BWS did not differ significantly between temperature regimens (Normal: 1969.13 g; Elevated: 2012.88 g; P = 0.1831), and there was no Diet × Temperature interaction for BWS (P = 0.6269).

By the end of Week 4, body weight at the end of the week (BWE) was significantly influenced by Temperature (P = 0.0334). Birds reared under elevated temperature achieved a higher final weight (2759.58 g) than those under normal temperature (2650.04 g). Diet did not significantly affect BWE (Low fat: 2717.41 g; High fat: 2692.20 g; P = 0.6157), and the Diet × Temperature interaction for BWE was not significant (P = 0.1652). Descriptively, the highest BWE was observed in HF–ET (2782.16 g), whereas the lowest was in HF–NT (2602.25 g).

For intake and efficiency, daily feed intake (DFI) was significantly higher under elevated temperature (169.83 g/d) compared with normal temperature (154.66 g/d; P = 0.0350), while Diet had no effect (P = 0.9432) and the interaction was non-significant (P = 0.2879). Feed conversion ratio (FCR) was significantly influenced by Diet (P = 0.0404), with the high-fat diet showing a lower (improved) FCR (1.566) than the low-fat diet (1.666). Temperature did not affect FCR (P = 0.8540), and the Diet × Temperature interaction was not significant (P = 0.3494). Overall, Week 4 outcomes highlighted temperature-driven increases in final BW and DFI, alongside a dietary improvement in FCR.

Table 4.5 Growth performance of broiler chickens during Week 4 of the experimental period, showing least-square means ± SE and P-values for diet, temperature, and Diet × Temperature interaction

Effect / Treatment	BWS (g)	BWE (g)	ADG (g/d)	DFI (g/d)	FCR
Diet					
Low fat	2030.13	2717.41	98.25	162.50	1.666
High fat	1951.88	2692.20	105.79	162.00	1.566
SE	22.87	35.26	4.35	4.93	0.0334
P (Diet)	0.0197	0.6157	0.2264	0.9432	0.0404
Temperature					
Normal	1969.13	2650.04	97.33	154.66	1.6123
Elevated	2012.88	2759.58	106.71	169.83	1.6211
SE	22.87	35.26	4.35	4.93	0.0334
P (Temp)	0.1831	0.0334	0.1344	0.0350	0.8540
Diet×Temp					
Low×Normal	2016.17	2697.83	97.42	158.66	1.6399
Low×Elevated	2044.08	2737.00	99.08	166.33	1.6934
High×Normal	1922.08	2602.25	97.25	150.66	1.5848
High×Elevated	1981.67	2782.16	114.33	173.33	1.5488
SE	32.34	49.87	6.15	6.97	0.0473
P (Diet×Temp)	0.6269	0.1652	0.2165	0.2879	0.3494

4.7 Overall Performance Summary (Week 1–4 Combined)

The overall growth performance of Week 1-4 is summarised in Table 4.6. For the main effect of Diet, overall ADG was similar between dietary treatments, with birds fed the low-fat diet recording 84.4 g/d compared with 83.5 g/d for birds fed the high-fat diet (P = 0.5889). The overall DFI was also not significantly different between Diet (Low fat: 123.71 g/d; High fat: 120.75 g/d; P = 0.2600). Similarly, overall FCR was not affected by Diet (Low fat: 1.466; High fat: 1.448; P = 0.3700), indicating comparable overall feed efficiency between dietary fat levels when averaged across the full experimental period.

In contrast, Temperature significantly influenced overall performance. Overall ADG was higher (85.9 g/d) for birds under elevated temperature compared to those under normal temperature (82.0 g/d), and the Temperature effect was significant (P = 0.0296). Higher temperature also led to higher overall DFI (124.88 g/d) than under the normal temperature treatment (119.58 g/d; P = 0.0470). However, there was no significant effect of Temperature on overall FCR (Normal: 1.460; Elevated: 1.453; P = 0.7310), indicating that the increased intake and growth rate under elevated temperature were generally in step throughout the entire period.

A Diet × Temperature interaction was observed for overall DFI in the stated analysis (P = 0.0380). The interaction means were 123.83 g/d for LF–NT, 123.58 g/d for LF–ET, 115.33 g/d for HF–NT, and 126.17 g/d for HF–ET. This interaction should be interpreted with great care, however, since temperature was set at the room level and there were only two independent rooms per temperature regimen, while the original analysis used cages as the experimental unit. Additionally, several outcomes and weekly comparison were tested increasing the likelihood of a chance significant result due to multiple testing. Therefore, the DFI interaction is considered exploratory rather than definitive evidence of a diet-dependent temperature response.

Table 4.6 Overall growth performance of broiler chickens across Weeks 1–4, showing least-square means ± SE and P-values for diet, temperature, and Diet × Temperature interaction

Effect / Treatment	**ADG (g/d)**	**DFI (g/d)**	**FCR**
Diet			
Low fat	84.4	123.71	1.466
High fat	83.5	120.75	1.448
SE	1.24	1.83	0.014
P (Diet)	0.5889	0.2600	0.3700
Temperature			
Normal	82.0	119.58	1.460
Elevated	85.9	124.88	1.453
SE	1.24	1.83	0.014
P (Temp)	0.0296	0.0470	0.7310
Diet×Temp			
Low×Normal	83.7	123.83	1.48
Low×Elevated	85.1	123.58	1.451
High×Normal	80.2	115.33	1.441
High×Elevated	86.7	126.17	1.455
SE	1.76	2.59	0.020
P (Diet×Temp)	0.1500	0.0380	0.2810

4.8 Digestibility and Apparent Metabolisable Energy:

Digestibility and AME results are summarised in Table 4.7. Diet had a significant effect on AME coefficient, with birds fed the low-fat diet recording a higher coefficient than those fed the high-fat diet (78.40 vs 72.81%; $P = 0.0001$). In contrast, temperature did not significantly affect AME coefficient (normal 74.94% vs elevated 76.27%; $P = 0.3271$), and the Diet × Temperature interaction was not significant ($P = 0.8462$). Apparent Metabolisable Energy content did not differ significantly due to diet ($P = 0.0648$), temperature ($P = 0.3761$), or their interaction ($P = 0.9084$), although the low-fat diet showed a numerically higher value than the high-fat diet (14.69 vs 14.20 MJ/kg DM).

For ileal digestibility, protein digestibility coefficient was significantly affected by diet ($P = 0.0024$), with the high-fat diet showing higher values than the low-fat diet (81.47 vs 77.51%). Temperature and the interaction effect were not significant for protein digestibility coefficient ($P = 0.9857$ and $P = 0.8485$, respectively). Protein digestible content was not significantly influenced by diet, temperature, or their interaction.

Fat digestibility coefficient was not significantly affected by diet, temperature, or the interaction, although diet showed a tendency ($P = 0.0721$). However, fat digestible content was strongly affected by diet ($P < 0.0001$), with markedly higher values in birds fed the high-fat diet than the low-fat diet (96.60 vs 40.75 g/kg DM). Temperature and interaction effects were not significant for this variable.

Starch digestibility coefficient was also significantly influenced by diet ($P < 0.0001$), with the low-fat diet producing higher values than the high-fat diet (95.53 vs 91.41%). Temperature and the interaction were not significant. A similar pattern was observed for starch digestible content, which was substantially higher under the low-fat diet than the high-fat diet (370.90 vs 269.46 g/kg DM; $P < 0.0001$), with no significant effect of temperature or Diet × Temperature interaction.

Overall, the results indicate that dietary fat level had a stronger effect than temperature on AME coefficient and ileal digestibility variables, while no significant interaction between diet and temperature was detected for any digestibility parameter

Table 4.7 AME and ileal digestibility of broilers fed low- and high-fat diets under normal and elevated temperature regimens

	AME		IDP		IDF		IDS	
Factor	**Coefficient (%)**	**Content (MJ/kg DM)**	**Coefficient (%)**	**Content (g/kg DM)**	**Coefficient (%)**	**Content (g/kg DM)**	**Coefficient (%)**	**Content (g/kg DM)**
Diet								
Low fat	78.40	14.69	77.51	173.50	93.43	40.75	95.53	370.90
High fat	72.81	14.20	81.47	176.20	94.56	96.60	91.41	269.46
SE	0.95	0.18	0.81	1.78	0.42	0.29	0.48	1.55
P (Diet)	0.0001	0.0648	0.0024	0.3097	0.0721	<0.0001	<0.0001	<0.0001
Temp								
Normal	74.94	14.33	79.50	174.90	93.96	68.74	93.91	321.66
Elevated	76.27	14.56	79.48	174.80	94.03	68.61	93.03	318.70
SE	0.95	0.18	0.81	1.78	0.42	0.29	0.48	1.55
P (Temp)	0.3271	0.3761	0.9857	0.9830	0.9150	0.7466	0.2113	0.1918
Diet×Temp								
Low fat Normal	77.87	14.59	77.63	173.80	93.09	40.60	95.93	372.44
Low fat Elevated	78.94	14.79	77.39	173.30	93.77	40.90	95.13	369.37
High fat Normal	72.01	14.07	81.37	176.00	94.83	96.88	91.89	270.89
High fat Elevated	73.60	14.33	81.58	176.40	94.28	96.32	90.92	268.04
SE	0.95	0.18	1.14	2.52	0.59	0.40	0.68	2.19
P(Diet×Temp)	0.8462	0.9084	0.8485	0.8484	0.3132	0.3034	0.8991	0.9597

Note. AME = apparent metabolisable energy; IDP = ileal digestibility of protein; IDF = ileal digestibility of fat; IDS = ileal digestibility of starch; DM = dry matter; SE = standard error; LF = low fat; HF = high fat; NT = normal temperature; ET = elevated temperature.

AME was determined from excreta samples collected at cage level (12 cages per Diet per Temperature treatment). For ileal digestibility, digesta from birds in each cage were pooled and samples from two cages under the same Diet × Temperature combination were then pooled together resulting in six ileal samples per Diet × Temperature combination (n = 6). For the dietary treatment, the cage was the experimental unit, but for the temperature treatment, the experimental unit was the room (two rooms per temperature regimen). Each two-cage composite sample was used as an analytical sample for ileal measurements. SE values are the standard errors that correspond to the least-squares means from the specified GLM analysis. The interpretation of Temperature and Diet × Temperature SEs and P-values should be done with caution because room level temperature assignment was not a specific factor in the original GLM.

4.9 Mortality / Survival:

The survival responses to diet and temperature are summarized in Table 4.8. Survival was numerically higher in birds fed the low-fat diet than in those fed the high-fat diet (87.10% vs 75.10%), but the diet effect was not statistically significant (P = 0.070). Survival was also numerically higher under the elevated-temperature regimen than under the normal-temperature regimen (86.12% vs 76.65%), although this difference was not statistically significant (P = 0.155). There was no significant interaction between the Diet and Temperature (P = 0.453). Survival was between 72.22% (HF–NT) and 91.67% (LF–ET) for the four treatment combinations. These results indicate that no statistically detectable effects of diet, temperature, or their interaction on survival were demonstrated. However, the absence of statistical significance should not be interpreted as evidence that these factors had no biological effect or acted independently.

Table 4.8 Survival rate (%) of broilers under different dietary fat levels and temperature regimens

Effect / Treatment	Logit mean	Logit SE	Survival (%)	P-value (Chisquare)
Diet				0.070
Low fat	1.910	0.368	87.10	
High fat	1.104	0.274	75.10	
Temperature				0.155
Normal	1.188	0.281	76.65	
Elevated	1.825	0.362	86.12	
Diet × Temperature				0.453
Low × Normal	1.421	0.421	80.56	
Low × Elevated	2.398	0.603	91.67	
High × Normal	0.956	0.372	72.22	
High × Elevated	1.253	0.401	77.78	

Survival rate was numerically higher in birds fed the low-fat diet (87.10%) than in those fed the high-fat diet (75.10%), although the diet effect was not statistically significant ($P = 0.070$). Temperature also did not significantly affect survival ($P = 0.155$), with elevated-temperature birds showing numerically higher survival (86.12%) than birds under normal temperature (76.65%). The Diet × Temperature interaction was not significant ($P = 0.453$), indicating that the effects of diet and temperature on survival were independent. The highest survival rate was observed in the low-fat × elevated-temperature group (91.67%), whereas the lowest survival rate was recorded in the high-fat × normal-temperature group (72.22%).

5. DISCUSSION

5.1 Overview of Major Findings

Three major limitations are important to mention when interpreting the results of the present analysis. The temperature and Diet × Temperature effects are not replicated independently as only two rooms were used for each temperature regimen, so should be interpreted with caution. Second, these dietary treatments were labelled as low-fat and high-fat, but the two diets differed in several ingredients and nutrient fractions (starch, fibre, protein) and dietary effects observed could not be attributed solely to fat levels. Third, the mortality rate was high and varied between treatment combinations, ranging from 72.22% to 91.67%, which could have affected the composition of the cages, estimates of feed intake and the performance measurements which followed. These limitations should be considered when interpreting the growth, digestibility and survival findings discussed below.

The current research focused on how dietary fat content and environmental temperature influence broiler growth performance, nutrient utilisation and survival. According to the literature reviewed in Chapter 2, it was expected that increased temperature would reduce feed intake, growth rate, and feed efficiency because heat stress commonly decreases appetite, increases panting and thermoregulatory energy expenditure, and disrupts nutrient metabolism (Bilal et al., 2021; Nawaz et al., 2021; Kpomasse et al., 2021). The current findings, however, were not entirely in accordance with this anticipated trend. The higher ADG and DFI observed with the higher temperature regime is not necessarily an indication that the birds were in the thermoneutral or tolerance range. Changes in composition of cages in relation to mortality could have also contributed to these responses, as could the moderate size of the temperature differential imposed, and room-level effects and prior thermal conditioning. This, then, indicates that the high-temperature treatment is not a thermoneutral or optimal environment, but rather a moderate thermal challenge. (Apalowo et al., 2024).

Chapter 2 discussed that dietary fat is energy-dense, with a lower HI than protein and carbohydrate, and may contribute to improved energy utilisation and feed efficiency, especially in warm environments (Brue and Latshaw, 1985; Choi et al., 2023; Onagbesan et al., 2023). However, in the current study, no significant enhancement in overall ADG, DFI or FCR was achieved between the high-fat diet and the low-fat diet. The most evident performance benefit of the high-fat diet

appeared only in Week 4, when birds fed the high-fat diet had significantly improved FCR. This partly confirms the literature indicating that fat could enhance energy efficiency although it also shows that the effect was not uniform throughout the entire experimental period.

A significant Diet × Temperature interaction was observed for overall DFI, showing that feed intake responses to temperature differed between low- and high-fat diets. This agrees with the argument in Chapter 2 that temperature can modify how birds consume and utilise dietary energy, making the Diet × Temperature relationship important in broiler nutrition (Mangan and Siwek, 2023; Onagbesan et al., 2023). However, as the interaction was not significant for overall ADG or FCR, there was no clear evidence of protection of growth performance or feed efficiency by the high-fat diet in the elevated temperature used in the present study.

The digestibility results showed that diet composition influenced nutrient utilisation more clearly than the temperature regimen used in this study. This is consistent with Chapter 2, where fat type, starch level, digestion, absorption and energy partitioning were described as major determinants of nutrient utilisation (Fébel et al., 2008; Ravindran and Abdollahi, 2021). Dietary fat only had a limited effect on overall growth performance but it did affect specific nutrient utilisation responses, such as the coefficient of AME, protein digestibility, fat digestible content and starch digestibility. Survival rate in the present experiment was not significantly affected by diet, temperature, or their interaction, which further suggests that the elevated temperature regimen was not severe enough to produce the mortality responses commonly associated with heat stress (Bilal et al., 2021; Apalowo et al., 2024).

5.2 Growth

In the current study, dietary fat level had a limited impact on overall performance, as the birds fed the low-fat and high-fat diets had similar overall ADG (84.4 vs. 83.5 g/d), DFI (123.71 vs. 120.75 g/d), and FCR (1.466 vs. 1.448). This indicates that, with the same AME (3150 kcal/kg) in both diets, the higher fat (8.08%) did not significantly enhance growth compared with the low-fat diets (4.3%). This is partly consistent with Dale and Fuller (1980), who found that when the temperature was held constant, chicks fed high-fat diets grew faster than those fed low-fat diets, but that there was no Diet × Temperature interaction. However, their contrast in dietary fat proportion was much stronger, with approximately 12.6% vs. 33.6% of calories from fat in one comparison and 14.5% vs. 27.7% in another, whereas the present study compared a smaller difference in analysed dietary

fat concentration, 46 g/kg DM in the low-fat diet and 93 g/kg DM in the high-fat diet. This smaller fat contrast may partly explain why the growth response in the present study was less pronounced.

The present study also showed a more distinct response to temperature than other studies. Birds under the elevated regimen had higher overall ADG (85.9 vs. 82.0 g/d) and DFI (124.88 vs. 119.58 g/d), while FCR was unchanged (1.453 vs. 1.460). This contrasts with Liu et al. (2020), whose meta-analysis showed that heat stress reduced body weight gain (BWG) by 151.40 g, reduced FI by 97.95 g, and increased FCR by 0.17 compared with thermoneutral controls. The likely reason is that the temperature challenge in the present study was not as severe: the elevated treatment decreased from 30°C at day 10 to 23°C at day 34, with a weekday cyclic increase of only ~1°C, while the studies included in Liu et al. often involved chronic or acute heat stress from 28°C to 38°C. Thus, the present regimen appeared less severe than the applied heat stress conditions in many of the previous studies, but the responses in performance cannot be used to determine if the regimen was within the physiologic tolerance range of the birds.

Furthermore, Martinez et al. (2022), using meta-regression showed that changes in BWG were negatively associated with changes in FCR ($p < 0.001$), i.e. better growth means better efficiency. In this study this was only partially observed: the elevated temperature improved growth, but not FCR. Therefore, together these comparisons suggest that the lack of a strong fat effect, and the unexpected positive effect of elevated temperature, were likely due to the moderate fat contrast and the relatively mild cyclic heat challenge used in this experiment, rather than a true absence of temperature or nutritional sensitivity in broilers.

Week-wise Dietary Fat Effects:

Despite the overall limited effect of dietary fat, the results of the weekly results indicated that the response to dietary fat varied across the growing period. During Week 1, birds receiving the low-fat diet had a much greater ADG compared to birds receiving the high-fat diet. This is contrary to the normal expectation that fat ought to enhance energy utilisation due to its lower HI (Brue and Latshaw, 1985). Another potential explanation is that younger broilers might not use higher fat levels as effectively as older birds because the fat digestion process requires digestive development, bile release, enzyme activity, and intestinal maturation (Ravindran and Abdollahi, 2021). Thus, in the early phase of the experiment, the low-fat diet, possibly, supported the faster growth due to the higher proportion of starch, and starch is generally highly digestible in broilers.

During Week 2, the level of dietary fat did not have a significant impact on the growth performance variables. This implies that the initial superiority that was present in the low-fat group was not always present during the early grower stage. The changes in digestive and metabolic capacity of broilers differ rapidly with growth and shift towards increased muscle accretion and fat storage (Maharjan et al., 2021). Accordingly, the insignificance of diet effect in Week 2 could represent a transitional period when the two diets provided adequate amounts of nutrient to satisfy growth needs.

In Week 3, birds fed the low-fat diet had a significantly higher end body weight, and ADG and DFI were also numerically in favour of the low-fat diet. This again suggests that the high-fat diet did not consistently enhance growth, despite the known energetic value of fat. The higher performance of the low-fat group could be linked to its more significant starch concentration and higher apparent energy utilisation as the starch is efficiently digested and can be used to provide readily available energy to grow fast. This finding also aligns with the belief that the effect of dietary fat does not only depend on the fat content but also on the entire dietary matrix where starch, protein, fibre, and ingredient composition play a role (Ravindran and Abdollahi, 2021).

By Week 4, though, the high-fat diet had a significant positive impact on FCR. It is a more agreeable result with the literature indicating that dietary fat could enhance feed efficiency by reducing HI and increasing the efficiency of the use of energy (Brue and Latshaw, 1985). Broilers at this later stage are larger, have a greater feed intake, and they have a more developed digestive system, which could allow them to make better use of higher dietary fat. Thus, the Week 4 response indicates that the advantage of high-fat feeding might be age-specific and more apparent in later stages of growth, even in cases where the overall response of the trial is not significant.

Overall, the weekly pattern indicates that dietary fat did not exert a uniform effect across the experiment. Low-fat feeding was more favourable in previous weeks and high-fat feeding increased feed efficiency during the last week. This confirms the inference that the broilers response to dietary fat is dependent on age, digestive maturity, source of dietary energy, and the ratio between the inclusion of dietary fat and the total dietary AME supply.

5.3 Digestibility:

The digestibility findings indicate that dietary composition had a greater effect on nutrient utilisation than did the ambient temperature. This implies that the digestive response was more

likely influenced by the differences in the structure of ingredients of the two diets (low fat and high fat) than by the moderate difference in temperature employed in this study. The starch digestibility coefficient was higher for the low-fat diet while the protein digestibility coefficient was higher for the high-fat diet. In contrast, the greater digestible fat concentration in the high-fat diet should not be interpreted as greater fat digestibility, because the fat digestibility coefficient itself was not significantly different between diets. This higher digestible fat concentration was mostly attributed to the higher analysed fat concentration of the high-fat diet. Therefore, digestibility coefficients reflect the digestibility of nutrients and digestible nutrient concentration reflects nutrient concentration in the diet and its digestibility coefficient. This suggests that when the diets are designed to achieve similar levels of energy and protein, then better individual nutrient utilisation does not necessarily result in better whole-bird performance. The overall lack of a temperature effect or a Diet × Temperature interaction indicates that the temperature used was not high enough to impair digestive function or alter the response to dietary fat level.

The limited temperature effect observed here contrasts with the broader heat-stress literature. Teyssier et al. (2022) showed that heat stress can depress dry matter digestibility by 1.6-3.9% and protein or nitrogen digestibility by 1.5-10%, but they also highlighted that the decrease is variable and typically accounts for only a small part of the loss of production under heat stress. This view is partly consistent with Ahmad et al. (2022) who reported that heat stress can reduce digestion and absorption efficiency through the induction of gut inflammation and damage to the epithelial cells. However, in the current study, the increased ambient temperature did not reduce digestibility, which suggests that the level of heat stress was probably insufficient to cause the gastrointestinal dysfunction often observed under chronic severe heat stress.

The diet-related differences are biologically plausible and align with digestive physiology. Ravindran and Abdollahi (2021) pointed out that starch is highly digestible in broilers, but the digestibility of fat and protein largely depends on the development of the digestive system, enzyme secretions, and intestinal maturation. They also argued that fat digestion is affected by fat type and emulsification ability. This explains why starch digestibility was very high in both diets but still higher for the low-fat diet, whereas digestible fat content primarily reflected the higher fat content of the high-fat diet. Virden and Kidd (2009) also suggested that physiological stress could reduce

nutrient digestibility, particularly of protein and carbohydrate, but the current results suggest that the level of stress under the circumstances of this study was low.

5.4 Mortality / Survival:

In the current study, the mortality was high and should be interpreted separately from the non-significant treatment P-values. Survival across the four treatment combinations was from 72.22% to 91.67% with considerable bird losses in some treatment combinations. No statistically significant differences due to diet, temperature or their interaction in survival were found, however, the size of mortality and its distribution in the cages may have affected cage composition, feed intake calculation and subsequent performance measurements. Therefore, the results of this study should be taken with a grain of salt. In contrast, Song and King (2015) found that high ambient temperatures tend to decrease feed intake, growth in body weight, feed efficiency and can increase mortality, especially in severe temperatures.

This finding is consistent with the conclusion of Wasti et al. (2020), who reported that heat stress induces mortality by promoting oxidative stress, acid-base disturbance, diminished immunity, and feed efficiency. They also reported that temperatures as low as 25°C can cause heat stress in poultry, particularly rapidly growing broiler chickens. Apalowo et al. (2024) likewise described heat stress as a major constraint in broiler production, linking high temperature with higher mortality, poorer growth, and disruption of intestinal and physiological homeostasis.

The absence of a significant temperature effect on survival contrasts with much of the heat-stress literature. Higher environmental temperature is generally linked to increased mortality in broilers because broilers have minimal capacity to lose heat through sweating and have to rely heavily on behavioural and respiratory control mechanisms such as panting and wing spreading, which dissipate excess heat (Bilal et al., 2021). In extreme heat stress, panting enhances respiratory load and may contribute to acid base imbalance, whereas prolonged thermal load increases oxidative stress, disrupts metabolism, impairs immune function, and increases disease and mortality (Bilal et al., 2021; Nawaz et al., 2021; Apalowo et al., 2024). The heat-stress pathway described by Bilal et al. (2021) also links high temperature with low feed intake, low feed efficiency, low growth rate, low body weight, immunosuppression, and increased risk of mortality.

In the current study, survival was not reduced in birds exposed to the elevated-temperature regimen. This suggests that the temperature difference between the normal and elevated groups

was not large enough to produce a measurable effect on survival. The higher temperatures to which the elevated group were subjected were moderate, and the challenge was conducted in controlled room conditions, involving ventilation, airflow management and constant access to water. These conditions are probably what enabled birds to keep thermal balance and prevent extreme panting, dehydration, oxidative stress, and immune suppression that are typically reported during chronic or acute heat stress (Kpomasse et al., 2021; Apalowo et al., 2024).

The numerically higher survival under elevated temperature may therefore reflect the fact that the birds were not exposed to harmful heat stress, but rather to a temperature range that remained tolerable for their age and physiological state. This theory aligns with the notion of the thermoneutral zone, where broilers will be able to maintain body temperature without using excessive energy to cool the environment (Apalowo et al., 2024). There is also a possibility that the controlled housing conditions decreased other stressors that most likely interact with heat, including poor ventilation, high humidity, high stocking density and limited access to water.

The numerical decrease in the survival of birds fed the high-fat diet must also be approached with care. Under certain conditions, excessive energy consumption or the change in lipid metabolism may enhance fat deposition and metabolic load (Brue and Latshaw, 1985; Fouad and El-Senousey, 2014). Nevertheless, due to the fact that the diet effect on survival was not significant, the current findings do not provide solid evidence that the high-fat diet decreased survivability. The overall results of the mortality experiments suggest that the level of dietary fat and the high-temperature treatment did not cause a sufficiently high level of stress to significantly influence the survival of the birds.

Thus, a lack of a significant response in mortality in the present study indicates that perhaps the increased temperature treatment was not high enough to induce the level of heat stress outlined in the reviews. Rosales (1994) also pointed out that mortality due to stress is often the result of the combination of stressors, such as management, nutrition, disease exposure, and extended physiological stress, rather than temperature. In this trial, the controlled environment and moderate ambient temperatures may not have resulted in severe stress, which possibly explains why mortality was not high and why there was no significant Diet × Temperature interaction.

Some of the main limitations of this study were that the independent replication of the temperature treatment was limited in the room, feeding times were given different formulations of diets besides

fat, that mortality was not even across treatment combinations, and separate weekly analyses were used which failed to consider repeated measurements of the same cages over time. Furthermore, the original cage-level GLM failed to take into account cages within rooms for temperature comparisons. These constraints limit the power of causal inference, especially for the Temperature and Diet × Temperature effects, and are taken into account for interpretation of results.

6. CONCLUSION

The present study was conducted to determine the effects of dietary fat level and environmental temperature on growth performance, nutrient utilisation, and survival of Ross 308 broiler chickens. The study aimed to solve one of the important problems existing in the modern broiler production system, which is rapid improvement in broiler genetics, which has also increased the sensitivity of the broiler to nutritional and environmental factors. Specifically, high environmental temperature is associated with decreased feed intake, poor weight gain, impaired feed conversion, altered nutrient metabolism, and increased mortality in broilers (Bilal et al., 2021; Nawaz et al., 2021; Kpomasse et al., 2021). Dietary fat is often regarded as a nutritional tool for use under warm conditions, as it provides a high-energy density diet and results in lower HI than carbohydrate and protein, which can lead to lower metabolic heat production and growth under thermal stress (Brue and Latshaw, 1985; Choi et al., 2023; Onagbesan et al., 2023). Therefore, this experiment examined whether increasing dietary fat could improve broiler performance under an elevated temperature regimen.

The study was laid out as a 2 × 2 factorial design of two dietary fat levels and two temperature regimens. Birds were fed either a low-fat or high-fat pelleted diet from day 10 to day 34, while being reared under either a normal or elevated temperature programme. The normal temperature regimen decreased from about 25°C at day 10 to 21°C at day 34, while the elevated temperature regimen decreased from about 30°C at day 10 to 23°C at day 34 with cyclic daytime increases of about 1°C on the weekdays. Both diets were designed to be equal for AME (3150 kcal/kg) and with a similar crude protein content, with the difference being in the amount of fat included. The low-fat diet had a calculated crude fat content of 4.3%, and the high-fat diet had a calculated crude fat content of 8.08% due to an increased soybean oil inclusion. Growth performance was evaluated as body weight, ADG, daily feed intake, and feed conversion ratio. In addition, AME, ileal digestibility of protein, fat and starch and survival rate were also assessed to gain a more comprehensive view of the influence of diet and temperature on broiler productivity.

The results indicated that dietary fat level was less important for growth performance than were environmental temperatures. The growth performance of birds exposed to the elevated-temperature regimen was not negatively affected as was initially thought from the literature on heat stress. However, the overall ADG was significantly greater for the elevated-temperature birds

than for the birds under the normal temperature regimen. Moreover, the daily feed intake was significantly increased and the overall feed conversion ratio was not significantly affected as a result of an increased temperature. This finding indicated that the high temperatures experienced in the current experiment might not have been considered extreme heat stress. However, the high level regimen might have been within an acceptable or near optimal range for Ross 308 broilers throughout the experiment. This interpretation is further supported by the fact that ventilation, the management of flow, and continuous access to water were provided under controlled housing conditions. Therefore, although the elevated group experienced warmer conditions than the normal group, the temperature challenge was probably not severe enough to cause the negative physiological and productive responses commonly reported under chronic or acute heat stress.

Dietary fat level had a limited effect on overall growth performance. Overall ADG, daily feed intake, and feed conversion ratio were comparable for birds fed the low-fat and high-fat diets throughout the entire experimental period. This means that during the conditions of this study, a higher fat level (high fat versus low fat) in the diet did not consistently improve growth or feed efficiency. However, the weekly results showed that the response to dietary fat changed with age. In Week 1, the ADG of birds in the low-fat diet was significantly higher than that of birds in the high-fat diet. In Week 3, low-fat birds had significantly higher end body weight. In contrast, during Week 4, the high-fat diet significantly improved feed conversion ratio. This indicates that a low-fat and high-starch diet may be more beneficial for younger birds as their digestive maturity may have influenced the digestion of fat, bile secretion, enzyme activity, and intestinal development (Ravindran and Abdollahi, 2021). At the later growth stages the birds perhaps were able to better utilise the higher fat diet which resulted in better feed efficiency in Week 4. Therefore, the effect of dietary fat was not uniform across the trial but appeared to depend on bird age and stage of digestive development.

An important Diet × Temperature interaction was found for overall daily feed consumption, indicating that the response of feed consumption to the temperature varied with the dietary fat level. Birds fed the high-fat diet showed a more pronounced increase in feed intake under elevated temperature compared with normal temperature, whereas feed intake in the low-fat groups was more similar across temperature regimens. However, this interaction did not extend to overall ADG or FCR. So, diet and temperature interacted with regard to feed intake, but this resulted in

no significant combined effect on growth rate or efficiency. This resulted in only partial support for the third hypothesis, which predicted a change in the performance response to increased temperature with a change in dietary fat. The interaction was significant for intake and not for the primary production efficiency measures.

The digestibility data gave additional information on the influences of diet and temperature. Dietary fat level had a stronger influence on nutrient utilisation than environmental temperature. The low-fat diet resulted in significantly higher AME coefficient and higher starch digestibility coefficient as compared to high-fat diet. This is probably associated with the increased starch content of the low-fat diet and the digestibility of starch is generally high in broilers. The high-fat diet significantly increased the ileal protein digestibility coefficient and digestible fat concentration. However, the greater digestible fat concentration largely reflected the higher analysed dietary fat concentration, because the fat digestibility coefficient itself was not significantly different between diets. The digestibility coefficient for fat itself was not significantly affected, but showed a tendency for a diet effect. The digestibility of protein, fat, starch, or AME and their digestible nutrient contents were not significantly affected by temperature or by Diet × Temperature interactions. These results suggest that the digestive function was not affected by the moderate elevated temperature regime, as is often seen in more extreme heat-stress conditions (Teyssier et al., 2022; Ahmad et al., 2022).

Survival rate was not significantly affected by dietary fat level, temperature, or their interaction. Numerically, survival was higher in birds fed the low-fat diet than in those fed the high-fat diet, and higher under elevated temperature than under normal temperature, but these differences were not statistically significant. The low-fat × elevated-temperature group had the highest survival rate, whereas the high-fat × normal-temperature group had the lowest survival rate. However, none of the survival effects were significant, so there was no conclusive evidence from the results that either of the dietary fat levels or the higher temperature regime directly affected survival. The absence of a statistically significant temperature effect on survival should not be interpreted as confirmation that heat stress was absent.

For the original hypotheses, the first hypothesis was partially supported as the overall ADG and daily feed intake were found to be significantly affected by environmental temperature, but with the opposite effect to what was expected with heat-stress. The second hypothesis, that the high-fat

diet would result in better feed efficiency, was partially supported by the data since high-fat feeding resulted in FCR improvements in week 4, but not in overall FCR. The third hypothesis was also partially supported with the presence of a Diet × Temperature interaction on overall daily feed intake, but not overall ADG and FCR.

Recommendations for Future Research:

Future research should examine whether higher or more prolonged environmental temperatures produce stronger diet × temperature interactions in broiler chickens. The digestibility, survival and overall feed conversion ratio in the present study was not affected by the elevated-temperature regimen which seemed to be moderate. Therefore, further studies should test dietary fat responses under more severe or chronic heat-stress conditions, including higher cyclic temperatures, longer exposure periods, and different humidity levels. In the future, other fat sources, including soybean oil, animal fat, and other more unsaturated vegetable oils could also be compared since fat source may affect digestibility, oxidative balance, carcass fat deposition, and heat tolerance. Additionally, larger studies that captured physiological measurements (body temperature, panting score, corticosterone, oxidative-stress markers, gut morphology) would help to understand biological mechanisms underlying growth and nutrient-utilisation responses.

Future studies should also incorporate more replications of the temperature treatments in independent rooms and measure relative humidity in addition to temperature to compute temperature-humidity index (THI). To ensure that the birds are reacting to the temperature, physiological measures of thermal stress, including body temperature, respiratory rate and appropriate blood and biochemical markers should be included. Primary outcomes should be preregistered prior to data analysis and longitudinal growth data should be analysed with a repeated-measures mixed model which includes repeated observations within cages and room-level assignment of temperature treatments.

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