Poultry thermoregulation in the heat: seasonal acclimatization and partitioning of evaporative water losses

By

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Declaration

I, Clinton Bukho Tshingilane hereby declare that this dissertation, which I submit for the degree MSc (Agric) Animal Science: Animal Physiology at the University of Pretoria, is my work and has not previously been submitted by myself or another individual for a degree at this or any other tertiary institution.

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of Variance
BC	Broiler chicken
BMR	Basal metabolic rate
CEWL	Cutaneous evaporative water loss
CRH	Corticotropin-releasing hormone
EHL	Evaporative heat loss
EWL	Evaporative water loss
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HPA	Hypothalamic pituitary adrenal
LH	Luteinizing hormone \
MHP	Metabolic heat production
M_{sum}	Summit metabolism
NAS	Natural & Agricultural Science
NRF	National Research Foundation
PIT	Passive integrated transponder
RER	Respiratory exchange ratio
REWL	Respiratory evaporative water loss
RMR	Resting metabolic rate
SAM	Sympathetic adrenal medullar
SANBI	South African National Biodiversity Institute
SD	Standard deviation
T_a	Air temperature
T_b	Body temperature
TEWL	Total evaporative water loss
TIC	Thai indigenous chicken
TICC	Thai indigenous cross-bred chicken
T_{lc}	Lower critical temperature

TNN	Transylvanian naked neck
TNZ	Thermoneutral zone
T_{uc}	Upper critical temperature
USA	United States of America
ZLTE	Zone of least thermoregulatory effort

Abstract

At high environmental temperatures (T_a), birds maintain homeostasis by dissipating heat via evaporative water loss (EWL). EWL is an important mechanism of heat dissipation by animals exposed to intense solar radiation and can be partitioned into respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) and the sum of both representing the total rates of evaporative water loss (TEWL) of an organism. Another important aspect of avian thermal physiology is variation within individuals via phenotypic plasticity, one category of which is seasonal acclimatization. Seasonal acclimatisation of physiological responses gives insight into the value of phenotypic adjustment and has been well studied in wild birds in thermoneutral and low temperatures. However, some physiological mechanisms such as resting metabolic rate (RMR) and total evaporative water loss (TEWL) are lacking in poultry science in particular the variations that exists among chicken breeds in part of the different genetic make-up and productive systems in which they are reared in. The study aimed to evaluate the thermoregulation of Boschveld chickens specifically the major pathways of heat dissipation at high temperatures by partitioning EWL into REWL and CEWL. Also adaptive changes in resting metabolic rate (RMR), body mass, and TEWL, in response to seasonal acclimatisation were investigated in this study. 19 female Boschveld chickens were bought and kept in outdoor aviaries where they were fed a commercial diet and provided water ad libitum. EWL was partitioned using a glass metabolic chamber and rates of REWL, CEWL and TEWL were measured over a range of 20-43 °C including T_b using a flow-through respirometry system from (November 2019 – January 2020) and (January 2020-February 2020). For seasonal acclimatisation, parameters such as body mass, TEWL, thermal conductance, evaporative heat loss/metabolic heat production, and RMR were measured using a flow-through respirometry system but maintained a T_a range between 20 and 35 °C in winter and summer from (June 2020- January 2021) measurements of body temperature (T_b) were taken using a passive integrated transponder (PIT) tag and weighed the birds. Body temperature increased with increasing T_a in experiment 1 but there was a sudden decrease at $T_a \approx 37$ °C of 42.18±1.19 °C, n=19 and a further decrease to the following $T_a = 39$ °C mean T_b of 41.96±0.67 °C. Seasonally, T_b did not vary significantly between seasons ($F_{1:115} = 0.023$, $P < 0.05$), with winter T_b (40.12 ± 1.44 °C, n =19) being similar to summer T_b (40.38 ± 1.22 °C, n =19). Seasonally, there were significant differences in body mass ($F_{1:116} = 132.73$, $P < 0.0001$) with winter birds (2101.57 ± 268.00 g) being heavier than summer birds (1948.14 ± 257.03 g). RMR varied over the experimental T_a range and minimum RMR (4.58 ± 1.67 W) was observed at $T_a \sim 35$ °C in experiment 1. The average winter RMR (3.94 ± 1.42 W, n =19) was significantly lower ($F_{1:115} = 75.64$, $P = 0.0001$) than the summer RMR (5.54 ± 2.25 W, n =19). There were significant differences in the thermal conductance of Boschveld chickens between seasons ($F_{1:115} = 47.68$, P< 0001) with average thermal conductance being high in summer (0.54 ± 0.28 W $°C^{-1}$, n= 19) than in winter (0.36 ± 0.17 W $°C^{-1}$, n= 19). CEWL was the major route of heat dissipation at 35 °C≥ T_a≤41 °C'; moreover, CEWL represented more than 60% of heat dissipation to the total rates of evaporative water loss and was for the most part a dominant route of heat dissipation except at 25 and 30 °C. Total evaporative water loss increased with increasing T_a at T_a above 25 °C and between T_a 35 and 39 °C. TEWL was closely similar, with mean TEWL 5.15± 1.8348 mg min^{-1} (T_a 35 °C). Seasonally, TEWL showed no significant differences between seasons ($F_{1:115}$= 1.98, P> 0.05). Overall, evaporative cooling efficiency (i.e., EHL/MHP) was significantly affected by season ($F_{1:114}$=15.93, P<0.001); with the ratio of EHL/MHP being higher in winter (0.90 ± 0.65) than in summer (0.75 ± 0.47). CEWL represents an important avenue of heat dissipation in Boschveld chickens and based on the climate change scenario, this chicken breed is suitable for extensive farm systems and is capable of adapting to the natural environment.

Chapter 1: General introduction

1.1 Background

Endotherms regulate an approximately constant T_b over a broad scale of ambient temperatures (Nilsson *et al*., 2016). Endothermic thermoregulation involves balancing heat losses to the environment with heat generated by metabolism (Richards, 1970). Among birds, poultry is thought to be particularly sensitive to temperature-related challenges, especially heat stress in part of their metabolic rate as a result of their artificial selection and fast growth rate (Lara & Rostagno, 2013; Nawaz *et al*., 2021). Although under moderate conditions heat dissipation can occur by physical exchange to the environment (Yahav *et al*., 1998), an action that can be greatly hindered when environmental temperatures exceed body temperature and as such, evaporative water loss (EWL) becomes the main direction of heat dissipation (Richards, 1970; Lin *et al*., 2005; Sandercock *et al*., 2006). There are two major pathways through which EWL occurs, respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) (Richards, 1976; McKechnie & Wolf, 2004; Minnaar *et al*., 2014). These two pathways together represent total evaporative water loss (TEWL), with minor contributions from the cloaca and ocular surfaces (Minnaar *et al*., 2014).

Partitioning of EWL into REWL and CEWL has largely been unexplored in chickens. As such, a limited amount of information is available about evaporative heat dissipation in poultry during high environmental conditions. It is often but not always, assumed that EWL via the respiratory tract using panting is the main avenue of heat dissipation in chickens (Richards, 1970; Lin *et al*., 2005; Sandercock *et al*., 2006; Luthra, 2017)**.** Few comparative studies suggest a pronounced heat dissipation via the mouth with little contribution via the skin as temperatures increase (Van Kampen, 1971; Richards, 1976). For instance, Richards (1976) reported a substantial role of respiratory evaporation in Babcock 390 hybrids *gallus domesticus* at air temperatures (T_a) ranging from 23 °C to 40 °C; while (Van Kampen, (1971) noted REWL in white leghorn fowl represented more than 80% of total evaporative water loss at $35 \leq T_a \leq 40$ °C.

It is worth mentioning that cutaneous water loss is not related to subcutaneous fat, although it has been reported that some chickens increase adipose tissue during heat exposure and others decrease (Lu *et al*., 2007), the extent to which adipose tissue affects the rates of evaporation is currently lacking in poultry and perhaps in need of research. Nevertheless, Nascimento *et al*. (2017) estimated respiratory evaporation in broiler chickens, but found heat loss via this mechanism was minimal, because chicks were kept under a thermo-neutral zone.

Only these studies, to the best of my knowledge, have quantified evaporative water loss partitioning in chickens. However, because genetic variations exist amongst breeds (Soleimani *et al*., 2011), these findings may not be generally applicable. Although the relative importance of EWL to thermal regulation when air temperatures approach or exceed the body temperature of chickens has been examined only occasionally, it has been more widely studied in wild birds.

Furthermore, the contribution of REWL versus CEWL to the total rates of evaporative water loss during thermoregulation in wild birds has been reported to vary amongst orders (McKechnie & Wolf, 2004; Wolf & Walsberg, 1996). For instance, rates of REWL have been estimated to represent more than 60% of the TEWL in passerines (songbirds); Verdins (*Auriparus flaviceps*) exposed to temperature exceeding 40 °C by Wolf & Walsberg (1996) with less than 15% contribution from CEWL. Similar observations were made by Tieleman & Williams (2002), who found four species of larks (Passeriformes: Alaudidae), greatly depended on REWL when temperatures were above 43 °C. On the contrary, few studies in ornithology reveal a substantial role of CEWL in evaporative cooling; such as that is observed in Columbiformes like pigeons and doves where it has been recognized to contribute more than 40% TEWL at air temperatures above 40 °C (Withers & Williams, 1990).

Another important aspect of avian thermal physiology is variation within individuals via phenotypic plasticity in response to natural climate conditions or seasonal acclimatisation. Seasonal acclimatisation of physiological responses gives insight into the value of phenotypic adjustment and has been well studied in wild birds in thermoneutral and low temperatures (Maldonado *et al*., 2008; Noakes *et al*., 2016). The evaluation of seasonal acclimatisation in birds appears in part to involve energetic adjustments such as "basal metabolic rate and summit metabolism" during winter (Maldonado *et al*., 2008; Noakes *et al*., 2016).

Frequently studied examples of seasonal acclimatisation involve small north-temperate birds with a high basal metabolic rate and summit metabolism in winter compared to summer (Doucette & Geiser, 2008; Noakes *et al*., 2017) which suggests enhanced cold tolerance in winter (McKechnie *et al*., 2015). Meanwhile, studies in subtropical regions indicate a widespread variation at least in basal metabolic rate (Van de Ven *et al*., 2013; Thompson, 2014). For instance, Smit & Mckechnie, (2010), reported five species of birds in the Kalahari Desert namely pearl-spotted owlets (*Glaucidium perlatum*), fork-tailed drongos (*Dicrurus adsimilis*), African scops-owls (*Otus senegalensis*), crimson-breasted shrikes (*Laniarius atrococcineus*) and white-browed sparrow-weavers (*Plocepasser mahali*) to have significantly lower mass-specific basal metabolic rate in winter than in summer.

On the other hand, Van de Ven *et al*. (2013) evaluated seasonal adjustments in two populations of the Southern red bishop *Euplectes orix*, one occurring in a warmer coastal site and the other at a colder inland site and noted differences in seasonal metabolic adjustments. They observed that birds occurring at a colder inland site had an upregulation of 58% basal metabolic rate, approximately 31% mass specific basal metabolic rate and 15% summit metabolism in winter than in summer. In contrast, birds in warmer coastal sites showed no significant seasonal change in basal metabolic rate, approximately 15% significant winter reductions in mass-specific basal metabolic rate and an 8% ~ summit metabolism (M_{sum}). An earlier study by Maddocks & Geiser (2000) showed a significantly lower resting metabolic rate (RMR) below the thermoneutral zone in winter than in summer in seasonally acclimatised silvereye *Zosterops lateralis*. Nevertheless, only the study by Arieli *et al*. (1979) has reported seasonal acclimatisation in a hen and the authors observed no variations in body weight between seasons and birds exhibited a somewhat high metabolic rate in winter. These observations provide a biased perspective since the authors observed these outcomes only in White leghorn x Rhode Island cross-bred chickens,

Therefore, this provides little insight into the physiological responses of various chicken breeds between seasons. It is possible since variations amongst chicken breeds exist that these physiological responses (i.e RMR, TEWL) might differ and the current study aims to confirm this or not. Currently available insight on the adjustment of TEWL between seasons indicates variations that exist between climatic sites (O'Connor *et al*., 2017). However, little effort has been done to assess physiological flexibility in TEWL in the field (acclimatisation) between seasons (Maldonado *et al*., 2008; Talbot *et al*., 2018).

1.2 Aims and Objectives

The broad objective of this study was to evaluate thermoregulation at high temperatures and between seasons in Boschveld chickens.

Specific objective:

Experiment 1: To Partition EWL into REWL and CEWL in indigenous chickens.

Experiment 2: To evaluate adaptive changes in RMR, body mass, and TEWL in response to seasonal acclimatisation.

1.3 Hypothesis

Experiment 1: the hypothesis of experiment 1 was that REWL is a major mechanism for heat dissipation in poultry and predicted that cutaneous CEWL represents small but important pathways of evaporative cooling at high air temperatures.

Experiment 2: the hypothesis of experiment 2 was that, during winter poultry show more pronounced winter upregulation of RMR than in summer.

Chapter 2: Literature Review

2.1 Introduction

Birds are known for their high body temperature, ~3 °C higher than those of mammals (Dawson, 1982) but maintain under warm temperatures a stable body temperature because of the insulating efficiency of the feathers and in such case, evaporation is at a minimum (Richards, 1970; Gerken *et al*., 2006). At high environmental temperatures (T_a), birds maintain homeostasis by dissipating heat via evaporative water loss (EWL) (Talbot *et al*., 2017). The amount of water lost through evaporation can be divided into two categories: respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) (Minnaar *et al*., 2014); the sum of both representing the total rates of evaporative water loss (TEWL) of an organism (McKechnie & Wolf, 2004; Minnaar *et al*., 2014). Studies on the EWL of chickens indicate that, as T_a nears normothermic T_b, chickens rely on REWL as a mechanism for heat dissipation (Van Kampen, 1971; Richards, 1976); however, because genetic variation exists amongst breeds (Soleimani *et al*., 2011), this may not necessary be the case.

A plethora of studies on the thermoregulation of chickens during heat stress involve commercial-type breeds (broiler, layers), focusing on thermoregulatory parameters like T_b and metabolic heat with little to nothing on EWL (Lin *et a*l., 2005; Gerken *et al*., 2006; Ribeiro *et al*., 2020; Nawaz *et al*., 2021; Ouchi *et al*., 2021). Related studies on indigenous chickens are few (Yahav *et al*., 1998; Aengwanich, 2008; Soleimani *et al*., 2011); highlighting the need to evaluate heat dissipation in these chickens is important because many are reared in extensive systems where they experience extreme environmental conditions.

During scavenging, it becomes impossible for these chickens to avoid heat stress, particularly in areas where temperatures near or exceed T_b. The relative importance of EWL to thermoregulation when air temperatures near or exceed the body temperature has widely been established in wild birds; while in chickens, it is limited to a few studies (Kampen, 1971; Richards, 1976). Studies in ornithology reveal variation among orders (Wolf & Walsberg, 1996; McKechnie & Wolf, 2004) in the contributions of REWL and CEWL to the total rates of evaporation at high T_a.

For instance, birds that belong to the order Columbiformes (e.g pigeons and doves) are reported to dissipate more than 40% of evaporative water loss via the skin (Withers & Williams, 1990; McKechnie & Wolf, 2004; Boyles *et al*., 2011; Minnaar *et al*., 2014;). Meanwhile, those members of the order Passeriformes are reported to rely heavily on REWL when T_a near or exceeds T_b (McKechnie & Wolf, 2004; Minnaar *et al*., 2014). Chickens are non-migratory and such birds often cope with seasonal changing environmental conditions (Maldonado *et al*., 2008). As such, it is expected of such organisms to show seasonal adjustments or reversible phenotypic variations as means of coping with the changing environment according to Maldonado *et al*. (2008). Studying the physiological response of birds following seasonal acclimatisation brings about an understanding of their phenotypic flexibility or ability to adapt and is well-studied in wild birds at temperatures (T_a) below the thermoneutral zone (TNZ) and within the TNZ (Noakes *et al*., 2016). The evaluation of seasonal acclimatization in birds appears in part to be energetic adjustments such as "basal metabolic rate and summit metabolism during winter" (McKechnie, 2008; Noakes *et al*., 2017). Some of the notable findings include a high basal metabolic rate coupled with high summit metabolism in those birds inhabiting north temperate zones according to a review by McKechnie, (2008).

On the other hand, studies on birds inhabiting subtropical climates indicate a variation of responses from either low mass-specific basal metabolic rate or resting metabolic rate (RMR) in winter than summer (Maddocks & Geiser, 2000; Smit & McKechnie, 2010) or high basal metabolic rate and summit metabolism and significant winter reductions depending on site (Van de Ven *et al*., 2013). The purpose of this review is to evaluate thermoregulation mechanisms in chickens during heat stress in terms of EWL, T_b, and metabolic heat production of chickens and seasonal adjustments of these thermoregulatory responses.

2.2 Heat stress

Stress is referred to as any stimuli either coming from extreme environmental temperatures (Lara & Rostagno, 2013), husbandry practices (Kruger *et al*., 2016), or metabolic disorders that disrupt the normal function of an animal. The intensity and duration of stress affect response in different animals (Armario *et al*., 2008). According to Nawaz *et al*. (2021), stress manifests in three phases, firstly, identification of stress by the body, secondly, activation of the immune system for adaptation, and finally, exhaustion as a result of persistent stress. One of the major environmental stressors affecting poultry production globally is heat stress brought on by rising temperatures as a result of climate change (Nawaz *et al*., 2021).

The inability of an animal to control its internal temperature or body temperature (T_b) as a result of rising temperatures results in heat stress (Lara & Rostagno, 2013). The effects of heat stress in poultry production have been associated with decreased growth rate, feed efficiency, damaged gut microflora and decreased meat quality (Zhang *et al*., 2020). Heat stress is categorized into acute heat stress (intense stress for a short period of time) and chronic heat stress (high environmental temperatures for a long period of time) (Nawaz *et al*., 2021).

Under high-temperature conditions, the activity of the neuroendocrine system is altered in poultry resulting in the activation of both the sympathetic-adrenal medullar (SAM) axis and the hypothalamic pituitary-adrenal (HPA) axis (Nawab *et al*., 2018). The mechanism of action of the neuroendocrine system is illustrated in (figure 2.1) (Nawab *et al*., 2018). The main role of the neuroendocrine system is to maintain homeostasis that is to keep an animal healthy.

As such, the alteration of this system and activation of SAM and HPA increases gluconeogenesis necessary for the survival of the animal. Some authors (Leinonen *et al*., 2014) have suggested that high temperatures elevate corticosterone levels which is the main mediator of heat stress. Although this may be, Cockrem, (2007) argues that the concentration of corticosterone is not the main stressor marker but rather HPA axis, because corticosterone levels can be elevated regardless of whether impulses are positive or negative as long as there is a presence of an invader. Corticosterone is one of the primary glucocorticoids and contrarily secreted from the HPA axis and pituitary gland (Pierre *et al*., 2016).

The SAM axis results in the secretion of adrenaline as a result of impulses received from the hypothalamus, with the secretion of adrenaline being important because it facilitates rapid response through increased heart rate and promotion of gluconeogenesis (Nawab *et al*., 2018). According to Sebho (2016) under stressful conditions, the rate of secretion of adrenaline is faster and therefore is not a good indicator of stress. On the other hand, corticosterone has a slower but more persistent effect on its levels which is why corticosterone is considered a good indicator of stress (Nawab *et al*., 2018).

One of the major flaws of having hormonal changes in birds during heat stress is that, homeostasis is compromised and as such, the health of the bird is compromised. For instance, high levels of corticosterone have been reported to have a negative effect on the fertility of birds, reduced immunity and depression (Hau *et al*., 2010). Some authors have indicated chronic heat stress generally greatly impact feed intake, and reduces body weight through muscle breakdown as a result of gluconeogenesis (Nawaz *et al*., 2021).

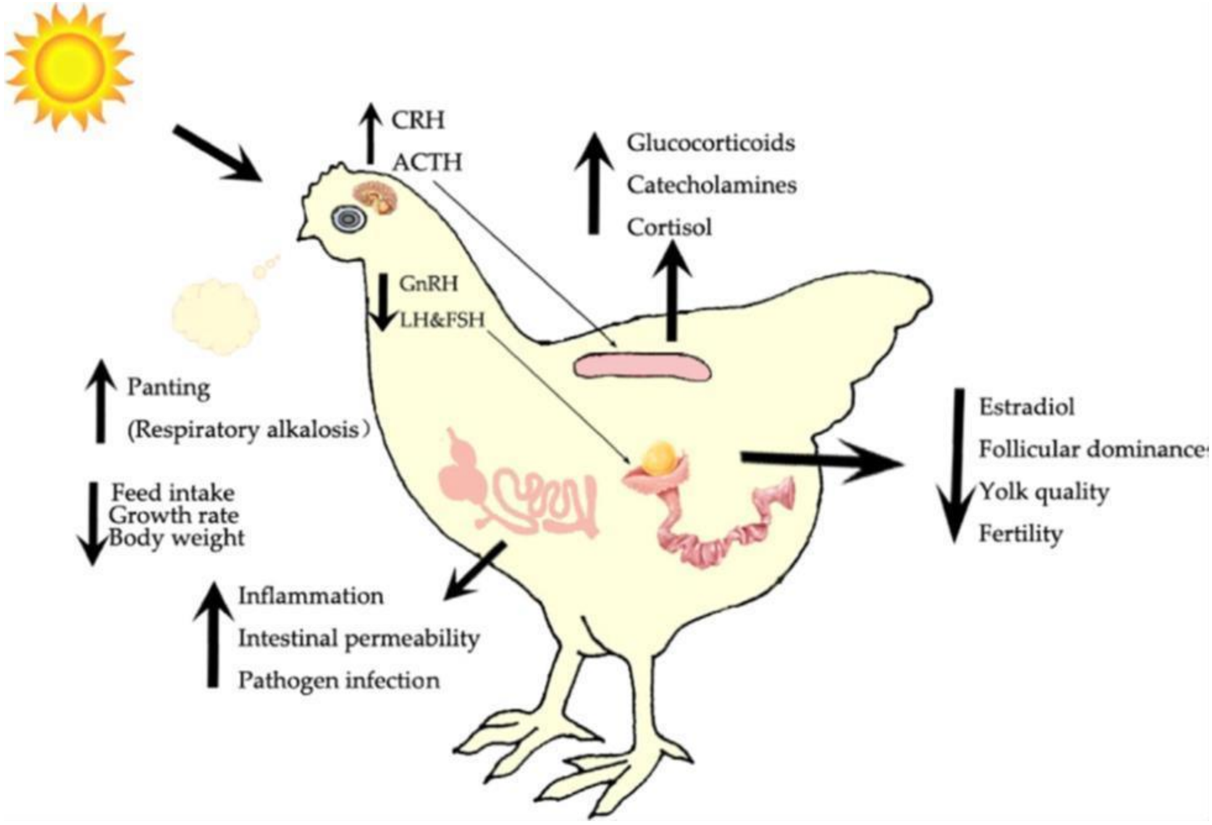

Figure 2.1 The mechanisms of action of the neuroendocrine system in a chicken during heat stress (source: Nawab *et al*., 2018)

2.3 Thermoregulatory responses of chickens to heat stress

2.3.1 Behavioural and physiological response

Aside from the neuroendocrine system's response to environmental stimuli (i.e extreme hot temperatures) to offset heat load, behavioural adaptation is considered the first defence of animals to offset heat load (Sejian *et al*., 2018). Some behavioural strategies used by chickens as means of coping with environmental heat stress include: avoiding social behaviour by distancing themselves, altering their posture with lifted wings from the body to allow for heat dissipation. Such behavioural adjustments have largely been observed in chickens confined in battery cages (reviewed by Daghir, 2008). Another behavioural response adopted by chickens during exposure to high T_a is to reduce feed consumption (Branco *et al*., 2021) and is a well-documented thermoregulatory response adopted by other animals as well (Sejian *et al*., 2018) in an effort to reduce metabolic heat production.

In addition, drinking of water during exposure to high T_a seems to be a common response not only in chickens (Branco *et al*., 2021) but other animals as well (Sejian *et al*., 2018) as a homeostatic response to water lost via evaporation (Branco *et al*., 2021). Reduced locomotion has been observed in broiler chickens, a behaviour aimed at reducing heat generated by movement according to Branco *et al*. (2021).

Furthermore, a pattern of lying down and lying laterally was observed by Branco *et al*. (2021), a behaviour the authors attributed to offsetting heat load via conduction. Wild birds, particularly those in arid habitats, have been observed to seek cooler microsites as a behavioural strategy of avoiding direct solar radiation (Wolf *et al*., 1996). Thermoregulatory behaviour of birds appears to differ with size, for instance, when evaluating the behaviour of eight species of birds of different sizes housed in outdoor aviaries, Xie *et al*. (2017), found small birds like the Budgerigars (*Melopsittacus undulates*) and Whitebrowed Woodswallows (*Artamus superciliosus*) wing-venting more than other birds like Red-collared Lorikeets (*Trichoglossus rubritorquis*) and Diamond Doves (*Geopelia cuneata*). This is because the surface-to-volume ratios of small birds are high, they gain more heat rapidly at high T_a as a result of limited water storage for evaporative cooling (Dawson, 1982) and thus in danger of heat stress (Xie *et al*., 2017). Wing-venting appears to be an essential method in birds to minimise heat gain and also the behavioural strategy for conserving water.

There are other behavioural strategies reportedly used by birds as means of maintaining homeostasis at high T_a, such behavioural strategies include those seen in adult herring gulls during nesting, where they change their orientation and face the sun this causes them to expose their most reflective plumage found in the neck, white head and breast, thus then reduce heat load (Lustick *et al*., 1978). Furthermore, gulls have unfeathered legs and can minimise heat gain by standing in the water (Lustick *et al*., 1978). One of the more specific behaviours among by birds to avoid rising of T_b to lethal levels is panting and gular fluttering. This mechanism is somewhat efficient because it increases evaporative water loss via the respiratory tract (Xie *et al*., 2017). How chickens physiologically adjust to heat stress can be described.

Firstly, the peripheral tissues increase their temperature as a result of increasing environmental temperature on the chicken. This in turn invokes several responses in which the chicken can counteract the mounting heat load in tissues such as consuming an overwhelming amount of water and depressing voluntary feed intake. Elevation of body temperature in chickens is directly related to the accumulation of heat to tissues and the length of time in which body temperature stabilises ranges between 3-5 days (reviewed by Daghir, 2008).

Secondly, as air temperatures increase above the limits of the chicken, birds tend to take advantage of specialized anatomical structures, such as the vascular system situated in the legs. This mechanism involves the arteriovenous network which allows for high amounts of blood to flow through uninsulated surfaces (legs) and in turn disperses heat under the regulation of shunts and the vascular system. Cooled blood is brought by the shunts via the veins and brings it in close contact with the arterial blood so that heat exchange can occur. Notably, this kind of heat transfer is adaptable by chickens confined in battery cages given the opportunity to roost on cool pipes where cold water circulates. According to Campbell *et al*. (2019), heat exchange via anatomical structures can occur via the eye, buccal cavity, beak and, nasal passages.

Finally, birds take advantage of feather loss as thermal windows for heat exchange. Feather loss in birds is not uncommon, especially in caged chickens like laying hens. The neck, breast region, and back are common parts in which feather loss occurs and is directly related to cage shape, size, and over stocking of chickens. Numerous studies in the late 1990s to early 2000s have demonstrated the advantage in which feather loss gives to chickens at high temperatures in both broilers (Zhou *et al*., 1999) and laying hens (Campbell *et al*., 2019).

2.3.2 Maintenance of body temperature during heat stress

One of the most important parameters for evaluating high T_a is Body temperature (T_b) which is a useful biomarker for assessing those animals that are tolerant to heat stress (Perini *et al*., 2021). Normally, the T_b of poultry varies from 41 °C to 42 °C (Cândido *et al*., 2020), but some authors have reported up to 43 °C (Perini *et al*., 2020). T_b is maintained at this range by physiologically adjusting responses to decrease heat loss. During heat stress, homeostasis is maintained by physiological activity and increases of T_b by1 to 2°C are maintained for an extended period before body temperature reaches the tolerance limit of the bird (Daghir, 2008).

On the other hand, in cold environments or cold stress, metabolic heat production is stimulated by the supplementation of feed to increase energy levels (Zhou *et al*., 2021) or birds generate heat by shivering (Hohtola, 2014) and, in some cases, huddle together to exchange body heat (Strawford *et al*., 2011). These observations on the responses of homeotherms/endotherms to environmental temperatures have been adopted in poultry science to manage the impact of fluctuating temperatures on the thermoregulation of birds as illustrated in Figure. 2.2. This diagram illustrates the relationship between body temperature and environmental temperatures (Daghir, 2008). The thermoneutral zone (TNZ) is a range that provides comfort and lies between the lower critical temperature (T_{lc}) and upper critical temperature (T_{uc}) (Pawar *et al*., 2016).

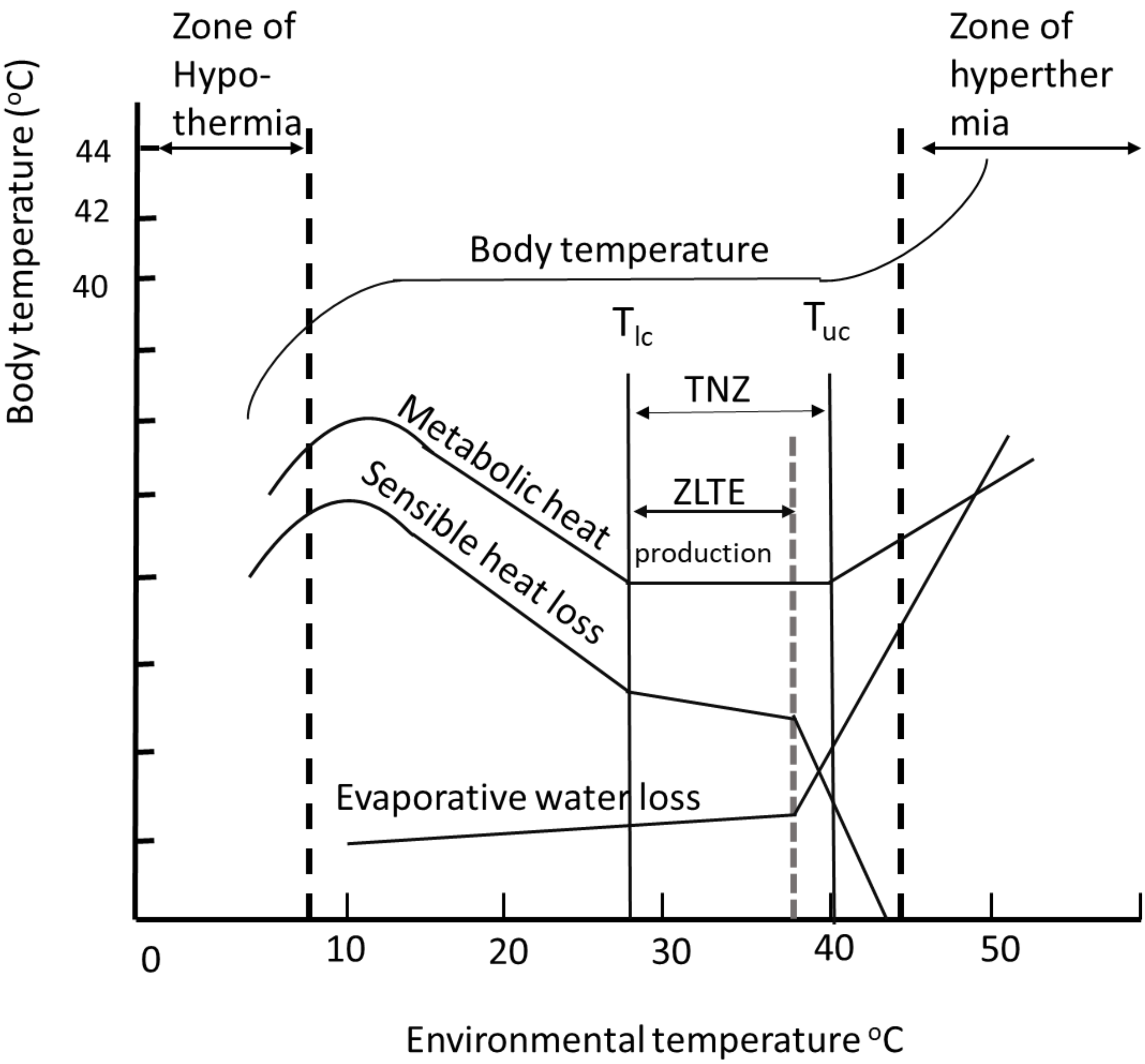

Figure 2.2 Illustration of thermoregulation in birds (Adopted from Daghir, 2008)

The zone of thermoneutrality is where normal body temperature is maintained with minimal involvement of the thermoregulatory mechanism and environmental temperatures below T_{lc} or above T_{uc} would either cause cold or heat stress (Youssef *et al.*, 2015). In addition, within the TNZ minimal metabolic production is achieved because the animal is neither gaining nor losing heat as part of the minimal physiological activity and behaviour thus limiting both sensible heat loss and evaporative water loss (Youssef *et al.*, 2015). The TNZ of broiler chickens reportedly ranges between 18-24 °C (Figure 2.2), but this range is subject to vary due to age, genetics, nutrition, environmental and growth management, among other factors (Cassuce *et al.*, 2013). For instance, when evaluating the TNZ of unacclimatised White Rock males, Kampen *et al.* (1979) found the thermoneutral zone between T_a ~

27.5 and 37.7 °C. In another study, the TNZ of female broiler breeders was around 22.5 and 30 °C (Pereira & Nääs, 2008). According to Pereira & Naas, (2008), TNZ of birds is affected by their adaptation and genetic response to different temperatures and humidity.

The zone of least thermoregulatory effort (ZLTE) is one of the three thermoregulatory responses of chickens which are TNZ, metabolic regulation that is below TNZ, and zone of latent heat control which is above TNZ (Figure 2.2) (DeShazer *et al.*, 2009). The ZLTE is within the TNZ and is reportedly ranging between 23 to 29°C (Meltzer, 1983) while some authors have reported a range between 18°C to 30°C reviewed by Luthra (2017). According to Luthra (2017), T_b is maintained at about 41 °C with minimal evaporative water loss (EWL) and no changes in metabolism and behaviour.

Typically, EWL is accommodated in this zone due to rising temperatures and can be elevated if environmental temperatures are higher than body temperature. As T_a rises above the upper critical temperature, metabolic heat rises to the zone of hyperthermia and, if persistent for long periods, it would result in heat exhaustion. The maintenance of T_b at high T_a is reported to vary amongst chicken breeds. Selection for growth has resulted in exotic breeds like broilers and layers having high metabolic heat production and thus making them susceptible to heat stress (Wang *et al.*, 2018). However, rural or indigenous poultry breeds are thought to withstand high T_a than exotic breeds (Aengwanich, 2008; Soleimani *et al.*, 2011).

One of the main arguments for variations that exist between the commercial broiler chicken vs indigenous type is body weight or size (Soleimani *et al.*, 2011). For instance, when comparing the T_b of broiler chicken (BC) to indigenous chickens (Thai indigenous chicken (TIC) and Thai indigenous crossbred chicken (TICC) at 28 days of experimentation at T_a= 38 °C, Aengwanich (2008) found T_b of broiler chicken to be significantly higher than that of indigenous type in both females and males. The author attributed this variation to body size of the broiler being larger than that of the indigenous type. Selection for fast growth rate and feed conversion efficiency has resulted in high metabolic rates in broiler chickens (Sandercock *et al.*, 2006) causing homeostasis dysregulation or a negative relationship between body size and thermoregulation (Soleimani *et al.*, 2011).

Furthermore, the study by Aengwanich, (2008) appears to demonstrate as well that these variations exist according to sex, with the male TIC and TICC chicken having lower T_b than male and female BC at T_a 38 °C, respectively. Similarly, a study by Soleimani *et al.* (2011), demonstrated that the Red jungle fowl and village fowl have lower T_b at high T_a 36 °C than commercial broiler chickens despite all birds having the same body weight. According to Soleimani *et al.* (2011), the domestication of indigenous chickens to a warmer climate has made them more heat tolerant than the exotic breeds.

Although, body size or weight in part causes variations amongst these chicken breeds, genetics also has been attributed as one causing variations in patterns of heat tolerance of chickens (Keambou *et al*., 2014). For instance, the Naked neck reportedly efficient in thermoregulation at high T_a was observed to have lower T_b compared to the normal feathered at T_a=35 °C (Yahav *et al*., 1998). This is because birds like the Naked neck chicken have the (*Na*) gene resulting into feather cover that is 20% to 40 % lower than the normally feathered chicken (Özkan *et al*., 2002), this gives them an advantage at high T_a by dissipating heat in featherless areas (Gerken *et al*., 2006). However, it can be noted both the red jungle fowl and village fowl are superior in the maintenance of T_b compared to the naked neck but the differences could be attributed to body weight, in the study by Soleimani *et al*. (2011), birds weighed about 930 grams. Meanwhile, the naked neck chicken in the study by Yahav *et al*. (1998) weighed more than 2 kg, it would be interesting if a comparison of the breeds in similar body weights would yield somewhat variations.

The maximum T_a or heat tolerance limit of indigenous chickens is currently lacking, because a majority of studies in poultry science concerning thermoregulation involve only two sets of T_a indicated as low and high (Yahav *et al*., 1998; Aengwanich, 2008; Soleimani *et al*., 2011). There are relatively few but old studies that have evaluated the thermoregulation of indigenous chickens at $T_a > 40$ °C. For instance, (Marder, 1973), investigated the thermoregulation of Bedouin fowl (*Gallus domesticus*), a bird native to Israel capable of tolerating high T_a, was found to maintain T_b well below 44 °C at $T_a \geq 45$ °C.

Another study, found four chicken breeds (Sinai, Leghorn, Sinai×Leghorn, and Leghorn×Sinai) to take T_a well above 43 °C (Arad & Marder, 1983). This somewhat implies the upper critical temperature of indigenous chicken stands way above 35 °C, a matter also suggested by Van Kampen (1978). However, the tolerance of Bedouin fowl at high T_a is largely attributed to them being acclimated to a wide range of high temperatures. Exposing birds to nonlethal temperatures enhances their ability to cope with high temperatures (Cândido *et al*., 2020; Ouchi *et al*., 2021). Noteworthy, as T_a increases, so does T_b (Arad & Marder, 1983), and birds can increase T_b to offset the costs of thermoregulation and can allow T_b to increase 5 °C above normothermic levels during acute heat exposure (Nilsson *et al*., 2016).

2.4 Evaporative water loss

Evaporative water loss is the primary route for heat dissipation when ambient temperatures are higher than body temperature (Richards, 1970; Lin *et al*., 2005; Sandercock *et al*., 2006; Albright *et al*., 2017). One of the most obvious signs of heat stress in birds is panting; a specialized form of heat that utilizes the surface of the mouth and air passages as means of evaporative cooling (Lin *et al*., 2005). The use of these surfaces is to eliminate water via evaporation to maintain body temperature below the critical limits (Ruvio *et al*., 2017). Evaporative water loss is a branch of poultry science (chickens) that is barely encountered in literature and is ultimately the most important when modelling the thermoregulation of chickens under global warming scenario.

Birds are globally distributed in different sets of environments ranging from arid to mesic and cold to hot, and evaporative heat loss plays an important role in the energy fluxes among birds and their environment and has a profound connection with their ecology and evolution (Tieleman & Williams, 2002). As temperatures rises above the regulated range, a cascade of events occurs in which the mounting heat load cannot be maintained within the normal range. As such, EWL becomes the main mechanism for offloading heat gain in which birds can avoid hyperthermia (McKechnie & Wolf, 2004).

Rates of evaporative heat loss greatly increase as environmental temperatures reaches the high end of the TNZ: for instance, 5 to 18 g/h of evaporative water loss occurring via panting in chickens has been attributed to air temperatures ranging from 29 to 35 °C (Daghir, 2008). For birds that frequently face hot weather, evaporative water loss is extremely important since the rate of evaporation increases to protect T_b from hyperthermia. However, in these circumstances, the fast rates of EWL required to prevent hyperthermia may result in dehydration.

Accelerated rates of EWL can result in a significant decline in the body's water pool that can surpass 5% of the body mass per hour when T_a exceeds T_b (Albright *et al*., 2017). In such cases, conflicts arise from maintaining a state of hydration while also avoiding hyperthermia (Minnaar *et al*., 2014). Furthermore, research indicates that rates of EWL vary according to body mass with small birds having higher mass-specific rates of EWL and thus, show greater demand for hydration (Albright *et al*., 2017).

For instance, Wolf & Walsberg (1996) demonstrated that a 7 g Verdine could lose almost 7% of its body mass per hour when T_a approaches 50 °C. These findings are similar to Albright *et al*. (2016) who found similar results when comparing 5 Passerines ranging of different body weights. The authors noted that birds of lower body mass such as Lesser Goldfinch (9.7 g) and House Finch (18.0 g) evaporated 8-9% of body mass per hour compared to birds with larger body mass Abert's Towhee (41.8 g) ~ 7% and 5% with Curve-billed Thrasher (71g) when T_a approached 50 °C (see figure 2.3). Under natural environments, birds would seek shade or microsites, limit physical activity and drink a lot of water to avoid dehydration risks. Indigenous chickens reared under extensive farming system are most likely to encounter high ambient temperatures with high rates of evaporative water loss than those reared under intensive farming.

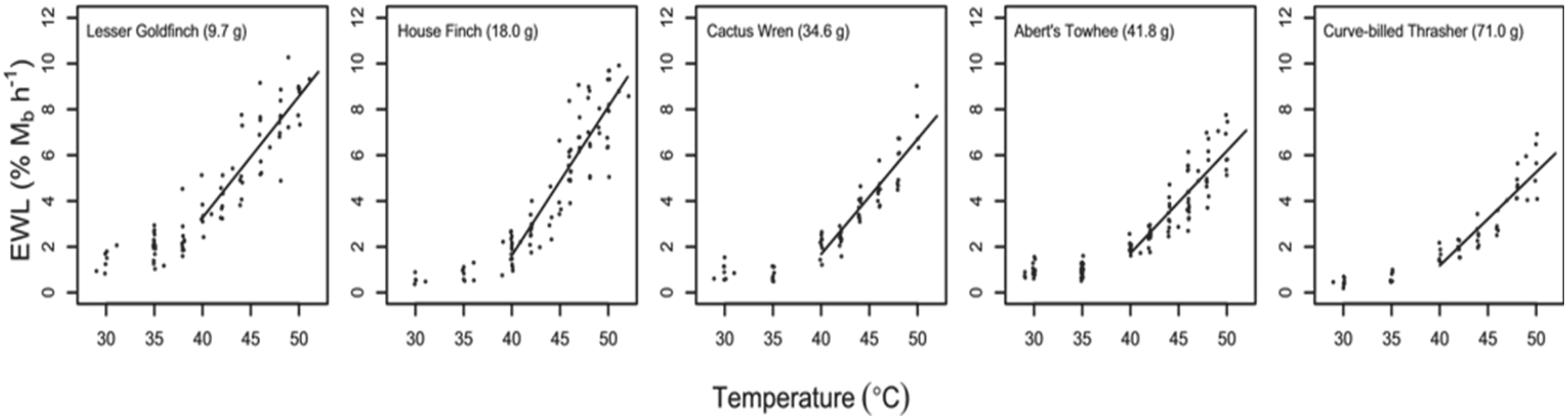

Figure 2.3 Evaporative water loss (EWL) of five passerines represented as a percentage of body mass (M_b) over a range of air temperature conditions (Albright *et al*., 2017

According to Ayo *et al*. (2011), protection of chickens reared extensively against heat is inadequate because it is a form of temporary light shade and radiation shield. Meanwhile, under intensive conditions birds are provided with automatic regulated microclimatic conditions acting as coolants. As such, the adverse impact of thermal stress is kept at a minimum because temperatures are kept constant at all time.

Although, EWL is a significant component between the bird's interaction and its environment, studies undertaken on the concept of EWL has for the most part focused in wild birds than chickens. Research on the EWL of chickens is very limited to a few and old studies and part of the reason is that there are no specific methods available in the literature in evaluating EWL and associated technical challenges of doing so may be one of the reasons why very few studies seem to have investigated this field. Therefore, it is important to assess how evaporative water loss can be partitioned.

2.4.1 Partitioning of evaporative water loss into REWL and CEWL

According to studies in Ornithology, EWL occurs via two major pathways respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) (Minnaar *et al*., 2014). The concept of partitioning EWL into REWL and CEWL can be achieved following the model described in the literature (Wolf & Walsberg, 1996; McKechnie & Wolf, 2004; Minnaar *et al*., 2014; Albright *et al*., 2017). This model of EWL data collection is performed in a laboratory and assumes that the bird is in a shaded and windless microsite with humidity, oxygen, and carbon dioxide kept at atmospheric levels which are conditions the bird would experience under its natural habitat with an average increase in air temperatures T_a.

Nascimento *et al*. (2017), described the method of partitioning EWL as noninvasive and involves separating the head from the neck. For instance, Wolf & Walsberg (1996), partitioned TEWL by placing the birds in a two-compartment metabolic chamber, separating the upper part of the bird (head chamber) from the lower half (body chamber) as illustrated in Figure 2.4. In another study, Tieleman and Williams (2002) used a mask and a glass chamber to evaluate both CEWL and REWL using an open-flow system for indirect calorimetry (Figure 2.5). Meanwhile, Minnaar *et al*. (2014) made use of a plastic mask to evaluate the mechanism of evaporation in Wahlberg's epauletted fruit bat. The technical challenges of partitioning EWL are that, the bird has to be first habituated during the experiment and data is collected when the bird is at rest. Also, the design of the system or chamber has to be built to the right size of the species.

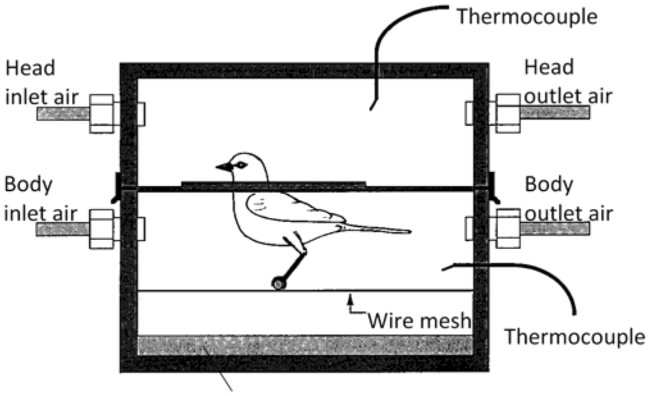

Figure 2.4 Separation of evaporative water loss in respiratory evaporative water loss and cutaneous evaporative water loss using a two-compartment metabolism chamber (Wolf & Walsberg, 1996)

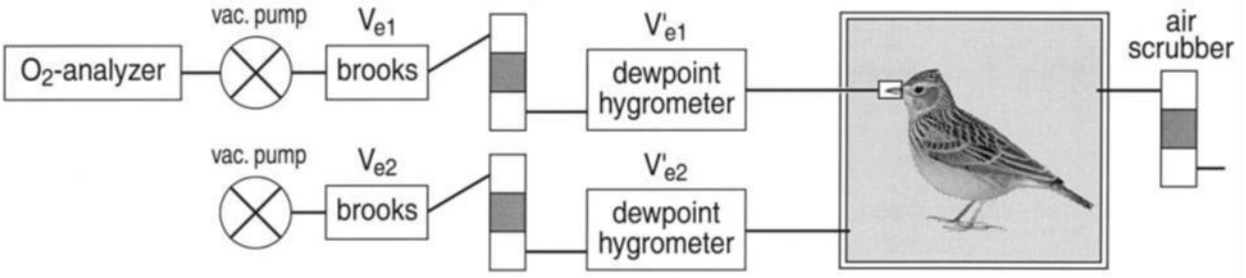

Figure 2.5 Partitioning evaporative water loss in the laboratory (Tieleman & Williams, 2002).

The evaluation of EWL in chickens seems to indicate that as T_a increases above the TNZ, chickens rely heavily on REWL. For instance, when partitioning evaporative water loss in domestic fowl (Babcock 390 hybrids) with an average weight of 2.04 Kg, Richards (1976), found more than 50% of evaporative cooling to occur via the mouth at $T_a \geq 25$ °C, meanwhile, at T_a below 25 °C, birds were observed dissipating more than 50% heat via the skin. Similar observations in Babcock 390 hybrids were made earlier by Menuam & Richards (1975).

The observations made by Van Kampen (1971; Richards (1976) that commercial chickens like the Babcock 390 hybrids and White Leghorn rely heavily on REWL as air T_a increases, is not surprising considering how very sensitive they are to heat stress (Lin *et al.*, 2005) and with a very narrow TNZ. Nevertheless, birds either accelerate REWL or CEWL as a major route of EWL at T_a above normothermic and not both (Wolf & Walsberg. 1996). The use of REWL as a mode of heat dissipation has been indicated to have energetic consequences compared to CEWL as a result of panting and gular flattening (Richards, 1970). This is because of the energy required for muscle activity needed for panting to occur consequently resulting to metabolic heat production. In poultry, respiratory alkalosis has been documented to be caused by panting (EL Hadi & Sykes, 1982). On the other hand, it appears that EWL via the skin plays an important role in maintaining T_b below the lethal limits when T_a is extremely high. Furthermore, evidence suggests CEWL to be energy efficient compared to REWL (McKechnie & Wolf, 2004). This is made possible by the skin of birds which comprises of a well vascularized dermis and a thin nonvascular epidermis that is double layered (Tieleman & Williams, 2002). This along with components that make up these structures help the bird to either increase CEWL during heat stress by vasodilation of the dermal capillary bed or alter the epidermal lipid permeability to reduce the water vapor across the skin in cases of dehydration according to Tieleman & Williams (2002). However, Tieleman & Williams (2002) noted that, consequences of hyperthermia and thermal distress could arise if drinking water is not available when using CEWL as a mode of heat dissipation.

The EWL of chickens has not been reported only in commercial type but also in indigenous chickens as well. Birds like the Bedouin fowl have been found to maintain T_b below 43 °C and between T_a 40 and 44 °C and this bird has been reported to dissipate its entire metabolic heat by evaporation coupled with small increase in metabolic rate making it suitable to adapt in harsh conditions (Marder, 1973). Noteworthy, the heat tolerance of the Bedouin fowl reported by Marder (1973) is the only one amongst indigenous birds to have been evaluated in patterns of evaporative water loss concerning thermoregulation in chickens however, the heat exposure of the birds to T_a between 25 and 48 °C prior to experimentation is in part the reason for this heat tolerance.

Thermal conditioning of chickens is one method to improve the heat tolerance of chickens to high T_a (Yahav & Hurwitz, 1996; Ouchi *et al.*, 2021). Nevertheless, according to Marder (1973) the 7.2 mg H_2O/gram-hour of EWL at T_a =48 °C represented only 0.8% of the body mass, which is quite small compared to 8-9% of body mass observed in passerines. It is worth mentioning that, it is not clear what major pathway of heat dissipation did birds use with rising T_a since (Marder 1973) in the study did not specify and as such this could be the TEWL of the bird. However, the author did indicate that thermal panting was observed at $T_a > 35$ °C with shallow panting similar to pigeons (Marder, 1973).

When evaluating EWL in birds, humidity is one aspect that must be considered since rates of evaporation from the bird is an interaction with its environment (van Dyk *et al.*, 2019), and increase humidity has been associated with high T_b and low EWL in broiler chickens (Lin *et al.*, 2005). However, the TEWL of Bedouin fowl at T_a 35 °C is notably lower than that of the Babcock 390 hybrids at T_a 35 °C. In the study by Marder (1973), the author maintained a relative humidity of no more than 30%, meanwhile, Richards (1975) kept the humidity at about 80% respectively. In the study by Richards (1975), the chickens maintained much higher T_b compared to those in the study by Marder, (1973), the effects of humidity between these chicken breeds cannot be concluded, and perhaps the underlying mechanism needs further investigation amongst indigenous breeds. In ornithology, the rates of evaporative water loss are reportedly vary between different sets of habitats. For instance, a study comparing total rates of evaporative water loss (TEWL) between species of larks living in arid and mesic habitats found EWL of those living in the desert was lower than those living in wet areas (Tieleman & Williams, 2002).

Notably, Tieleman & Williams (2002) stated that the reduced CEWL in arid desert birds (hoopoe larks (*Alaemon alaudipes*) and Dunn's larks (*Eremalauda dunni*) compared to those in mesic area skylarks (*Alauda arvensis*) and woodlarks (*Lullula Arborea*) had an overall impact on the TEWL of the species. These findings coincide with the earlier works observed in different species living in different habitats having different body sizes ranging from hummingbird to Ostriches *(Struthio camelus*) by Williams (1996).

According to Williams (1996), the reduction in TEWL in arid birds may be presumably of natural selection acting on the physiological mechanisms of the birds such as causing a reduction in CEWL by increasing the lipid layer on the skin of the birds causing water vapour resistance. Furthermore, the authors attributed this reduction in TEWL to the adaptability of the birds to minimize water loss by expiration. Tieleman & Williams (2002) suggested that because natural selection presumably influences TEWL, variations of TEWL among species may exist. The evaluation of chickens and their interaction with their habitat in patterns of EWL is missing in poultry science and one aspect that needs attention, especially to indigenous chickens when considering the climate change scenario.

2.5 Seasonal acclimatisation

Non-migratory birds often deal with changing environmental temperatures as a result of climatic seasonality (Maldonado *et al*., 2008). As such, those organisms exposed to such conditions are expected to adjust their physiological response through reversible phenotypic variation (Maldonado *et al*., 2008). The concept of phenotypic flexibility represents adjustments that can be caused by predictable (usually inter-seasonal) or unpredictable (usually intra-seasonal) changes in the environment (Piersma & Drent, 2003). This phenomenon has been a major topic of interest in many ornithology studies and has been examined under two physiological models.

Firstly, examining the behaviour and physiological response of birds by using artificial environmental conditions in the lab (Boratyński *et al*., 2016). Secondly, evaluating the physiological response of birds under natural conditions in different seasons or seasonal acclimatisation (McKechnie, 2008). Testing for physiological changes in response to the natural environment or acclimatisation is of great importance in poultry in particularly those birds that regularly interact with their environment like the indigenous chickens. This is because the ability of an animal to physiologically adjust to changing environment provides insight into ecological and evolutional properties of many traits of that animal.

Some of the commonly investigated phenotypic traits associated with acclimatisation include body mass and basal metabolic rate/ resting metabolic rate (RMR), these phenotypic traits are not fixed but fluctuate throughout an individual lifetime and are used by physiologists to observe inter/ intraspecific variations among avian species (McKechnie, 2008).

However, the plasticity of physiological traits in different seasons is not clearly defined in poultry. Firstly, some authors such as Sykes & Fataftah (1986), have misconstrued the concept of acclimatisation and have misinterpreted it with acclimation. For instance, the study by Sykes & Fataftah (1986) implies that the conditioning or acclimation of white egg laying hen (Euribrid Hisex) and brown egg laying hen (Isabrown) is actually acclimatising those birds. Notably, groups of birds were first kept in controlled environmental temperature before experimentation at T_a of 38 °C and relative humidity kept well below 26%.

Assessing the nature of seasonal acclimatisation, means much consideration has to be made on laboratory tests since they imitate what might possibly happen to birds under natural conditions (Hart, 1962). Nevertheless, the approach of grouping animals to their own respective T_a to assess their adaptive changes in the lab can be considered acclimation (Thompson *et al*., 2016) than acclimatization and has been used to assess short term adaptive changes in Ornithology (McKechnie & Wolf, 2004).

Secondly, a plethora of studies in poultry science have given much attention on the effects of season on production rather than on the adaptation stand point, as such the most evaluated chicken breeds consist of the commercial broiler or layer type chickens (Richards, 1976). Nevertheless, body mass in small wild birds has been reported to show seasonal variations (Wu *et al*., 2014). These variations are reportedly more pronounced in those birds that live in north temperate regions (Maddocks & Geiser, 2000) because of their energetic requirements for migration (Vézina *et al*., 2007), depressed feed availability in winter as such body mass is reportedly higher in winter than in summer as a result of fat stores (Swanson & Vézina, 2015). From the energy demand point of view, it appears winter is more stressful in small wild birds as a result of unfavourable conditions, long nights, limited food availability and energy costs associated with thermoregulation (Maddocks & Geiser, 2000; Wu *et al*., 2014).

In poultry, body mass is reportedly affected by season with chickens showing a much higher body mass in winter than in summer according to the study by Osti *et al*. (2017). Furthermore, the authors noted that those birds (broiler) in tropical and subtropical climate were evidently heavier than those in temperate regions. The reason behind the high body weight in winter is not clearly indicated by the authors but they did state temperature and humidity were relatively close to the comfort zone in subtropical zone as such, feed intake was enhanced.

The seasonal variation in body mass in the study by Osti *et al*. (2017) as a result of acclimatisation is not clearly defined because the study is based on production performance rather than a physiological point of view, therefore birds were fed a commercial diet with additives. The effect of feed on growth performance attributes such as body weight is well established in poultry science particularly in broiler type chicken because of their economic value.

A study by Rajkumar *et al*. (2011), comparing performance of indigenous chicken (Naked neck) and normal chicken between winter and summer season in tropical climate, indicated a contrasting difference in body weight between the naked neck and the normal chicken. The authors noted naked neck chickens were heavier in summer than in winter compared to the normal chicken and attributed this fact to the N_a gene suggesting genetic differences may cause differences in adaptation.

Winter is considered a stressful period in small wild birds (Wu *et al*., 2014) and a similar outcome can be expected for indigenous chickens since they are kept in a free-range production system and feed sources of these birds come from scavenging of food scraps and insects coupled with unbalanced supplements offered once a day in a household (Badubi *et al*., 2006). Therefore, increased feed intake and uptake of nutrients may be considered a response in wintering birds (Wu *et al*., 2014). As such, the plasticity of a trait would be repeatable changes in body composition, organ size and digestive processes and exhibit considerable metabolic variations of which body mass is a major source according to Wu *et al*. (2014).

However, this is not indicated or missing in the majority of poultry studies as such, the variations in patterns of adaptation that chicken’s exhibit following seasonal acclimatisation is not clear. Finally, only the study by Arieli *et al*. (1980) attempted to evaluate seasonal acclimatisation in White Leghorn x Rhode Island cross-bred hens and tested their thermoregulatory function at the lab at T_a 0 °C and 32 °C in both winter and summer season respectively. The authors indicated that T_b is better regulated in summer than in winter acclimatised birds.

Furthermore, the authors noted that the high T_b at T_a 32 °C in winter acclimatised birds was due to the bird’s inability to modify insulation and metabolic rate during short term exposure to high T_a. In addition, the authors noted no variations in body weight following acclimatisation. The disparity, between the study by Arieli *et al*. (1980) and previously mentioned studies (Rajkumar *et al*., 2011) concerning body weight in chickens between seasons is in part of the approach or method of assessing seasonal acclimatisation in poultry.

In the study by Arieli *et al*. (1980), adult birds used ranging between 1.5 to 2.5 years and with a low laying percentage, the authors indicated that this approach eliminated the effect of growth and interference of egg laying during T_b measurements. It can also be noted that, Arieli *et al*. (1980)

measured seasonal acclimatisation in post-absorptive birds, similar to that adopted in wild birds (Vézina *et al*., 2007; Van de Ven *et al*., 2013). Restricted feeding in pigeons has been reported to cause shallow hypometabolic response (Phillips *et al*., 1991) and reductions in rectal and foot surface temperatures in broiler breeders according to Savory *et al*. (2006). Although a plethora of studies mainly focus on productivity rather than adaptation, it would require therefore, to assess the effects of seasonal acclimatisation in hens, to do so, the element of production has to be eliminated more emphasis put on their physiological performance, thus a proper procedure or guideline preferably those indicated in ornithology and Arieli *et al*. (1980) would be best suited for seasonal acclimatisation studies.

2.5.1 Resting metabolic rate

Birds exhibit seasonal adjustments in body mass and energy which are necessary for survival particularly for those birds in harsh environmental conditions (Wu *et al*., 2014). The rate of energy is considered to varying among and within species (Van de Ven *et al*., 2013), and these variations are commonly examined under a comparative measure of metabolic rate called basal metabolic rate (BMR) usually when the bird is at rest, post absorptive state, in non-reproductive phase and at temperatures within the thermoneutral zone (Bushuev *et al*., 2018).

Historically, flexible traits like BMR have led to a notion that those birds inhabiting north temperate region provide a more natural experiment for phenotypic flexibility since they are energetically engaged to their environment in behaviours such as migration (Liknes & Swanson, 2011). Meanwhile, those birds inhabiting tropical climates are considered to have long life span, slow pace of life and resources are primarily used for maintenance (Bushuev *et al*., 2018). As such, the vast majority of studies on the seasonal acclimatisation of birds come from north temperate regions indicating that these species increase their resting metabolic rate in winter (McKechnie *et al*., 2015).

Only recently has studies embarked on assessing the flexibility of traits in birds inhabiting tropical or subtropical latitudes and the consensus amongst these studies indicate these species of birds exhibit reductions in metabolic rate in winter. For instance, Maddocks & Geiser, (2000) indicated that small passerines like the silvereyes *Zosterops lateralis* showed lower avian BMR in winter (Figure 2.8*a* below) than in summers following acclimatization (Figure 2.8*b* below).

Similarly, Smit & McKechnie (2010) found that five species in the Kalahari Desert of Southern Africa had large winter declines in BMR. The authors stated that this trend was the result of selection for winter energy conservation during chilly and dry seasons. The general consensus on the variations that exists between birds inhabiting north temperate latitudes and tropical or subtropical climates in the flexibility of metabolic rate amongst ornithology studies is that these variations exist because of selection for cold tolerance in seasonally cold environments such as north temperate latitudes. Thus, the upregulation of BMR in winter in north temperate latitudes is selection for winter energy conservation, meanwhile, in subtropical habitats which are characterized by moderate, dry winters, results to downregulation of BMR (Smit & McKechnie, 2010; McKechnie *et al*., 2015).

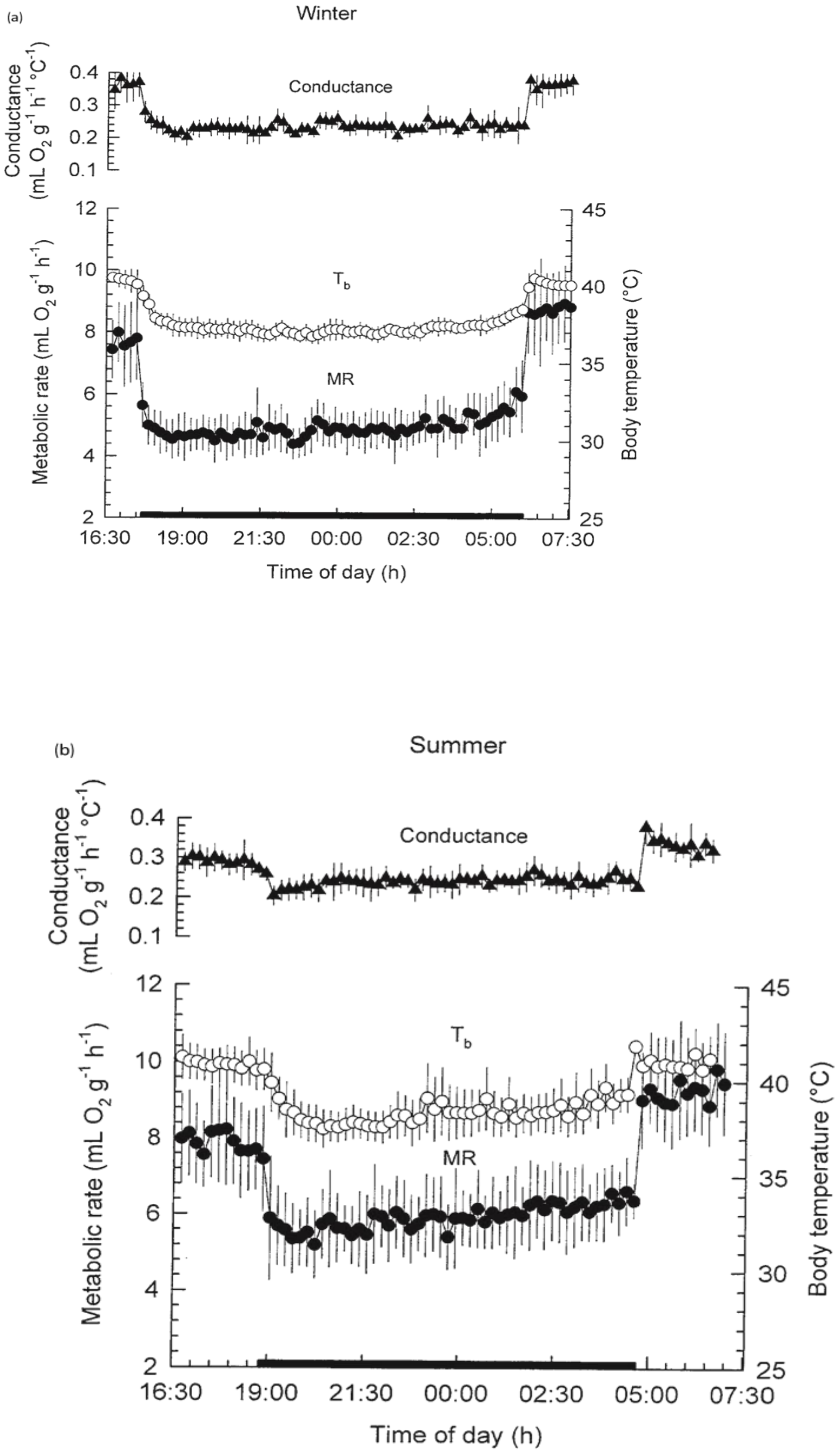

Figure 2.6 illustration of seasonal fluctuation of metabolic rate, body temperature (T_b) and thermal conductance of a small bird passerine Zosterops lateralis at ambient temperature (T_a) 16 °C during winter (Figure a) and summer (Figure b) (Maddocks and Geiser, 2000)

Although, the adjustment of metabolic rate following seasonal acclimatisation is well established in wild birds, it is somewhat limited to small birds and lacking in poultry science. Nevertheless, the study by Arieli *et al*. (1980) is in agreement with the assertion made in ornithology that metabolic rate varies with season within a population and habitat. Furthermore, the authors appeared to demonstrate that adult White Leghorn x Rhode Island cross-bred hens elevate metabolic rate more in winter than in summer without any variations in body weight between seasons.

Intensive breeding for high productivity at low energy cost, according to Arieli *et al*. (1980), leads to decreased body size, reducing the capacity to modulate energy loss through insulation. Strain differences are well known in poultry therefore it is not clear how far this outcome can be reflected in other breeds in response to seasonal acclimatisation. There is therefore a need to evaluate seasonal acclimatisation and flexibility of metabolic rate in other chicken breeds.

Despite the importance of RMR for measuring energy expenditure, the rate at which RMR increases with decreasing air temperature is influenced by insulation provided by feathers, and thermal conductance is an indication of this influence (Mortensen & Blix, 1986). According to Wolf & Walsberg (2000), plumage or feathers of bird's act as an important thermal buffer between the animal and its environment by holding back convective and radiative heat flow from an animal's skin surface to the environment. In wild birds, conductance has been reported to be low in winter compared to summer season in those birds inhabiting subtropical/tropical climates (Maddocks & Geiser, 2000). Thermal conductance of poultry has received very little attention compared to wild birds particularly with varying seasons.

The study by Arieli *et al*. (1980), is perhaps the only study to have reported thermal conductance in poultry following seasonal acclimatisation with findings revealing seasonal differences in conductance with somewhat higher conductance in summer than in winter attributed to modified feathering and body fat distribution. Concerning strain difference in patterns of plumage in poultry, Ward *et al*. (2001) found no differences in the insulation properties of the plumage of broiler chickens and free-range chickens at different environmental conditions but suggested, differences may arise from different regions of the body like the pectoral region probably as a result of the living conditions of the birds (e.g cages vs out door aviaries). Nevertheless, there is a need to evaluate thermal conductance of poultry following acclimatisation since thermogenic response is a form of adaptation, and thus this warrants more evaluation to other chicken breeds as well.

2.5.2 Total Evaporative Water Loss

Another physiological response that appears to be influenced by environmental temperature together with water availability is total evaporative water loss (TEWL) (Maldonado *et al*., 2008). TEWL is an important component of an animal's history and is reportedly varying amongst the orders (Tieleman & Williams, 2002). The rate of evaporation is influenced by metabolic rate, ambient temperature and relative humidity (Gavrilov, 2017). Furthermore, there is some indication that TEWL is strongly influenced by acclimatisation but the effort to understand the underlying mechanisms is at best minimal. Moreover, the concept of TEWL with varying seasons is missing in poultry science, and as such the current available research on TEWL in varying season is performed on small wild birds.

This fact perhaps is due to the method used to quantify evaporation. Nevertheless, the study by Noakes *et al*. (2016) evaluated TEWL during summer and winter on white-browed sparrow-weaver (*Plocepasser mahali*) at three different sites and found no significant differences between seasons but did find variations between sites. The obvious lack of data on the variations of evaporative water loss between seasons is troubling not only in poultry but also in wild birds, since evaporative water loss represents the adaptation and evolution of an animal.

2.6 Summary of the review

Chickens play an important role economically to many countries especially in poor resourced communities. Normally, chickens raised in communal areas are indigenous chickens, most common breeds being the Naked neck, Ovambo, Boschveld chicken, etc. The physiological performance at high T_a particularly evaporative cooling of chickens is somewhat under-reported in poultry, nonetheless well established in wild birds. Another important phenomenon that is rather missing in poultry science however established in ornithology is phenotypic flexibility which involves reversible phenotypic adjustments studied under acclimation or acclimatisation. It is important to evaluate the concept of acclimatisation in poultry, in particular those that are free ranging since they interact with their environment more often than the commercial type chickens and such assessment can help develop selection criteria of those breeds that are genetically capable of adapting to climate change scenario.

Chapter 3: Materials and Methods

3.1 Ethical Approval

Ethical clearance was obtained through the Natural & Agricultural Sciences (NAS) Ethics Committee: Ethics clearance number (NAS154/2019) and the Research Ethics and Scientific Committee of the South African National Biodiversity Institute (SANBI) (NZG/ P19-20).

3.2 Study site

The study was carried out at the University of Pretoria Zoology laboratory, Pretoria, South Africa (latitude 25°44’46'S, longitude 28°11’17'E). The study area receives an average rainfall of 670 mm annually, between the months of October and March with average minimum temperatures reported in July being 15 °C and average maximum temperature reported in January being 30 °C (Kabeya *et al*., 2017). Frost spells occur during some winters (Mengistu *et al*., 2016).

3.3 Birds and management

A total of 19 female Boschveld chickens with a body mass of approximately 1.32±50 kg, were bought at Boschveld Free Range Chickens farm in Bela-Bela, Limpopo. Boschveld chickens, a composite cross of the Venda, Ovambo and Matabele breeds, are hardy and well-adapted to a variety of environmental conditions, reaching sexual maturity at 143 days (Grobbelaar *et al*., 2010). The birds were housed in open-sided housing (outdoor aviaries) with wood shavings on the floor at the University of Pretoria Hatfield Experimental Farm. A commercial diet was obtained from the Agricultural research council for laying hens and water were provided *ad libitum* using tube feeders and water fonts.

3.4 Partitioning of evaporative water loss

3.4.1 Measurements of gas exchange

To measure oxygen consumption (V_{O2}), rates of EWL, and carbon dioxide production ($\dot{V}_{CO2}$), a flow-through respirometry system (McKechnie and Wolf, 2004) was used. Each bird was placed individually in a perspex 31.4 L metabolism chamber with a 3 cm layer of mineral oil at the bottom to prevent evaporation from excrement from affecting measurements of evaporative water loss (McKechnie & Wolf, 2004). An aluminium footed stand with a plastic garden mesh green surface was placed 10.2 cm above the mineral oil to prevent the birds from contact with the oil.

Separation of evaporative water loss into CEWL and REWL was done using a similar approach to that followed by McKechnie & Wolf (2004). Some modifications on the system were done such that the dimensions of the lower and upper parts of the chamber were different. The lower part was (50 cm long × 30.7 cm high × 20.7 cm wide); while the upper part was made by tilting the front upper part of the chamber 10 cm above the surface of the chamber to create a 60° angle to accommodate the head compartment. This was to ensure that the chicken would mimic the normal resting posture during measurements. To partition REWL and CEWL, a 7.5 cm diameter hole was drilled at the centre of the front-upper sloped section of the chamber, and a clear plastic container (1 L Lock & Lock), dimensions of 11.4 cm long × 11.4 wide cm × 13.2 cm high was used as a separate compartment for the head. The 1 L clear plastic container (which acted as a head compartment) was then secured to the glass chamber by placing the lid of the container over the opening made in the glass chamber and opening similar dimensions of the opening in the chamber to the lid, the lid was kept intact using 8 (8 × 30 mm Hex bolts and nuts) bolted around the lid. Before placing the lid, a 0.25 mm elastic latex sheet (Semantodontics Dental Dam, Phoenix, AZ, USA) similar to Minnaar *et al*. (2014) was placed underneath the lid and over the opening of the chamber; and both the lid and elastic latex were bolted together to the glass chamber. An opening smaller than the dimensions of the chamber opening was made in the latex to allow the head of the chicken to protrude and to provide a snug fit for the neck (Wolf & Walsberg, 1996).

To prevent birds from pulling their necks back during measurements of EWL, a 25 cm oval-shaped plastic-coated wire was used as a neck brace. After placing the chicken inside the metabolism chamber, the chamber was then placed inside a darkened temperature-controlled cabinet (Model KMF 720, Binder, Tuttlingen, Germany) (Minnaar *et al*., 2014). The behaviour of the chicken inside the darkened temperature-controlled cabinet was observed through a HIKVISION Bullet camera with 3.6 mm lens infrared light source connected to a monitor. Atmospheric air was supplied by a gas generator through the chamber using 3 mass flow controllers (model FMA5400/5500, Omega Engineering, Bridgeport, NJ). One mass flow controller was used for the head at a flow rate of 3 L min^{-1} and two mass flow controllers at flow rates of 9.03 L min^{-1} and 5.2 L min^{-1} respectively connected in parallel.

Noteworthy, the flow rates of the experiment were adjusted, not regularly, but depending on the bird's behaviour and air temperature. The flowmeters were calibrated against a soap bubble flow meter. The flow rate of the baseline channel was split from the experimental channel and controlled using a needle valve. Atmospheric air was supplied via a clear thermoplastic tubing. Inlet and outlet of the head compartment were made parallel at the base of the plastic container and inlet and outlet of the body compartment was placed in similar manner as Whitfield *et al*. (2015) at the back top and at the bottom front of the chamber, respectively. This was done to maximise mixing of air as previously done in other studies (Minnaar *et al*., 2014; Freeman *et al*., 2020).

Excurrent air from the head and body compartments as well as baseline channel were subsampled using Multiplexer (model MUX3-1,101-18 M, Sable Systems, Las Vegas, NV) set up in a manual mode and an SS-3 subsampler (Sable Systems). To measure the carbon dioxide (CO_2) and water vapor (H_2O), subsampled air was pulled through a CO_2/ H_2O analyser (model LI-840A, LICOR, Lincoln, NE, USA), zeroed using nitrogen gas (Afrox, Johannesburg, South Africa) and CO_2 spanned using a calibration gas cylinder with 1900 ppm concentration (AFROX, Johannesburg, South Africa). The H_2O of the LI-COR was spanned using a dewpoint generator (DG-4, Sable Systems, Las Vegas NV) and Oxygen (O_2) was subsampled using an O_2 analyser (FC-10, Sable Systems). All data from the analysers was acquired and recorded using Expedata software.

3.4.2 Body Temperature and Air temperature measurements

Body temperature (T_a) was measured using temperature-sensitive passive integrated transponder (PIT) tags (Bio-Thermo 12-mm microchip, Destron Fearing, St. Paul, MN; resolution: 0.1 °C) injected sub-cutaneously on the right flank of the birds. Body temperature (T_b) signals from the PIT tag were received via a loop antenna that was placed adjacent to the chamber and attached to a receiver and data logger system (HPR +, Biomark, Boise ID, USA), thus measurements were captured electronically. Air temperature (T_a) in the chamber was measured by inserting a thermistor probe (Sable Systems, Las Vegas, NV, USA) sealed using a rubber grommet on the side of the chamber, a lubricant was applied around the thermistor probe for ease of movement and to prevent injury during insertion. All data from the thermistor probe and PIT tag were recorded using Expedata software. Readings of T_a and T_b were checked every 5 minutes.

3.5 Seasonal acclimatisation

3.5.1 Measurements of Gas Exchange

Rates of EWL, oxygen consumption ($\dot{V}_{O2}$), and carbon dioxide production ($\dot{V}_{CO2}$) were measured using the same approach as earlier described in section 3.4.1 with a slight modification to the metabolism chamber. The 7.5 cm diameter×8 cm high opening that was made at the centre of the front-upper part of the chamber was sealed by placing a 0.25 mm elastic latex (Semantodontics Dental Dam, Phoenix, AZ, USA) over the chamber opening, the lid of a (1 L Lock & Lock) clear plastic container (dimensions 11.4 cm long × 11.4 wide cm × 13.2 cm high) was then placed and bolted over the latex membrane and over the chamber opening using 8 (8 × 30 mm Hex bolts and nuts) bolted around the lid to secure a tight seal. As such both lid, Latex was bolted into the chamber.

It is important to mention that, because there was no need for the head compartment during measurements, focus was solely on total evaporative water loss (TEWL), metabolic rate, and T_b. movement of individuals inside the metabolism chamber was restricted by placing a green plastic garden mesh approximate dimensions (20 cm long × 19 cm high × 20 cm wide) at the back of the chamber and on top of the aluminium footed stand. The chicken stood 10 cm above the mineral oil. Atmospheric air was supplied to the chamber using the same set up as above, however only two parallel connected mass flow controllers (model FMA5400/5500, Omega Engineering, Bridgeport, NJ). Air moved at a flow rate of 9.56 min^{-1} and 9.23 L min^{-1} from each mass flow meter to the chamber respectively. The inlet of the body compartment was positioned at the back top of the chamber and outlet was positioned at the bottom front of the chamber. Ex-current air from the metabolism chamber and baseline channel were subsampled using the same system analysers section 3.4.1 followed by the same calibration method. All data from the metabolism chamber was received and recorded using Expedata software.

3.5.2 Body Temperature and Air temperature measurements

Because the same individuals were used also in experiment 2, body temperature and air temperature measurements were carried out using the same procedure as section 3.4.2 and data from the metabolism chamber was received and recorded using Expedata software.

3.6 Experimental Protocol

3.6.1 The partitioning of evaporative water loss

The partitioning of evaporative water loss experiment was carried out during the summer days from (November 2019-February 2020) with average environmental temperatures during this period ranging between 28.1 and 29 °C with humidity ranging between 72.3% to 73% (source: South African Weather Service). All data was collected when chickens were at rest or appeared calm and post-absorptive or when they had been fasted (Cavieres & Sabat, 2008).

The measurements for EWL, RMR, and T_b were taken over a T_a of 20, 25, 30, 35, 37, 39, 41, and 43 °C over a period of an 1 hour, except at $T_a = 41$ and 43 °C, where measurements were taken for 30 minutes because chickens exhibited signs of hyperthermia. Birds were weighed before experimentation and body masses recorded. Experiments began as described by Minnaar *et al.* (2014), subsampled air was drawn from the baseline air channel for 5-10 minutes until stable O_2, CO_2, and H_2O readings were obtained. Then air from head compartment was subsampled for 10-15 minutes then followed by the body compartment and switching between the two channels when stable readings were obtained.

Each run ended with a 5-10 minutes baseline reading, a similar approach to Minnaar *et al.* (2014). In cases where O_2, CO_2, and H_2O readings from the head compartment deviated from normal as a result of either a tear from the latex membrane caused by the beaks of the chickens during the protrusion of the head of the chicken in the initial stages of experimentation or during measurement as a result of pulling the head back, such output was deleted and a new membrane used with a proper fit of the neck brace and the process restarted again. Behaviour of individuals during experimentation was observed using an infrared light source video camera connected to a monitor. Thus, only data of calm individuals was included in the analysis. It is worth mentioning, that if individuals exhibited signs of agitation such as continuously pulling their head back, standing during measurements and flipping their wings, such runs were terminated for the day and the chicken given a resting period of 2 days and this period was extended to 3 days at $T_a \geq 35$ °C. After a successful run or termination of the run birds were immediately given water and feed. Measurements were obtained from all 19 birds at each T_a, with each bird resting for at least 3 to 4 days between runs.

3.6.2 Seasonal Acclimatisation

Measurements for seasonal acclimatisation followed similar approach as experiment 1, except that maximum T_a birds tolerated during measurements in winter was 35 °C. This is because chickens could not take T_a >35 °C in part of them being completely acclimatised to the winter season as such measurements were taken over a T_a of 20, 25, 30, and 35 °C. One bird was measured at a time and the sequence of T_a values randomized (Minnaar *et al.*, 2014), while winter experiments were taken from June 2020- August 2020 for 3 hours at each T_a value. The average daily maximum and minimum temperatures during this period were 21.3 and 5.1 °C with average humidity of 57.9%. Measurements were taken as early as 4:00 am until 6:00 pm and all measurements were taken to post absorptive individuals (Freeman *et al.*, 2020). In summer measurements were carried at T_a of 20, 25, 35, 37, 39 and 40 °C. At $T_a \geq 35$ °C, T_a was increased in increments of 5 °C to the desired T_a every 10-15 minutes in both seasons. In summer, experiments occurred between (November 2020 and January 2021) average daily maximum was 28.46 °C, average daily minimum 16.6 °C and average relative humidity was 69.13%, meanwhile, in winter experiments occurred between (June 2020 and August 2020) humidity was 62.45, daily maximum and minimum were 21.3 and 5.1 °C (source: South African Weather Service, 2021) after measurements, birds were given water and feed.

3.7 Statistical analysis

3.7.1 The partitioning of evaporative water loss

Carbon dioxide and H_2O traces from analysers were corrected for drift and lag using applicable algorithms in Expedata as done by other studies (Freeman *et al.*, 2020). To obtain values of oxygen consumption ($\dot{V}_{O2}$), and carbon dioxide production ($\dot{V}_{CO2}$), REWL ($V_{H2O\ head}$), CEWL ($V_{H2O\ body}$) and T_b in resting or calm individuals, an average of the most stable 5 minute period from CO_2 traces at each T_a run was obtained using Expedata software a similar approach with the study by (O'Connor *et al.*, 2017). In cases where individuals were not calm often depicted by deviation of CO_2 from normal that data was discarded from the analyses. Furthermore, in cases where the desired T_a in the chamber was not reached that data was discarded from the analyses. To calculate excurrent flow rates from the glass metabolism, equation 9.3 of Lighton (2008) was used. Furthermore, rates of oxygen consumption ($\dot{V}_{O2}$), and carbon dioxide production ($\dot{V}_{CO2}$), REWL ($V_{H2O\ head}$) and CEWL ($V_{H2O\ body}$) were calculated using Equations 9.4, 9.5 and 9.6 of Lighton (2008) as follows:

Equation 9.3

$$FR_e = FR_i\ (1- (F_iO_2/100) - (F_iCO_2/100) - (F_iH_2O/BR))\ /\ (1-(F_eO_2/100) - (F_eCO_2/100) - (F_eH_20/BR)$$

Where FR_e is the excurrent flow rate in (ml min^{-1}); FR_i is the incurrent flow rate (ml min^{-1}); $F_i\ O_2$ is the baseline O_2 obtained for 5-10 minutes; F_iCO_2 is the baseline CO_2 obtained for 5-10 minutes and F_iH_2O is the baseline water vapour obtained for 5-10 minutes. BR is the barometric pressure in kilopascals obtained in the baseline. (F_eO_2) (ml min^{-1}) is the average excurrent or channel O_2 obtained in the most stable 5-minute period, (F_eCO_2) (ml min^{-1}) is the average excurrent or channel CO_2 obtained in the most stable 5-minute period and (F_eH_2O) is the excurrent water vapour from the head compartment of the metabolic chamber obtained in the most stable 5-minute period.

Equation 9.4

$$\dot{V}_{O2} = (FR_i\,(F_i\ O_2/100) - (FR_e\,(F_eO_2/100)$$

Where ($\dot{V}_{O2}$) is the oxygen consumption in milliliters/minute (ml min^{-1}).

Equation 9.5

$$\dot{V}_{CO2} = (FR_e\,(F_eCO_2/\ 100)) – (FR_i\,(F_iCO_2/\ 100))$$

Equation 9.6

$$V_{H2O\ head} = ((FR_e\ (F_eH_2O/\ BR)) – (Fr_i\ (F_iH_2O/\ BR)))\ 0.803$$

Where ($V_{H2O\ head}$) is the calculated water vapour in milligrams per minute (mg min^{-1}). It is important to note, that volume H_2O in ml H_2O was obtained from the mass flow controller and converted to mg H_2O by multiplying 0.803 mg per H_2O ml water vapour (Lighton 2008).

$$V_{H2O\ body} = ((FR_e\ (F_eH_2O/\ BR)) – (Fr_i\ (F_iH_2O/\ BR)))\ 0.803$$

Obtaining the rate of evaporative water loss via the body ($V_{H2O\ body}$) follows the same equation as 9.6 with similar calculations. Estimates of resting metabolic rate (RMR) in Watts (W) were obtained from $\dot{V}_{O2}$ and respiratory exchange ratio (RER) $\dot{V}_{CO2}/\dot{V}_{O2}$), $\dot{V}_{O2}$ was converted to metabolic rate by assuming an RER value of 0.71 following a similar approach to Minaar *et al.* (2014). After all the data were recorded, averages were then taken and plotted the data on excel (see chapter 4.1). Noteworthy, statistical models were not generated when evaluating (EWL) but expedata software was used to collect the data.

3.7.2 Seasonal Acclimatisation

Carbon dioxide and H_2O traces from analysers were corrected for drift and lag using relevant algorithms in Expedata (O'Connor et al., 2017). Resting values of oxygen consumption ($\dot{V}_{O2}$), and carbon dioxide production ($\dot{V}_{CO2}$), EWL (V_{H2O}) and T_b were taken similarly to 3.6.1 (above). Moreover, the same equation 9.3 above was used to calculate FR_e and equations 9.4, 9.5, 9.6 of Lighton (2008), to get the rates of oxygen consumption ($\dot{V}_{O2}$), and carbon dioxide production ($\dot{V}_{CO2}$) and EWL (V_{H2O}). Estimated rates of RMR (W) were calculated using RER of 0.71 and $\dot{V}_{CO2}$. Calculated rates of evaporative heat loss (EHL) (W) were obtained by converting rates of EWL assuming a latent heat of vaporization of water of 2.406 J mg^{-1} a similar approach to Freeman *et al*. (2020).

Data were analysed in R, using general linear mixed models. For each response variable, T_a was included as a continuous predictor and season as a categorical predictor. Identity of individuals was also included as a random effect to account for repeated measures. Then the ANOVA was run on each model to generate F ratios and P values. Data was significant at ($P < 0.05$), values are presented as means ±SD.

Chapter 4: Results

4.1 Partitioning of evaporative water loss

4.1.1 Body Temperature

Body temperature (T_b), of Boschveld chickens increased with increasing air temperature (T_a) between 20 and 35 °C, however, there was a sudden decrease in T_b at $T_a \approx$ 37 °C of 42.18±1.19 °C and T_b continued to decrease to the following T_a 39 °C mean T_b =41.96 ± 0.67 °C. At $T_a >$ 39 °C, T_b increased with increasing T_a to a maximum of 42.12 ± 0.33 °C (Figure 4.1). At $T_a \geq$ 35 °C, chickens began panting and gular fluttering with their wings open.

4.1.2 Resting metabolic rate

Between 10 and 25 °C, RMR followed the expected endothermic pattern of decreasing with increasing T_a (Figure 4.2). RMR varied throughout the experimental T_a range, and at T_a 35 °C, RMR was at a minimum of 4.58 ± 1.67 W at T_a.

4.1.3 Evaporative water loss

Both REWL and CEWL were low with a slightly elevated REWL at T_a 25 and 30 °C, however, between $T_a \approx$ 35 and 41 °C there was a rapid increase in CEWL and a steady decline in REWL between $T_a \approx$35 and ≈ 39 °C (Figure 4.3). For instance, the rate of CEWL at T_a 35 °C was 49.21±19.48 mg min^{-1} and at T_a 41 °C were 83.80 ± 40.26 mg min^{-1} respectively. On the other hand, the rate of REWL at $T_a \approx$ 35 °C was 36.57±20.91 mg min^{-1} and at $T_a \approx$39 °C was 25.90±8.92 mg min^{-1}. It is important to mention that between 37 and 39 °C the rate of CEWL was almost equal and furthermore it appeared that at $T_a \sim$ 41 °C CEWL became stable and began to decrease at maximum $T_a \sim$ 42 °C, at the same time REWL increased. Between T_a 41 and 43 °C CEWL decreased by a factor of 1.56 and REWL increased by a factor of 14.16. Rates of CEWL were greater than those of REWL at all T_a values except at T_a 25 and 30 °C the fractional contribution of REWL to TEWL ranged from 58.43 ± 8.96% ($T_a \approx$25 °C) to 54.95 ± 14.58% ($T_a \sim$ 30 °C) (insert graph). At $T_a \sim$ 43 °C, however, CEWL accounted for the majority of evaporative heat loss, namely, 58.56 ± 13.06% of TEWL (Figure 4.4). TEWL increased with increasing T_a at $T_a >$ 25 °C and between T_a 35 and 39 °C TEWL was approximately equal, with mean TEWL 5.15± 1.83 mg min^{-1} (T_a 35 °C), 5.19 ± 0.81 mg min^{-1} ($T_a \approx$ 37 °C) and 4.93±1.55 mg min^{-1} ($T_a \approx$ 39 °C). At maximum $T_a \approx$ 43 °C TEWL was 8.33±2.48 mg min^{-1}.

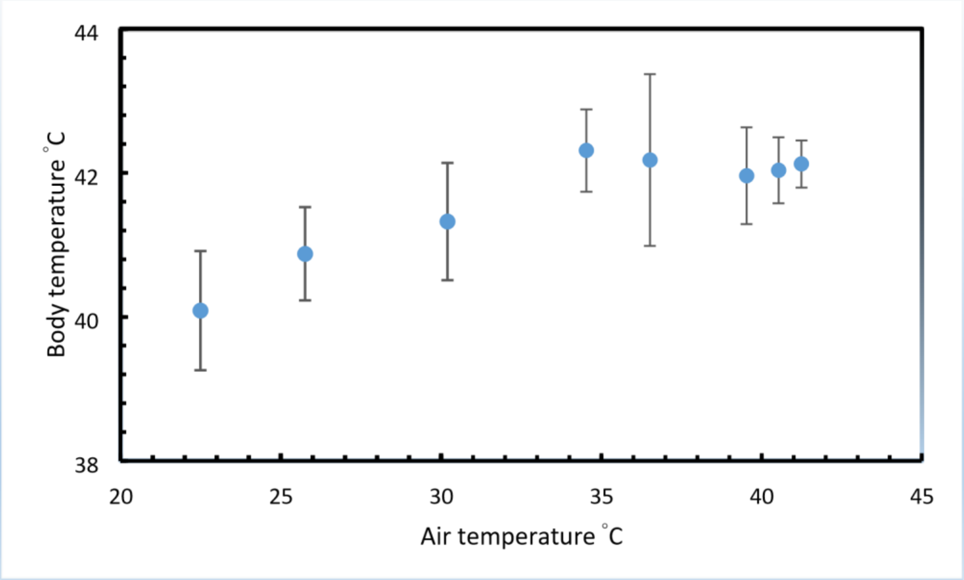

Figure 4.1 Body temperature (T_b) of Boschveld chickens over a range of air temperatures (T_a), maintained in outdoor enclosures in Pretoria, South Africa.

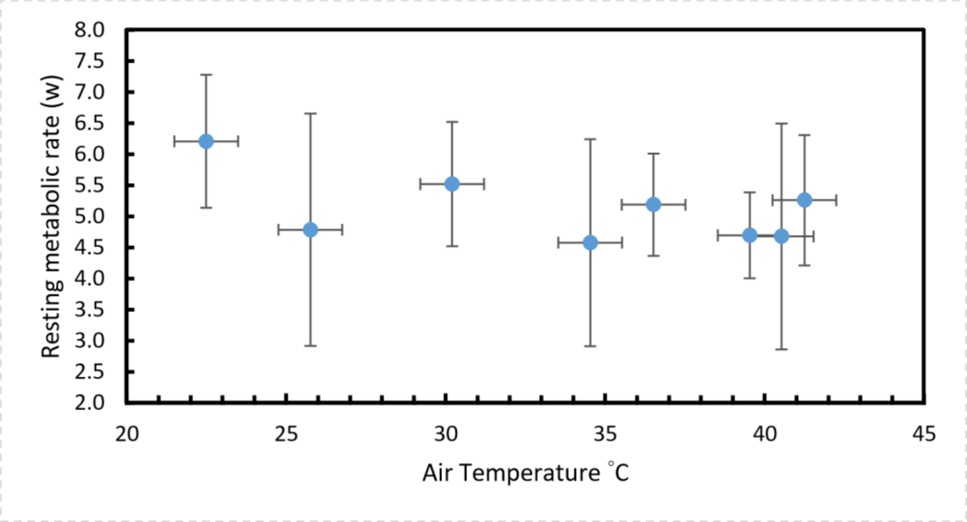

Figure 4.2 Resting metabolic rate (RMR) of Boschveld chickens over a range of air temperatures (T_a) maintained, in outdoor enclosures in Pretoria, South Africa.

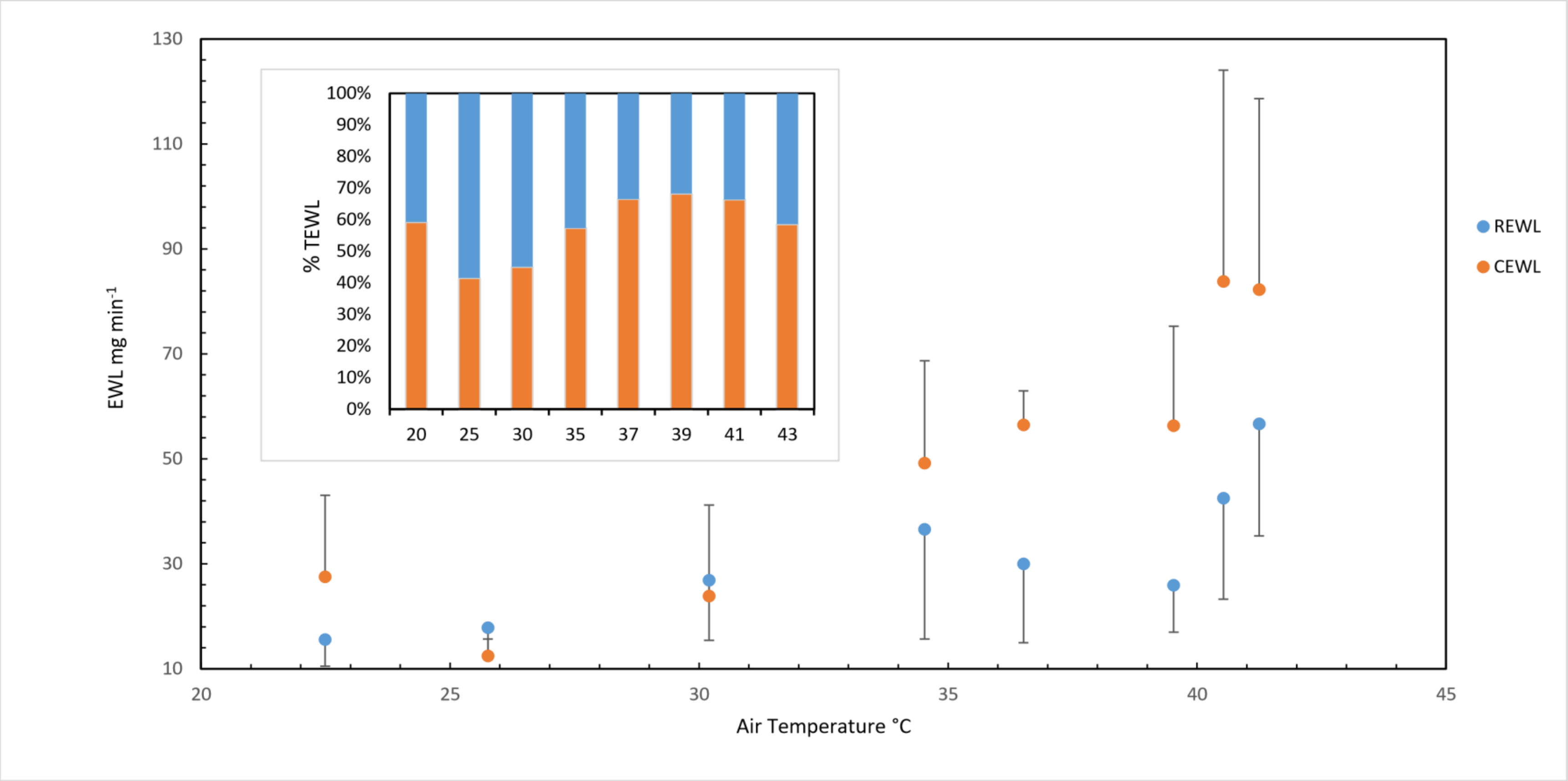

Figure 4.3 Respiratory evaporative water loss (REWL) and cutaneous evaporative water loss (CEWL) in Boschveld chickens over a range of air temperatures (T_a), maintained in outdoor enclosures in Pretoria, South Africa.

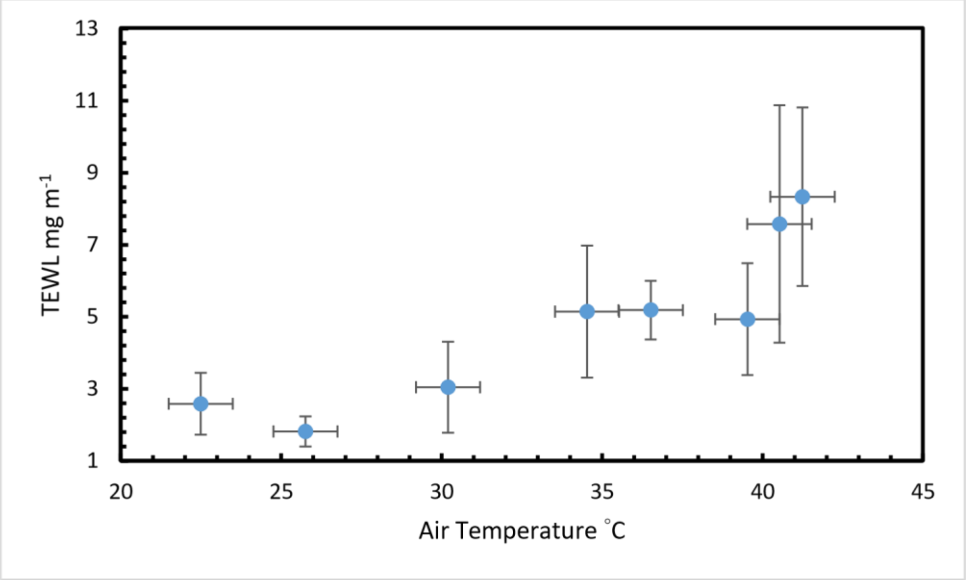

Figure 4.4 Total evaporative water loss (TEWL) in Boschveld chickens over a range air temperature (T_a), maintained in outdoor enclosures in Pretoria, South Africa.

4.2 Seasonal acclimatization

4.2.1 Body mass

Seasonally, there were significant differences in body mass (M_b) of Boschveld chickens ($P < 0.0001$). During summer, birds had lower body mass (1948.14 ± 257.03 g) compared to those in winter (2101.57 ± 268.00 g).

4.2.2 Body temperature

Body temperature (T_b) of Boschveld chickens did not vary significantly between seasons ($P > 0.05$), with winter T_b of birds (40.12 ± 1.44 °C) being similar to summer T_b (40.38 ± 1.22 °C). Seasonal comparison of T_b was possible only at $T_a \leq 35$ °C. During winter, chickens became very agitated in metabolic chambers at $T_a = 40$ °C, precluding measurements. However, in summer they remained calm at 40 °C with an average T_b (41.66 ± 0.55 °C) (Figure 4.5). Relationships between T_b and T_a were similar in both seasons, with T_b increasing with T_a from 38.96 ± 1.41 °C at $T_a \sim 22$ °C to 41.5 ± 0.68 °C at T_a ~35 °C in winter and from 39.43 ± 0.91 °C at T_a~22 °C to 41.66 ± 0.55 °C at $T_a \approx 40$ °C in summer-acclimatized birds (Figure 4.5).

4.2.3 Resting metabolic rate

Resting metabolic rate in birds established in winter RMR (3.94 ± 1.42 W) was significantly lower ($P = 0.0001$) than the summer RMR (5.54 ± 2.25 W). Measurements of seasonal RMR were done only at $T_a \leq 35$ °C. Typical endothermic pattern of RMR decreasing with increasing T_a in summer was observed below the thermoneutral zone; maximum RMR in summer were observed at T_a ~20 °C (6.82 ± 2.09 W) and minimum RMR was observed at $T_a \approx 25$ °C (5.27 ± 1.97 W). Minimum RMR in winter was observed at $T_a = 30$ °C (3.51 ± 1.39 W), whereas maximum RMR was observed at $T_a = 20$ °C (4.62 ± 1.15 W) (Figure 4.6).

4.2.4 Thermal conductance

Thermal conductance of Boschveld chickens varied significantly between seasons ($F_{1:115} = 47.68$, P< 0001) with average thermal conductance being high in summer than in winter (0.54 ± 0.28 W $°C^{-1}$) (0.36 ± 0.17 W $°C^{-1}$, n= 19) (Figure 4.7). Conductance increased with temperature in summer.

4.2.5 Total evaporative water loss

Total evaporative water loss of chicken showed no significant differences between seasons ($P>0.05$). Total evaporative water loss increased with increasing temperature in both seasons but birds showed slightly higher evaporative heat loss in summer. Some individuals showed higher TEWL in winter than others, for instance at T_a between 30 °C and 35 °C (Figure 4.8).

4.2.6 Evaporative heat loss/ Metabolic heat production

Overall, evaporative cooling efficiency (i.e., EHL/MHP) was significantly affected by season ($P<0.001$). The ratio of EHL/MHP was especially high in winter (0.90 ± 0.65) and low in summer (0.75 ± 0.47). In both seasons EHL/MHP increased with increasing T_a and reached a maximum of 1.32 ± 0.83 in winter and a maximum 0.75 ± 0.18 at $T_a = 35$ °C respectively. Seasonal comparison of EHL/MHP between winter and summer were only possible at $T_a \leq 35$ °C because during winter, EHL/MHP of Boschveld chickens reached a plateau at $T_a \sim 35$ °C suggesting the maximum evaporative capacity for heat dissipation had been reached.

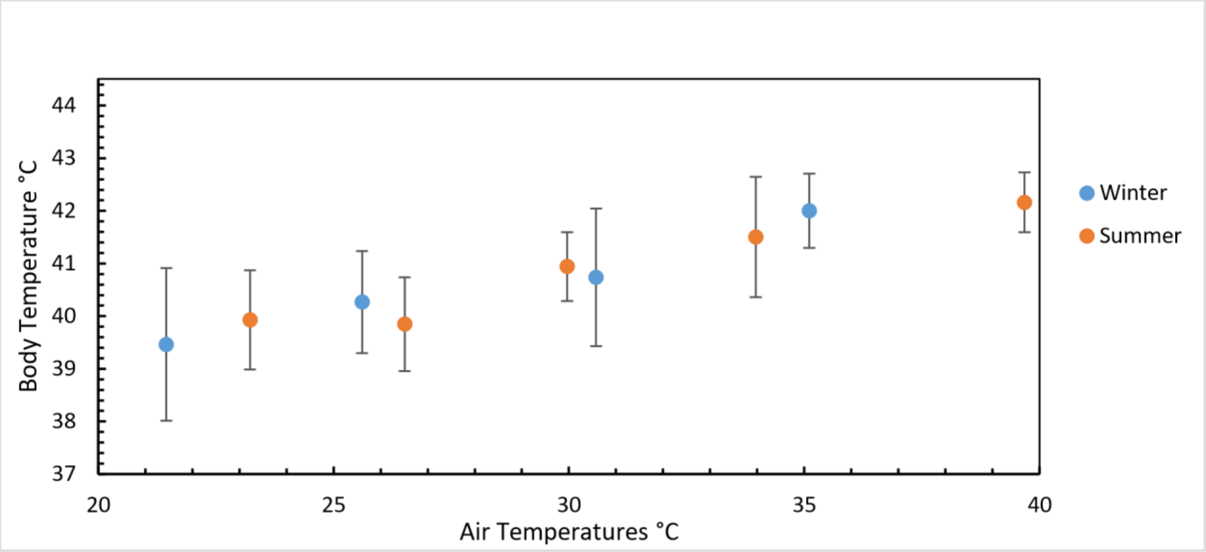

Figure 4.5 Body temperature (T_b) of seasonally-acclimatised Boschveld chickens.

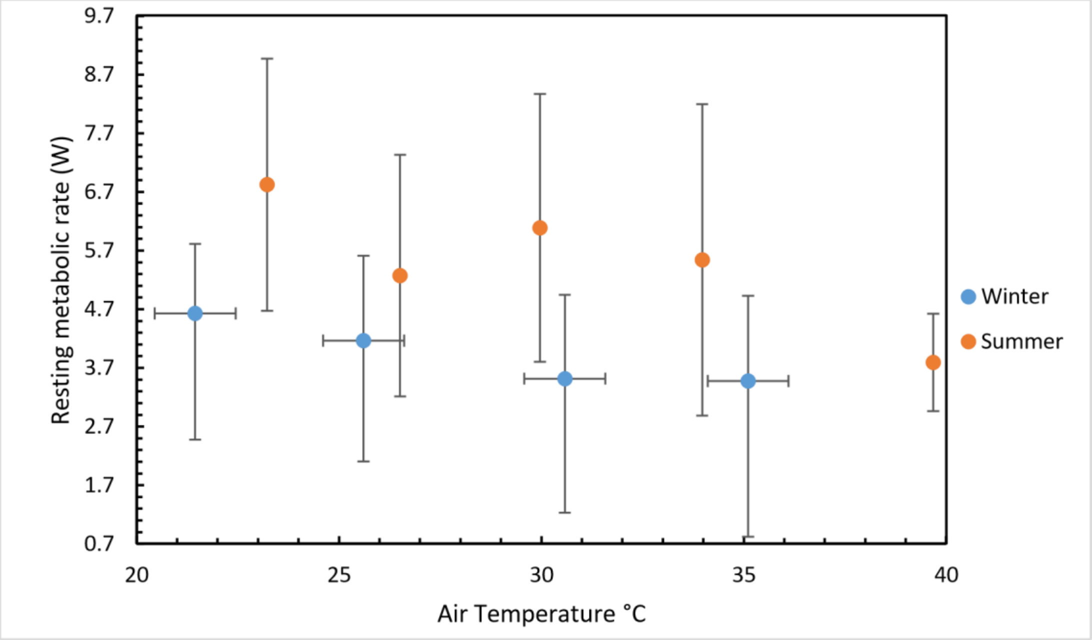

Figure 4.6 Resting metabolic rate (RMR) of seasonally-acclimatised Boschveld chickens.

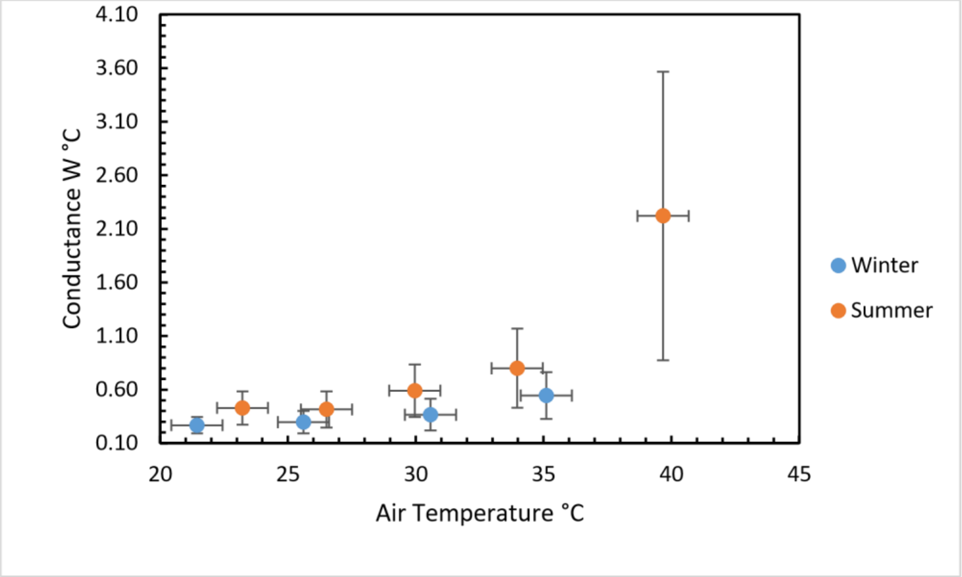

Figure 4.7 Thermal conductance of Boschveld chickens as a function of air temperature (T_a) following acclimatisation**.**

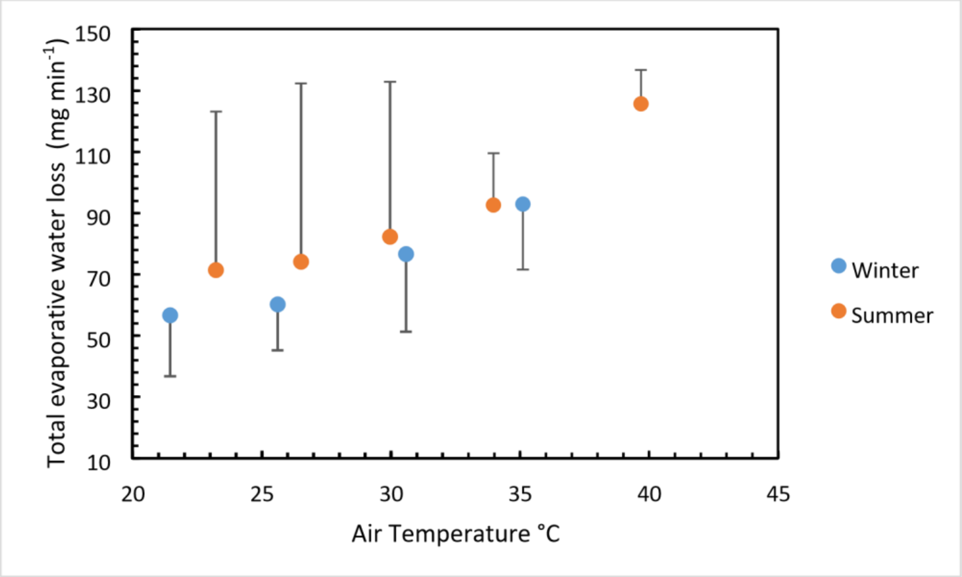

Figure 4.8 Total evaporative water loss of Boschveld chickens following acclimatisation in chickens kept in outdoor aviaries.

Chapter 5: Discussion

5.1 Partitioning of evaporative water loss

The results of this study do not support the hypothesis in experiment 1 that REWL represents the major avenue of heat dissipation in Boschveld chickens at T_a approaching or exceeding normothermic T_b. Instead CEWL accounts for 58.56 - 66.34 % of evaporative heat dissipation at $T_a \geq 39$ °C.

5.1.1 Body Temperature

The positive relationship between T_b and T_a at $20 \geq T_a \geq 35$ °C in the current study appears to be a common occurrence amongst birds (Figure 4.1) and has been found in broilers and other indigenous genotypes (Aengwanech, 2007; Yahav *et al.*, 1998), as well as in wild birds (Tieleman & Williams, 1999; O'Connor *et al.*, 2017). According to Nilsson *et al.* (2016), birds allow T_b to increase with T_a in an effort to offset the costs of thermoregulation with T_b increasing to 5 °C above normothermic levels during acute heat exposure.

In the current study, T_b appeared to reach its peak at $T_a \approx 35$ °C, T_b=42.31 °C± 0.57; however, the cause of this plateau is unclear and not easy to compare with other poultry studies. A plethora of poultry studies have evaluated T_b thermoregulation within two temperature set points ranging between 20-38 °C (Lin *et al.*, 2005; Soleimani *et al.*, 2011; Aengwanich, 2007; Ribeiro *et al.*, 2020). The T_b at $T_a \approx 35$ °C in the current study is higher than T_b observed in Red Jungle Fowl (T_b= 41.62 °C), indigenous village fowl (T_b= 41.91 °C) however lower than in broiler chicken (T_b= 43.65 °C) at T_a= 36 °C according to the study by Soleimani *et al.* (2011).

This somewhat suggests a more efficient thermoregulation of T_b by Red Jungle Fowl and indigenous village fowl compared to the Boschveld chicken and does indicate variations that exists amongst the breeds in patterns of thermoregulation (Soleimani *et al.*, 2011). On the other hand, T_b=42.31 °C at $T_a \approx 35$ °C in the current study is similar to T_b (42.3 °C) at T_a =35 °C observed in Naked neck chickens (Yahav *et al.*, 1998).

However, T_b = 41.96 °C of Boschveld chickens at $T_a \approx 39$ °C is notably lower than T_b of Naked neck chickens at T_a 35 °C. Although this evidence suggests efficient thermoregulation of T_b by Boschveld chickens at higher T_a this is not entirely conclusive since Yahav *et al.* (1998) only evaluated thermoregulation in Naked neck chickens between two T_a set points i.e., 15 and 35°C.

Evidence suggests that the Naked neck (*Na*) gene reduces feather mass which in turn improves heat dissipation and high tolerance at high T_a (Singh *et al.*, 2001), however, the extent of the physiological performance of these birds at $T_a > 35$ °C is unknown. Of course, it is possible that the thermoregulatory efficiency of Naked necks extends well above T_a 35 °C, perhaps a comparative study on thermoregulation and heat dissipation of Boschveld chickens and Naked necks is warranted, Nevertheless, the differences between the two birds in patterns of thermoregulation could have not only been as a result of genetics but also method employed.

In the current study, T_b was measured using temperature-sensitive passive integrated transponder (PIT) tags injected subcutaneously on the right flank of the birds. This method is considered non-invasive and has been used in a numerous study in avian thermoregulation (Minnaar *et al*., 2014; Oswald *et al*., 2018; Noakes & Mckechnie, 2020). Moreover, relative humidity was maintained at 15%, considering that it has a significant influence on rectal temperature at relatively high temperatures of 35°C, but not at 30 °C in four week-old broiler chickens (Lin *et al*., 2005). However, the authors concluded that the effects of humidity on chickens can be affected by ambient temperature and age of the animal. In the current study, at $20 \geq T_a \leq 39$ °C, T_b measurements were carried out for 1 hour, meanwhile at $T_a > 39$ °C, T_b measurements were carried out for 30 minutes, because chickens began exhibiting signs of hyperthermia which were panting, guller fluttering, pale comb, wattle, diarrhoea and wings outstretched. This is in contradiction to what Marder (1973) observed in adult Bedouin hens (*Gallus domesticus),* who reported the ability of Bedouin fowl to regulate T_b at high environmental T_a ranging between 38 and 45 °C; even during long term exposure birds were able to mainatin T_b well below the critical range. This is possibly the only study that has evaluated thermoregulation of chickens at T_a exceeding 40 °C, showing Bedouin fowl to be heat tolerant than the Boschveld chicken used in this study.

5.1.2 Resting metabolic rate

Information on the relationship between metabolic rate and T_a in domestic fowl is only limited to a handful of studies, although it well studied in wild bird species. Our current understanding of RMR in chickens is largely based on broiler chickens (Meltzer, 1983; Meltzer *et al*., 1982) rather than indigenous chickens and as such potentially providing a biased perspective. Genetic modification for rapid growth coupled with unrestricted food supply is thought to be linked to unusually high metabolic rates and has made chickens more vulnerable to environmental stressors and the cost of growth in commercial chickens (Tickle & Codd, 2019; Song & King, 2015; Nawaz *et al*., 2021).

Marder (1973) evaluated thermoregulation of Bedouin fowl to high T_a ranging between 25 and 50 °C and found metabolic rates were 2.41 and 2.24 $\text{cal/g}^{-1}\ \text{h}^{-1}$ at 30 and 40 °C, respectively. This metabolic rate is notably lower than RMR observed in Boschveld chickens at $T_a \approx 30$ °C= 5.52 W and $T_a \approx 40$ °C=4.68 W in the current study (Figure 4.2). According to Marder (1973), the low metabolic rates observed in Bedouin hens are due to them being acclimatized to their natural environment and as well as acclimated to a variety of high T_a. The exposure of the hens to repeated stress increased their response to high T_a (Marder, 1973) a pattern that has been seen and documented in female Thai indigenous chicken by Aengwanech (2007).

The slight increase in RMR at $T_a \sim 42$ °C suggests that Boschveld chickens experienced somewhat metabolic costs during hyperthermia and that if temperature keeps rising as a result of global warming, these birds would suffer from heat exhaustion (Nawaz *et al.*, 2021). Those birds such as desert passerines that rely on panting have increased EWL at the cost of high metabolic rates using respiratory muscles (McKechnie *et al.*, 2017; Smith *et al.*, 2017; Talbot *et al.*, 2018). At $T_a \geq 35$ °C, Boschveld chickens were panting and gular fluttering between $T_a \sim 39$ and $T_a \sim 41$ °C.

However, there was no discernible increase in RMR, a pattern associated with gular fluttering or CEWL as means of heat dissipation (O'Connor *et al.*, 2017); previously reported in desert doves, elf owl, western screech owl and pigeons (McKechnie *et al.*, 2017; Smith *et al.*, 2017; Talbot *et al.*, 2018) as well as previously in Bedouin fowl by Marder, (1973). This is because gular fluttering has a low energy cost (Campbell, 2014), which aids in inhibiting endogenous heat production, allowing for more coherent evaporative cooling (O'Connor *et al.*, 2017).

5.1.3 Evaporative water loss

The high CEWL at $T_a \approx 20$ °C and elevated REWL between 25 and 30 °C in the current study are somewhat similar observations to those reported by Richards (1976) in Babcock 390 hybrid chickens. The author noted a lower REWL at $T_a \leq 20$ °C but a relative importance in REWL with increasing T_a. In the current study, the relative importance of REWL seem to be restricted between 25 and 30 °C that contributed about 58 and 54% of TEWL, respectively, nonetheless, Boschveld chickens showed a much a higher rate of evaporation via the skin at $T_a \geq 35$ (Figure 4.3). Although these birds had similar body weight (1.1-2.4 kg) like Babcock 390 hybrid (mean 2.04 kg), it is not clear what caused differences in heat dissipation at T_a approaching T_b aside from the fact that there could be seasonal, genetics and experimental variations that exists between the two studies.

Notably, Richards (1976) performed the experiments of partitioning evaporative water loss during the winter season meanwhile in the current study experiments were done during the summer season, however the extent and effect of season on heat dissipation of domestic fowls is lacking in poultry science as such our assumptions are not conclusive. Nevertheless, CEWL in doves has been observed to be higher in those birds acclimated at high T_a than those in cool environments (McKechnie & Wolf, 2004). Furthermore, in the current study, relative humidity was kept at 15% over T_a ranging between 20 - 43 °C, consequently, the use of CEWL coupled with gular fluttering appears to be an effective mechanism for dissipating heat at high T_a and low humidity, allowing for large increases in EWL with small or negligible increases in RMR and has been observed in heatacclimated Rock Pigeons (*Columba livia*) (O'Connor *et al.*, 2017).

Respiratory evaporative water losses (REWL) have been associated with panting leading to dehydration in many heat-stressed birds. Since REWL is a linear function of respiratory frequency (Menuam & Richards, 1975), ventilation parameters such respiratory frequency can change within the TNZ (Withers & Williams, 1990).

As such, the sudden decline in REWL between $T_a \approx 35$ and ≈ 39 °C in this study is not surprising because chickens less thermally tolerant than Boschveld chickens, like the Babcock 390 hybrid (Richards, 1975) would have a significant increase in evaporative water loss at $T_a > 35$ °C (Withers & Williams, 1990) and birds like Monk Parrot (*Myiopsitta monachus*) have been observed to pant and use lingual flutter to elevate heat dissipation at $T_a > 35$ °C (Weathers *et al*., 1978; Withers & Williams, 1990).

According to Richards (1975), overt panting in domestic fowls was observed between T_a 27-30 °C, nonetheless, in the current study, panting was observed at $T_a > 33$ °C but it appeared that within the TNZ that ranged between 35 and 39 °C (Figure 4.3), REWL does not seem to be the primary avenue of heat dissipation in Boschveld chickens suggesting a higher thermal tolerance than that observed in broiler and layer type chickens.

Although the current study is the first to demonstrated a substantial role in CEWL at T_a approaching normothermic T_b of chickens, it has on the other hand, been reported to account for more than 40% or exceeding 50% of the TEWL in Spinifex Pigeon (*Geophaps plumifera)* at 35 °C $\leq T_a \geq$ 45 °C by Withers and Williams (1990). Furthermore, the contribution of REWL compared to CEWL to the total rates of EWL during thermoregulation in wild birds have been reported to vary amongst orders (McKechnie & Wolf, 2004; Wolf & Walsberg, 1996). For instance, rates of REWL also have been estimated to represent more than 60% of TEWL in passerines; Verdins (*Auriparus flaviceps*) exposed to temperature exceeding 40 °C by Wolf & Walsberg (1996) with less than 15% contribution from CEWL.
The relationship between TEWL and T_a followed the same pattern of increasing with increasing T_a between T_a 25 and 35 °C, then at 35 $\geq T_a \leq$ 39 °C TEWL appeared to have reached an equilibrium, however at T_a>39 °C, there was a marked increase in TEWL by 4-fold (Figure 4.4). At T_a above 39 °C, CEWL accounted for 50% - 66% of the TEWL (insert graph). Based on the current observations, it appears that CEWL represents an important factor in maintaining thermoregulation in Boschveld chickens under hot ambient conditions, as well as for water conservation (McKechnie & Wolf, 2004).

5.2 Seasonal acclimatization

The results of this study do not support the hypothesis that RMR is upregulated in winter, instead chickens showed a much higher upregulation of RMR in summer than in winter.

5.2.1 Body mass

Poultry tolerate a wide range of environmental temperatures such as 18-24 °C, which is the thermoneutral zone of chickens (Rajkumar *et al*., 2011). Temperature-associated environmental challenges, especially adverse environmental condition (hot and cold climate) affect poultry productive performance in both intensive and extensive production systems (Garcês *et al*., 2001; Giloh *et al*., 2012; Iyasere *et al*., 2017; Osti *et al.,* 2017*)*. Although there are numerous stressors with similar outcomes that poultry may exhibit (Belda *et al*., 2015), changing environmental temperatures directly affects their physiology and welfare with detrimental effects on productivity (Iyasere *et al*., 2017). It is reported that weight gain and feed intake in birds are optimal under thermoneutral zone (May & Lott, 2001), however vary among breeds and age groups. So therefore in certain geographical areas, the interaction of genetics and environment may impact on the overall performance traits (Okere, 2014).

This study reveals that, during winter, Boschveld chickens increase their average body mass (M_b) compared to summer. These outcomes are in contrast to those reported by Rajkumar *et al*. (2011) in naked-neck chickens fed maize-soybean meal *ad libitum*, suggesting that there could be genetic differences between these two breeds. For instance, when evaluating the performance of naked-neck and normal chicken under winter and summer temperatures, the nakedneck was found to be heavier than the normal chicken in summer, whereas they were similar in weight in winter (Rajkumar *et al*. (2011). The authors attributed this increase to the (*Na*) gene of the Naked neck, which under high temperatures reduces feather cover up to 40%, and as such reduces stress by having better heat dissipation because of increased surface area which ultimately increases growth performance.

It is worth noting that, genetics and environment do play a role on the overall performance of the chicken, however it seems other factors such as plumage (Noubandiguim *et al*., 2021), and the type of feed provided also play a role on the overall weight a chicken can have. Nevertheless, high body mass of birds during winter has previously been reported in chickens when comparing seasonal differences. For instance, when investigating seasonal effects on the growth performance of 3060 broiler chickens reared in an open-house system, Osti *et al*. (2017) found that these birds had a higher body weight in winter than in summer.

Furthermore, when comparing climate variations, the authors found, those that were grown in tropical and subtropical latitudes had a significantly higher M_b than those reared in the temperate zone. It should be mentioned that, although these results are somewhat similar to the current study, most of the available data on chickens concerning M_b is largely based on feed effects, feed intake, growth performance, in simple terms commercial rather than ecological (Osti *et al*., 2017). This study was not based on the effect of feed, nonetheless, body mass data were obtained when birds were likely post-absorptive, and feed fed to chickens was maintenance feed. A similar approach was made also by Arieli *et al*. (1979) in seasonally acclimatised White Leghorn x Rhode Island cross-bred hens but the authors observed no variations in body mass between seasons in hens. Although this might also suggest genetic differences between the two breeds, it does need a further evaluation since other factors such as geographical region might play a role in the body mass of birds. At low air temperatures, birds increase their body temperature mainly via increasing feed intake to elevate heat production, with some studies reporting up to 4% increase in feed consumption when temperatures decrease from 20 to 12 °C (May & Lott, 2001). Osti *et al*. (2017), found that energy intake was higher at temperatures below 18 °C accompanied by increases in weight and fat deposits and lower at temperatures above 26 °C. Similarly, in wild birds, increases in body mass during winter are largely attributed to fat stores following winter acclimatisation as these fat stores act as energy reserves needed to regulate body temperature when food is available in inadequate amounts and also increase insulation against energy loss (Maddocks & Geiser, 2000).

5.2.2 Body temperature

The body temperature (T_b) of meat-type chickens lies between 40.6 and 41.4 °C at T_a 21-24 °C normally referred to as the comfort zone (Christensen *et al*., 2012; Shakeri *et al*., 2020). Also, layer chickens thermal comfort zone has been reported to be 21 °C at six weeks of age (Pereira & Nääs, 2008). However, when T_a lies between 26–35 °C, birds can use evaporative cooling (Shakeri *et al*., 2020) to limit body temperature increases by 1 to 2 °C but this can be maintained for an extended period before body temperature reaches the heat tolerance limit of the bird (Ruuskanen *et al*., 2021). The thermoneutral zone (TNZ) found in this study lies between T_a = 20-25 °C and chickens had an average T_b of 39.5 °C (Figure 4.5).

The results obtained in the current study are somewhat similar to T_b of laying hens reported by Ribeiro *et al*. (2020). The authors observed cloacal T_b using a digital thermometer to lie between 39.4–39.9 °C, however, the TNZ in which the authors found homeostasis was 25.9 to 29.9 °C for air dry-bulb temperature which is quite higher than was observed in the current study. This is not surprising considering that the upper critical temperature limit differs depending on genetics, age and diet and has earlier been suggested to range between 29–32 °C for layer chickens (Pereira & Nääs, 2008). Furthermore, these differences are not only due to genetic stocks but also geographical regions in which these trials were carried out according to Pereira & Nääs (2008).

This study demonstrates that T_b of boschveld chickens does not vary between summer (40.38 ± 1.22 °C) and winter (40.12 ± 1.44 °C). However, these outcomes are in contradiction with the study by Arieli *et al*. (1979) who found that, T_b is better regulated in summer than in winter acclimatised hens. The authors also noted T_b to be high at higher T_a in winter than in summer as a result of the bird's inability to modify insulation and metabolic rate during short term exposure to high T_a but no discernible increases in T_b in both seasons were observed in the current study.

In addition, T_b of Boschveld chickens obtained in the current study are lower than those reported by Abioja *et al*. (2020). The authors reported an average rectal T_b of 41.5 °C of hens in three chicken genotypes (Transylvanian naked neck (TNN) FUNAAB-α and Nigerian indigenous chicken breed) during the dry period under humid tropical conditions.

There are couple of reasons for such instances, such as the method of experimentation, in which in the current study, T_b measurements were taken when birds were post-absorptive. Fasting causes shallow hypometabolic responses in pigeons (Phillips & Berger, 1991), adult laying hens, and reductions in rectal and foot-surface temperatures in broiler breeders (Savory *et al*., 2006; Christensen *et al*., 2012). Notably, Abioja *et al*. (2020) did not indicate whether birds were post-absorptive or not during measurements. Another possible reason is the method in which T_b measurements were taken, which can potentially cause a rise in body temperature through stress induced hyperthermia which is common in many species and is evident when core body temperature increases by 0.5 °C and a decrease in surface temperature by 1.5 °C within 10 to 15 min of the onset of an acute stressor (Edgar *et al*., 2013). In the past, T_b has typically been measured using various methods considered either invasive or non-invasive.

For instance, the use of digital thermometers has limitations, though less invasive (Chen & White, 2006) and used to measure an animal's rectal temperature (Abioja *et al*., 2020). They can lead to stress-induced hyperthermia (Dallmann *et al*., 2006) because the animal must be restrained and held (Torrao *et al*., 2011). Meanwhile, the use of surgically implanted radio-telemetry data loggers and telemetry devices are considered reliable but invasive (Iyasere *et al*., 2017). This is because**,** to use data logger's invasive surgery has to be used which could bring about risk of infection and coupled with the healing process of the animal brings about welfare concerns of the animal (Flecknell and Waterman-Pearson, 2000).

In the current study, T_b was measured using temperature-sensitive passive integrated transponder (PIT) tags (BioThermo 12-mm microchip, Destron Fearing, St. Paul, MN; resolution: 0.1 ^{o}C) injected subcutaneously on the right flank of the birds, a method considered non-invasive and has been used in a numerous study of avian thermoregulation (Minnaar *et al*., 2014; Oswald *et al*., 2018; Noakes & Mckechnie, 2020).

5.2.3 Resting metabolic rate

Summer and winter variations in avian metabolic rate and environmental condition correlates thereof have been well studied in wild bird species (Thompson *et al*., 2015) compared to poultry. Furthermore, it can be understood, resting metabolic rate in chickens is largely based on broilers rather than indigenous chickens, potentially providing a biased perspective as the metabolic rate of broilers is comparatively higher in other galliform species (Tickle & Codd, 2019). In addition, selection for rapid growth coupled with unrestricted food supply is thought to be linked to unusually high resting metabolic rate (RMR) and the cost of growth in commercial chickens (Tickle & Codd, 2019).

The study by Arieli *et al*. (1979) is perhaps the only study that has indicated variations in metabolic rate in White Leghorn x Rhode Island cross-bred hens with birds showing higher metabolic rates in winter than in summer without any variations in body weight between the two seasons. These results are in contradiction with the current study, wherein Boschveld chickens showed reduced RMR in winter than in summer, a similar pattern observed in several small passerines from subtropical latitudes (Maddocks & Geiser, 2000; Bush *et al*., 2008; Smit & Mckechnie, 2010).

Moreover, variations exist in patterns of body mass following acclimatisation. In the current study, summer metabolic measurements were taken during the laying season of chickens, however, to avoid misinterpretation or error with data collection all individuals that were reproductively active (i.e., egg laying) during experimentation were excluded and rested for the day and as such, the accuracy of the data can be guaranteed. A similar approach was followed by Arieli *et al*. (1979) using birds that were less reproductive, with variations in metabolic rate most likely from intensive breeding for high productivity at low energy cost resulting in decreased body size so as to reduce the capacity to modulate energy loss by insulation.

Indigenous chickens such as the Boschveld breed, are low-input chickens typically kept under a free-range production system that are left to scavenge for food (scraps and insects) compared to the broiler type (Badubi *et al*., 2006; Dessie *et al*., 2011), feed resources vary depending on season (Mtileni *et al*., 2009). The current study, demonstrates that winter season was not cold enough to cause chickens to have high energy demands for maintenance. As such, the reduced RMR following acclimatisation in chickens is most likely to conserve energy during the winter period which is common in Afrotropical birds (Bush *et al*., 2008). Although the functional significance of lower RMR during winter is still unknown in poultry, this study does conform to the assertion made by Smit and McKechnie (2010) that, increases in metabolic rate such as Summit metabolism (M_{sum}) during winter due to cold tolerance is not necessary for those birds inhabiting subtropical latitudes. In addition, the birds study (Boschveld chicken) experienced similar winter daytime temperatures (20 °C) to those observed by Smit and McKechnie (2010).

Smit and McKechnie (2010), noted that mild winters combined with low caloric requirements and inactive reproductive behaviour can possibly reduce basal metabolic rate (BMR) in birds compared to those subjected to long extremely cold weather such as those inhabiting north temperate regions. Furthermore, afrotropical birds exhibit low RMR in winter than in summer as a way of conserving energy (Bush *et al*., 2008). In the current study, RMR at T_a of 40 °C in summer birds is not higher than RMR at T_a of 35 °C (Figure 4.6). Furthermore, in the current study relative humidity during metabolic measurements was kept at ~15% which is low, it has been demonstrated before to have a significant effect on RMR by van Dyk *et al*. (2019). These authors found increases in humidity increased the rates of RMR of white browed sparrow-weaver (*Plocepasser mahali*) with increasing T_a.

However, the effects of humidity on RMR have been suggested to vary considerably among avian taxa (van Dyk *et al*., 2019) such as those reported by Powers (1992) for Anna's hummingbird (*Calypte anna*). In the current study panting was observed at $T_a > 33$ °C, and as T_a increased, gular fluttering was also observed, which is quite common to heat stressed chickens (Campbell, 2014).

5.2.4 Thermal conductance

Resting metabolic rate is an important parameter of measuring energy expenditure, however, the rate at which RMR increases with decreasing air temperature is affected by insulation provided by feathers and thermal conductance is a sign of this influence (Mortensen & Blix, 1986). In the current study, thermal conductance of Boschveld chickens differed significantly ($P < 0.05$) between seasons, with winter-acclimatised birds having lower conductance than summer-acclimatised birds. Similarly, Arieli *et al*. (1979), found seasonal differences in conductance with somewhat higher conductance in summer than in winter which they attributed it to modified feathering and body fat distribution.

In the current study, birds were observed to be featherless in the pectoral area, nonetheless, it is unknown to what extent these observations had to the outcomes of this study in patterns of conductance. Maddocks and Geiser (2000) stated that, decreases in conductance are presumably caused by increases in feather insulation of winter acclimatized birds. Plumage or feathers of bird's act as an important thermal buffer between the animal and its environment by holding back convective and radiative heat flow from an animal's skin surface to the environment (Wolf & Walsberg, 2000).

Ward *et al*. (2001), found no differences in the insulation properties of the plumage of broiler chickens and free-range chickens at different environmental conditions but suggested that differences may arise from different regions of the body like the pectoral region and these differences may be as a result of the living conditions of the birds (e.g cages vs out door aviaries). However, it is important to note that, birds used by Ward *et al*. (2001) were a breed different from that used in the current study, it is unknown if any quantitative studies on the genetic differences on the thermal insulation properties of plumage in chickens have been conducted so far, but can be argued that, the use of the same breed housed under different living conditions may provide a biased perspective.

Nevertheless, during cold stress, chickens can decrease heat loss from featherless areas by behavioural thermoregulation such as cover bare areas with the surrounding feathers or sit in clusters or on their legs for warmth (Wathes & Clark, 1981; Mitchell, 1985; Ward *et al*., 2001). Although free-range chickens may be exposed to low temperatures coupled with high wind speed, behaviours such as selection for warm microclimates can help reduce heat loss during cold and wet days which can ultimately contribute to heat conservation than plumage insulation (Ward *et al*., 2001).

5.2.5 Total Evaporative water loss

Data in this study revealed that total evaporative water loss (TEWL) was not significantly (P<0.05) different between seasons. According to Gavrilov & Gavrilov (2019), TEWL is greatly influenced by acclimatisation and natural selection. However, the lack of obvious differences in the current study in heat dissipation between summer and winter (Figure 4.8) is not entirely clear but it might suggest that they are able to acclimate well in warm climates. Notably, comparative analysis in the current study between both seasons were only possible at $T_a \approx 35$ °C, it is possible at T_a above 35 these birds would need to rely greatly on evaporative cooling to maintain homeostasis (Song & Beissinger, 2020) and this can be noted with the increased RMR in summer compared to winter (Figure 4.6).

In the current study, a relatively low humidity 15% was maintained in both seasons, although the effects of humidity were not well studied on the EWL of chickens. Much of the available data has largely based on production effects (e.g live weight, feed conversion ratio etc.) (Weaver & Meijerhof, 1991; Purswell *et al*., 2012; Xiong *et al*., 2017). On the other hand, data on other species of birds suggests that humidity has strong effects on EWL of birds experiencing high T_a depending on the taxa (Powers, 1992; Gerson *et al*., 2014). Nevertheless, seasonal heat balance is significantly influenced by predictable changes in metabolic heat production.

5.2.6 Evaporative heat loss / metabolic heat production

The ratio of EHL/MHP is often used to quantify maximum evaporative cooling capacity of individuals when $T_a > T_b$ (O'Connor *et al*., 2017). According to O'Connor *et al*. (2017), high ratios of EHL/MHP suggest more evaporative cooling efficiency commonly seen in those species birds that either gular flutter or predominantly use CEWL as a mechanism for heat dissipation. According to O'Connor *et al.* (2017), there are differences in the efficiency of evaporative heat dissipation between taxa. In the present study, Boschveld chickens had EHL/MHP ratios ranging from 0.57 at thermoneutral zones to higher values of 1.32 at $T_a \sim 35$ °C in winter and at T_a 40 °C in summer.

Although, these values somewhat conform with those of O'Connor *et al*. (2017), EHL/MHP values greater than 1 were observed at $T_a \sim 35$ °C in winter which is lower than T_b and this was in part of the chickens being completely acclimatized in winter and thus could not handle T_a above 35 °C. However, values of EHL/MHP greater than 1.0 were observed in summer when T_a approached T_b at the test level 40 °C. Nevertheless, results in this study are in contradiction with those observed by Noakes *et al*. (2016), who found sparrow-weavers in two different sites (Polokwane and Frankfort) to have maximum EHL/MHP values higher in summer than in winter, that ranged from 1- 2.31 at $T_a \geq 40$ °C.

The high EHL/MHP in summer might have been because birds were tested at $T_a \geq 40$ °C and as such the authors observed high TEWL and low RMR. In the current study, the low EHL/MHP in summer is most likely due to T_a not being high enough to exceed T_b. Furthermore, the high RMR in summer drastically reduced the value of EHL/MHP. Arguably, had T_a equalled or exceeded 40 °C, the ratio of EHL/MHP would have been higher in summer than winter as such the low RMR at T_a of 40 °C (Figure 4.6) and gradual increase of TEWL with increasing T_a (Figure 4.8) is an indication of this fact.

5.3 Limitations

This study demonstrates that Boschveld chickens, a composite cross of the Venda, Ovambo and Matabele breeds; are an adaptable breed of chickens. This study was conducted only on female Boschveld chickens it is not clear whether sex has any influence on heat dissipation or seasonal acclimatisation. Furthermore, with limited research conducted on thermoregulatory abilities of non-commercial chicken breeds, coupled with the sample size of 19 birds, the current results are not sufficient to conclude that the Boschveld has superior adaptive traits than other like breeds (e.g. Naked neck).. Nonetheless, these results do affirm their hardiness compared to the commercial chicken hybrids, such as broiler and layer genotypes. This research in part addresses the importance of indigenous breeds concerning climate change scenario. For instance, the lack of an increase in RMR at $T_a = 40$ °C coupled with low humidity 15% suggests evaporative cooling is quite efficient in these birds and they have a better chance of coping with higher temperatures in future if sufficient water is available. This study does raise questions on the effects of humidity on evaporative cooling of indigenous chickens. It has been suggested that, high T_b is correlated to high relative humidity and impairs evaporative cooling in commercial chicken hybrids (Lin *et al*., 2005). Notably, there is a paucity of studies quantifying the interaction of evaporative cooling and relative humidity in chicken's particularly indigenous chickens at high T_a, considering genetic differences amongst the stocks these interactions are most likely to differ.

Chapter 6: Conclusion and recommendation

The data in this study suggests that cutaneous evaporative water loss (CEWL) represents an important avenue of heat dissipation in Boschveld chickens and as such is suitable for extensive farming system. There was no evidence found in this study to support the initial hypothesis that RMR is elevated in winter compared to summer. The reduced RMR in summer at $T_a \approx 40$ °C suggest a much higher heat tolerance for these chickens. The ratio of EHL/MHP in the current study was measured at $T_a \leq 40$ °C, maximum evaporative cooling efficiency would have been possible had the chickens been tested at T_a greater than 40 °C. There is an inherent need to further evaluate importance of heat dissipation in poultry between seasons to establish phenotypic flexibility and the RMR of indigenous chickens because the current available data is not enough to conclude the metabolic heat production of these chickens at high T_a. As such, a quantitative analysis of the evaporative cooling efficiency of free-range chickens at $T_a >$ 40 °C is needed to further understand the results of this study.

References

Abioja, M. O., Omotara, O. P., Iyasere, O. S., Abiona, J. A., Egbeyale, L. T., & Akinjute, O. F. (2020). Comparative study of adaptation in three chicken genotypes under humid tropical conditions of Nigeria. *Journal of Animal Physiology and Animal Nutrition*, *104*(5), 1401-1409.

Aengwanich, W. 2008. Effects of high environmental temperature on the body temperature of Thai Indigenous, Thai indigenous crossbred and broiler chickens. *Asian Journal of Poultry Science,* 2: 48-52

Albright, T. P., Mutiibwa, D., Gerson, A. R., Smith, E. K., Talbot, W. A., O'Neill, J. J., & Wolf, B. O. (2017). Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proceedings of the National Academy of Sciences*, *114*(9), 2283-2288.

Arad, Z., & Marder, J. (1983). Acid-base regulation during thermal panting in the fowl (*Gallus domesticus*): Comparison between breeds. *Comparative Biochemistry and Physiology Part A: Physiology*, 74(1), 125-130.

Arieli, A., Meltzer, A., & Berman, A. (1979). Seasonal acclimatisation in the hen. *British Poultry Science*, *20*(6), 505-513.

Armario, A., Escorihuela, R. M., & Nadal, R. (2008). Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals. *Neuroscience & Biobehavioral Reviews*, *32*(6), 1121-1135.

Ayo, J. O., Obidi, J. A., & Rekwot, P. I. (2011). Effects of heat stress on the well-being, fertility, and hatchability of chickens in the northern Guinea savannah zone of Nigeria: a review. *International Scholarly Research Notices*, *2011*.

Badubi, S. S., Rakereng, M., & Marumo, M. (2006). Morphological characteristics and feed resources available for indigenous chickens in Botswana. *Livestock Research for Rural Development*, *18*(1), 205-211.

Belda, X., Fuentes, S., Daviu, N., Nadal, R., & Armario, A. (2015). Stress-induced sensitization: the hypothalamic–pituitary–adrenal axis and beyond. *Stress*, *18*(3), 269-279.

Boratyński, J. S., Jefimow, M., & Wojciechowski, M. S. (2016). Phenotypic flexibility of energetics in acclimated Siberian hamsters has a narrower scope in winter than in summer. *Journal of Comparative Physiology B*, *186*(3), 387-402.

Boyles, J. G., Seebacher, F., Smit, B., & McKechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integrative and Comparative Biology*, *51*(5), 676-690.

Branco, T., Moura, D. J. D., de Alencar Nääs, I., da Silva Lima, N. D., Klein, D. R., & Oliveira, S. R. D. M. (2021). The Sequential Behavior Pattern Analysis of Broiler Chickens Exposed to Heat Stress. *AgriEngineering*, *3*(3), 447-457.

Bush, N. G., Brown, M., & Downs, C. T. (2008). Seasonal effects on thermoregulatory responses of the Rock Kestrel, Falco rupicolis. *Journal of Thermal Biology*, *33*(7), 404–412.

Bushuev, A., Tolstenkov, O., Zubkova, E., Solovyeva, E., & Kerimov, A. (2018). Basal metabolic rate in free-living tropical birds: the influence of phylogenetic, behavioural, and ecological factors. *Current Zoology*, *64*(1), 33-43.

Campbell, D. L. M., De Haas, E. N., & Lee, C. (2019). A review of environmental enrichment for laying hens during rearing in relation to their behavioural and physiological development. *Poultry Science*, *98*(1), 9–28.

Campbell, G. (2014). Effects of temperature on gular fluttering and evaporative water loss in four sympatric cormorants in southern Africa. MSc in Conservation Biology, University of Cape Town (UCT).

Cândido, M. G. L., Tinôco, I. F. F., Albino, L. F. T., Freitas, L. C. S. R., Santos, T. C., Cecon, P. R., & Gates, R. S. (2020). Effects of heat stress on pullet cloacal and body temperature. *Poultry Science*, *99*(5), 2469–2477.

Cassuce, D. C., Tinôco, I. D. F., Baêta, F. C., Zolnier, S., Cecon, P. R., & Vieira, M. D. F. (2013). Thermal comfort temperature update for broiler chickens up to 21 days of age. *Engenharia Agrícola*, *33*, 28-36.

Cavieres, G., & Sabat, P. (2008). Geographic variation in the response to thermal acclimation in rufous-collared sparrows: Are physiological flexibility and environmental heterogeneity correlated? *Functional Ecology*, *22*(3), 509–515.

Chen, P. H., & White, C. E. (2006). Comparison of rectal, microchip transponder, and infrared thermometry techniques for obtaining body temperature in the laboratory rabbit (*Oryctolagus cuniculus*). *Journal of the American Association for Laboratory Animal Science*, *45*(1), 57-63.

Christensen, K., Thaxton, Y. V., Thaxton, J. P., & Scanes, C. G. (2012). Changes in body temperature during growth and in response to fasting in growing modern meat type chickens. *British poultry science*, *53*(4), 531-537.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(2), 169-178.

Daghir, N. J. (Ed.). (2008). Poultry production in hot climates. 2nd ed. Cambridge, MA: Cabi.

Dallmann, R., Steinlechner, S., Von Hörsten, S., & Karl, T. (2006). Stress-induced hyperthermia in the rat: Comparison of classical and novel recording methods. *Laboratory Animals*, *40*(2), 186– 193.

Dawson, W. R. (1982). Evaporative losses of water by birds. *Comparative Biochemistry and Physiology Part A: Physiology*, *71*(4), 495-509.

Deeb, N., & Cahaner, A. (1999). The effects of naked neck genotypes, ambient temperature, and feeding status and their interactions on body temperature and performance of broilers. *Poultry Science*, *78*(10), 1341–1346.

Dessie, T., Taye, T., Dana, N., Ayalew, W., & Hanotte, O. (2011). Current state of knowledge on phenotypic characteristics of indigenous chickens in the tropics. *World's Poultry Science Journal*, *67*(3), 507–516.

Doucette, L. I., & Geiser, F. (2008). Seasonal variation in thermal energetics of the Australian owlet nightjar (*Aegotheles cristatus*). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *151*(4), 615–620.

Edgar, J. L., Nicol, C. J., Pugh, C. A., & Paul, E. S. (2013). Surface temperature changes in response to handling in domestic chickens. *Physiology and Behaviour*, *119*, 195–200.

Freeman, M. T., Czenze, Z. J., Schoeman, K., & Mckechnie, A. E. (2020). Extreme hyperthermia tolerance in the world's most abundant wild. *Scientific Reports*, 1–6.

Garcês, A., Casey, N. H., & Horst, P. (2001). Productive performance of naked neck, frizzle and dwarf laying hens under various natural climates and two nutritional treatments. *South African Journal of Animal Sciences*, *31*(3–4), 174–180.

Gavrilov, V. M. (2017). Total evaporative water loss in birds at different ambient temperatures: Allometric and stoichiometric approaches. *Zoological studies*, *56*.

Gavrilov, V. M., & Gavrilov, V. V. (2019). Scaling of total evaporative water loss and evaporative heat loss in birds at different ambient temperatures and seasons. *International Journal of Avian & Wildlife Biology*, *4*(2), 40-53.

Gerken, M., Afnan, R., & Dörl, J. (2006). Adaptive behaviour in chickens in relation to thermoregulation. *70*(5), 199–207.

Gerson, A. R., Smith, E. K., Smit, B., McKechnie, A. E., & Wolf, B. O. (2014). The impact of humidity on evaporative cooling in small desert birds exposed to high air temperatures. *Physiological and Biochemical Zoology*, *87*(6), 782–795.

Giloh, M., Shinder, D., & Yahav, S. (2012). Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. *Poultry Science*, *91*(1), 175–188.

Grobbelaar, J. A. N., Sutherland, B., & Molalakgotla, N. M. (2010). Egg production potentials of certain indigenous chicken breeds from South Africa. *Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animales*, *46*, 25-32.

El Hadi, H., & Sykes, A. H. (1982). Thermal panting and respiratory alkalosis in the laying hen. *British Poultry Science*, *23*(1), 49-57.

Hart, J. S. (1962). Seasonal acclimatization in four species of small wild birds. *Physiological Zoology*, *35*(3), 224-236.

Hau, M., Ricklefs, R. E., Wikelski, M., Lee, K. A., & Brawn, J. D. (2010). Corticosterone, testosterone and life-history strategies of birds. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1697), 3203-3212.

Hohtola, E. (2004, July). Shivering thermogenesis in birds and mammals. In *Life in the cold: evolution, mechanisms, adaptation, and application. 12th International Hibernation Symposium* Institute of Arctic Biology. (pp. 241-252).

Iyasere, O. S., Beard, A. P., Guy, J. H., & Bateson, M. (2017a). Elevated levels of the stress hormone, corticosterone, cause "pessimistic" judgment bias in broiler chickens. *Scientific Reports*, *7*(1), 1–12.

Iyasere, O. S., Edwards, S. A., Bateson, M., Mitchell, M., & Guy, J. H. (2017b). Validation of an intramuscularly-implanted microchip and a surface infrared thermometer to estimate core body temperature in broiler chickens exposed to heat stress. *Computers and Electronics in Agriculture*, *133*, 1–8.

James A. DeShazer, G. LeRoy Hahn, & Hongwei Xin. (2009). Chapter 1: Basic Principles of the Thermal Environment and Livestock Energetics. *Livestock Energetics and Thermal Environment Management*, 1–22.

Kabeya, K. P., Robbertse, P. J., Marais, D., & Steyn, J. M. (2017). Plant anatomy as a tool for evaluating the effect of different levels of nitrogen, plant population density, row spacing and irrigation on kenaf (Hibiscus cannabinus) fibre development. *South African Journal of Plant and Soil*, *34*(5), 351–360.

Kampen, V., Mitchell, B. W., & Siegel, H. S. (1979). Thermoneutral zone of chickens as determined by measuring heat production, respiration rate, and electromyographic and electroencephalographic activity in light and dark environments and changing ambient temperatures. *The Journal of Agricultural Science*, *92*(1), 219–226.

Keambou, T. C., Hako, B. A., Ommeh, S., Bembide, C., Ngono, E. P., Manjeli, Y., Wamonje, F., Nzuki, Wanjala, B., Wamalwa, M., Cho, C. Y., Skilton, R. A., & Djikeng, A. (2014). Genetic diversity of the cameroon indigenous chicken ecotypes. *International Journal of Poultry Science*, *13*(5), 279–291.

Kruger, L. P., Nedambale, T. L., Scholtz, M. M., & Webb, E. C. (2016). The effect of environmental factors and husbandry practices on stress in goats. *Small Ruminant Research*, *141*, 1–4.

Lara, L. J., & Rostagno, M. H. (2013). Impact of heat stress on poultry production. *Animals*, *3*(2), 356–369. https://doi.org/10.3390/ani3020356

Leinonen, I., Williams, A. G., & Kyriazakis, I. (2014). The effects of welfare-enhancing system changes on the environmental impacts of broiler and egg production. *Poultry Science*, *93*(2), 256–266. https://doi.org/10.3382/ps.2013-03252

Lin, H., Zhang, H. F., Du, R., Gu, X. H., Zhang, Z. Y., Buyse, J., & Decuypere, E. (2005). Thermoregulation Responses of Broiler Chickens to Humidity at Different Ambient Temperatures. II. Four Weeks of Age.

Lin, H., Zhang, H. F., Jiao, H. C., Zhao, T., Sui, S. J., Gu, X. H., Zhang, Z. Y., Buyse, J., & Decuypere, E. (2005). Thermoregulation responses of broiler chickens to humidity at different ambient temperatures. I. One week of age. *Poultry Science*, *84*(8), 1166–1172.

Lu, Q., Wen, J., & Zhang, H. (2007). Effect of Chronic Heat Exposure on Fat Deposition and Meat Quality in Two Genetic Types of Chicken 1.

Luskick, S., Battersby, B., & Kelty, M. (1978). Behavioral thermoregulation: orientation toward the sun in herring gulls. *Science*, *200*(4337), 81-83.

Luthra, K. (2017). Evaluating thermal comfort of broiler chickens during transportation using heat index and simulated electronic chickens.

Maddocks, T. A., & Geiser, F. (2000). Seasonal variations in thermal energetics of Australian silvereyes (*Zosterops lateralis*). 327–333.

Maldonado, K. E., Cavieres, G., Veloso, C., Canals, M., & Sabat, P. (2009). Physiological responses in rufous-collared sparrows to thermal acclimation and seasonal acclimatization. *Journal of Comparative Physiology B*, *179*(3), 335-343.

Marder, J. (1973). Temperature regulation in the bedouin fowl (Gallus domesticus). *Physiological Zoology*, *46*(3), 208–217.

May, J. D., & Lott, B. D. (2001). Relating weight gain and feed:Gain of male and female broilers to rearing temperature. *Poultry Science*, *80*(5), 581–584.

McKechnie, A. E. (2008). Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: A review. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *178*(3), 235–247.

McKechnie, A. E., Chetty, K., & Lovegrove, B. G. (2007). Phenotypic flexibility in the basal metabolic rate of laughing doves: responses to short-term thermal acclimation. *Journal of Experimental Biology*, *210*(1), 97–106.

McKechnie, A. E., Gerson, A. R., McWhorter, T. J., Smith, E. K., Talbot, W. A., & Wolf, B. O. (2017). Avian thermoregulation in the heat: Evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *Journal of Experimental Biology*, *220*(13), 2436–2444.

McKechnie, A. E., Noakes, M. J., & Smit, B. (2015). Global patterns of seasonal acclimatization in avian resting metabolic rates. *Journal of Ornithology*, *156*, 367–376.

McKechnie, A. E., & Wolf, B. O. (2004). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *Journal of Experimental Biology*, *207*(2), 203-210.

Meltzer, A. (1983). Thermoneutral Zone and Resting Metabolic Rate of Broilers. *British Poultry Science*, *24*(4), 471–476. https://doi.org/10.1080/00071668308416763

Meltzer, A., Goodman, G., & Fistool, J. (1982). Thermoneutral Zone and Resting Metabolic Rate of Growing White Leghorn-Type Chickens. *British Poultry Science*, *23*(5), 383–391. https://doi.org/10.1080/00071688208447972

Mengistu, M. G., Steyn, J. M., Kunz, R. P., Doidge, I., Hlope, H. B., Everson, C. S., Jewitt, G. P. W., & Clulow, A. D. (2016). A preliminary investigation of the water use efficiency of sweet sorghum for biofuel in South Africa. *Water SA*, *42*(1), 152–160.

Menuam, B., & Richards, S. A. (1975). Observations on the sites of respiratory evaporation in the fowl during thermal panting. *Respiration Physiology*, *25*(1), 39–52.

Minnaar, I. A., Bennett, N. C., Chimimba, C. T., & McKechnie, A. E. (2014). Partitioning of evaporative water loss into respiratory and cutaneous pathways in Wahlberg's epauletted fruit bats (Epomophorus wahlbergi). *Physiological and Biochemical Zoology*, *87*(3), 475-485.

Mitchell, M. A. (1985). Effects of Air Velocity on Convective and Radiant Heat Transfer from Domestic Fowls at Environmental Temperatures of 20° and 30°C. *British Poultry Science*, *26*(3), 413–423.

Mortensen, A., & Blix, A. S. (1986). Seasonal changes in resting metabolic rate and mass-specific conductance in Svalbard ptarmigan, Norwegian rock ptarmigan and Norwegian willow ptarmigan. *Ornis Scandinavica*, 8-13.

Mtileni, B. J., Muchadeyi, F. C., Maiwashe, A., Phitsane, P. M., Halimani, T. E., Chimonyo, M., & Dzama, K. (2009). Characterisation of production systems for indigenous chicken genetic resources of South Africa. *Applied Animal Husbandry & Rural Development*, *2*(1), 18-22.

Nascimento, S. T., Maia, A. S. C., Gebremedhin, K. G., & Nascimento, C. C. N. (2017). Metabolic heat production and evaporation of poultry. *Poultry Science*, *96*(8), 2691–2698.

Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., & Liu, W. (2018). Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. *Journal of Thermal Biology*, *78*(June), 131–139.

Nawaz, A. H., Amoah, K., Leng, Q. Y., Zheng, J. H., Zhang, W. L., & Zhang, L. (2021). Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. *Frontiers in Veterinary Science*, 814.

Nilsson, J. Å., Molokwu, M. N., & Olsson, O. (2016). Body temperature regulation in hot environments. *PloS one*, *11*(8), e0161481.

Noakes, M. J., & McKechnie, A. E. (2020). Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. *Physiological and Biochemical Zoology*, *93*(2), 140-152.

Noakes, M. J., Wolf, B. O., & McKechnie, A. E. (2016). Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird. *Journal of Experimental Biology*, *219*(6), 859–869.

Noakes, M. J., Wolf, B. O., & McKechnie, A. E. (2017). Seasonal metabolic acclimatization varies in direction and magnitude among populations of an afrotropical passerine bird. *Physiological and Biochemical Zoology*, *90*(2), 178–189.

O'Connor, R. S. O., Wolf, B. O., Brigham, R. M., & Mckechnie, A. E. (2017). Avian thermoregulation in the heat: efficient evaporative cooling in two southern African nightjars. *Journal of Comparative Physiology B*, *187*(3), 477–491.

Osti, R., Bhattarai, D., & Zhou, D. (2017). Climatic variation: effects on stress levels, feed intake, and bodyweight of broilers. *Brazilian Journal of Poultry Science*, *19*, 489-496.

Oswald, K. N., Lee, A. T. K., & Smit, B. (2018). Seasonal physiological responses to heat in an alpine range-restricted bird: The Cape Rockjumper (chaetops frenatus). *Journal of Ornithology*, *159*(4), 1063–1072.

Ouchi, Y., Chowdhury, V. S., Cockrem, J. F., & Bungo, T. (2021). *Effects of Thermal Conditioning on Changes in Hepatic and Muscular Tissue Associated with Reduced Heat Production and Body Temperature in Young Chickens*. *7*(January), 1–9.

Özkan, S., Smith, W. K., & Bath, H. M. (2002). The development of thermal resistance of the feather coat in broilers with different feathering genotypes and feeding regimes. *British Poultry Science*, *43*(3), 472–481.

Pawar, S. S., Sajjanar, B., Lonkar, V. D., Kurade, N. P., Kadam, A. S., Nirmal, A. V., ... & Bal, S. K. (2016). Assessing and mitigating the impact of heat stress in poultry. *Adv. Anim. Vet. Sci*, *4*(6), 332-341.

. Pereira, D. F., & Nääs, I. D. A. (2008). Estimating the thermoneutral zone for broiler breeders using behavioral analysis. *Computers and electronics in agriculture*, *62*(1), 2-7.

Perini, F., Cendron, F., Rovelli, G., Castellini, C., Cassandro, M., & Lasagna, E. (2020). Emerging genetic tools to investigate molecular pathways related to heat stress in chickens: A review. *Animals*, *11*(1), 46.

Phillips, D. L., Rashotte, M. E., & Henderson, R. P. (1991). Energetic responses of pigeons during food deprivation and restricted feeding. *Physiology & behavior*, *50*(1), 195-203.

Pierre, K., Schlesinger, N., & Androulakis, I. P. (2016). The role of the hypothalamic-pituitaryadrenal axis in modulating seasonal changes in immunity. *Physiological genomics*, *48*(10), 719738.

Piersma, T., & Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology & Evolution*, *18*(5), 228-233.

Ribeiro, B. P. V. B., Junior, T. Y., de Oliveira, D. D., de Lima, R. R., & Zangeronimo, M. G. (2020). Thermoneutral zone for laying hens based on environmental conditions, enthalpy and thermal comfort indexes. *Journal of Thermal Biology*, *93*, 102678.

Powers, D. R. (1992). Effect of temperature and humidity on evaporative water loss in Anna's hummingbird (Calypte anna). *Journal of Comparative Physiology B*, *162*(1), 74-84.

Purswell, J. L., Dozier III, W. A., Olanrewaju, H. A., Davis, J. D., Xin, H., & Gates, R. S. (2012). Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. In *2012 IX International Livestock Environment Symposium (ILES IX)* (p. 3). American Society of Agricultural and Biological Engineers.

Rajkumar, U., Reddy, M. R., Rao, S. V., Radhika, K., & Shanmugam, M. (2011). Evaluation of growth, carcass, immune response and stress parameters in naked neck chicken and their normal siblings under tropical winter and summer temperatures. *Asian-Australasian Journal of Animal Sciences*, *24*(4), 509-516.

Richards, S. A. (1976). Evaporative water loss in domestic fowls and its partition in relation to ambient temperature. *The Journal of Agricultural Science*, *87*(3), 527-532.

Ruuskanen, S., Hsu, B. Y., & Nord, A. (2021). Endocrinology of thermoregulation in birds in a changing climate. *Molecular and Cellular Endocrinology*, *519*, 111088.

Ruvio, J. F., Schiassi, L., Araújo, H. B., Damasceno, F., & Junior, T. Y. (2017). Estimation of heat dissipation in broiler chickens during the first two weeks of life. *Brazilian Journal of Agriculture-Revista de Agricultura*, *92*, 248-260.

Sandercock, D. A., Hunter, R. R., Mitchell, M. A., & Hocking, P. M. (2006). Thermoregulatory capacity and muscle membrane integrity are compromised in broilers compared with layers at the same age or body weight. *British Poultry Science*, *47*(3), 322-329.

Savory, C. J., Kostal, L., & Nevison, I. M. (2006). Circadian variation in heart rate, blood pressure, body temperature and EEG of immature broiler breeder chickens in restricted-fed and ad libitum-fed states. *British poultry science*, *47*(5), 599-606.

Sebho, H. K. (2016). Exotic chicken status, production performance and constraints in Ethiopia: a review. *Asian Journal of Poultry Science*, *10*(1), 30-39.

Sejian, V., Bhatta, R., Gaughan, J. B., Dunshea, F. R., & Lacetera, N. (2018). Adaptation of animals to heat stress. *Animal*, *12*(s2), s431-s444.

Shakeri, M., Oskoueian, E., Le, H. H., & Shakeri, M. (2020). Strategies to combat heat stress in broiler chickens: unveiling the roles of selenium, vitamin E and vitamin C. *Veterinary sciences*, *7*(2), 71.

Singh, C. V., Kumar, D., & Singh, Y. P. (2001). Potential usefulness of the plumage reducing Naked Neck (Na) gene in poultry production at normal and high ambient temperatures. *World's Poultry Science Journal*, *57*(2), 139-156.

Smit, B., & McKechnie, A. E. (2010). Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Functional Ecology*, *24*(2), 330-339.

Smith, E. K., O'Neill, J. J., Gerson, A. R., McKechnie, A. E., & Wolf, B. O. (2017). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert songbirds. *Journal of Experimental Biology*, *220*(18), 3290-3300.

Soleimani, A. F., Zulkifli, I., Omar, A. R., & Raha, A. R. (2011). Physiological responses of 3 chicken breeds to acute heat stress. *Poultry science*, *90*(7), 1435-1440.

Song, D. J., & King, A. J. (2015). Effects of heat stress on broiler meat quality. *World's Poultry Science Journal*, *71*(4), 701-709.

Song, S., & Beissinger, S. R. (2020). Environmental determinants of total evaporative water loss in birds at multiple temperatures. *The Auk*, *137*(1), ukz069.

Strawford, M. L., Watts, J. M., Crowe, T. G., Classen, H. L., & Shand, P. J. (2011). The effect of simulated cold weather transport on core body temperature and behavior of broilers. *Poultry science*, *90*(11), 2415-2424.

Swanson, D. L., & Vézina, F. (2015). Environmental, ecological and mechanistic drivers of avian seasonal metabolic flexibility in response to cold winters. *Journal of Ornithology*, *156*(1), 377388.

Sykes, A. H., & Fataftah, A. R. A. (1986). Acclimatisation of the fowl to intermittent acute heat stress. *British Poultry Science*, *27*(2), 289-300.

Talbot, W. A., Gerson, A. R., Smith, E. K., McKechnie, A. E., & Wolf, B. O. (2018). Avian thermoregulation in the heat: metabolism, evaporative cooling and gular flutter in two small owls. *Journal of Experimental Biology*, *221*(12), jeb171108.

Talbot, W. A., McWhorter, T. J., Gerson, A. R., McKechnie, A. E., & Wolf, B. O. (2017). Avian thermoregulation in the heat: evaporative cooling capacity of arid-zone Caprimulgiformes from two continents. *Journal of Experimental Biology*, *220*(19), 3488-3498.

Thompson, L. J. (2014). *Flexibility in metabolic rate in a small Afrotropical bird Zosterops virens* (Doctoral dissertation).

Thompson, L. J., Brown, M., & Downs, C. T. (2015). The potential effects of climate-change associated temperature increases on the metabolic rate of a small Afrotropical bird. *Journal of Experimental Biology*, *218*(10), 1504–1512. https://doi.org/10.1242/jeb.116509

Thompson, L. J., Brown, M., & Downs, C. T. (2016). Thermal acclimation in a small Afrotropical Bird. *Behavioural processes*, *128*, 113-118.

Tickle, P. G., & Codd, J. R. (2019). Thermoregulation in rapid growing broiler chickens is compromised by constraints on radiative and convective cooling performance. *Journal of thermal biology*, *79*, 8-14.

Tieleman, B. I., & Williams, J. B. (2002). Cutaneous and respiratory water loss in larks from arid and mesic environments. *Physiological and Biochemical Zoology*, *75*(6), 590-599.

Tieleman, B. I., & Williams, J. B. (1999). The role of hyperthermia in the water economy of desert birds. *Physiological and Biochemical Zoology*, *72*(1), 87-100.

Torrao, N. A., Hetem, R. S., Meyer, L. C. R., & Fick, L. G. (2011). Assessment of the use of temperature-sensitive microchips to determine core body temperature in goats. *Veterinary Record*, *168*(12), 328-328.

Van de Ven, T. M., Mzilikazi, N., & McKechnie, A. E. (2013). Seasonal metabolic variation in two populations of an Afrotropical euplectid bird. *Physiological and Biochemical Zoology*, *86*(1), 19-26.

Van Dyk, M., Noakes, M. J., & McKechnie, A. E. (2019). Interactions between humidity and evaporative heat dissipation in a passerine bird. *Journal of Comparative Physiology B*, *189*(2), 299-308.

Van Kampen, M. (1971). Some aspects of thermoregulation in the White Leghorn fowl. *International Journal of Biometeorology*, *15*(2), 244-246.

Vézina, F., Jalvingh, K. M., Dekinga, A., & Piersma, T. (2007). Thermogenic side effects to migratory predisposition in shorebirds. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *292*(3), R1287-R1297.

Wang, Y., Saelao, P., Chanthavixay, K., Gallardo, R., Bunn, D., Lamont, S. J., ... & Zhou, H. (2018). Physiological responses to heat stress in two genetically distinct chicken inbred lines. *Poultry science*, *97*(3), 770-780.

Ward, J. M., Houston, D. C., Ruxton, G. D., McCafferty, D. J., & Cook, P. (2001). Thermal resistance of chicken (Gallus domesticus) plumage: a comparison between broiler and free-range birds. *British poultry science*, *42*(5), 558-563.

Wathes, G. M., & Clark, J. A. (1981). Sensible heat transfer from the fowl: thermal resistance of the pelt. *British Poultry Science*, *22*(2), 175-183.

Weathers, W. W., & Caccamise, D. F. (1978). Seasonal acclimatization to temperature in monk parakeets. *Oecologia*, *35*(2), 173-183.

Weaver Jr, W. D., & Meijerhof, R. (1991). The effect of different levels of relative humidity and air movement on litter conditions, ammonia levels, growth, and carcass quality for broiler chickens. *Poultry science*, *70*(4), 746-755.

Whitfield, M. C., Smit, B., McKechnie, A. E., & Wolf, B. O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African aridzone passerines. *The Journal of experimental biology*, *218*(11), 1705-1714.

Williams, J. B. (1996). A phylogenetic perspective of evaporative water loss in birds. *The Auk*, *113*(2), 457-472.

Withers, P. C., & Williams, J. B. (1990). Metabolic and respiratory physiology of an arid-adapted Australian bird, the spinifex pigeon. *The Condor*, *92*(4), 961-969.

Wolf, B. O., & Walsberg, G. E. (2000). The role of the plumage in heat transfer processes of birds. *American Zoologist*, *40*(4), 575-584.

Wolf, B., & Walsberg, G. (1996). Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *The Journal of experimental biology*, *199*(2), 451457.

Wu, M., Xiao, Y., Yang, F., Zhou, L., Zheng, W., & Liu, J. (2014). Seasonal variation in body mass and energy budget in Chinese bulbuls (Pycnonotus sinensis). *Avian Research*, *5*(1), 1-10.

Xie, S., Turrell, E. J., & McWhorter, T. J. (2017). Behavioural responses to heat in captive native Australian birds. *Emu-Austral Ornithology*, *117*(1), 51-67.

Xiong, Y., Meng, Q. S., Jie, G. A. O., Tang, X. F., & Zhang, H. F. (2017). Effects of relative humidity on animal health and welfare. *Journal of integrative agriculture*, *16*(8), 1653-1658.

Yahav, S., & Hurwitz, S. (1996). Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. *Poultry Science*, *75*(3), 402-406.

Yahav, S., Luger, D., Cahaner, A., Dotan, M., Rusal, M., & Hurwitz, S. (1998). Thermoregulation in naked neck chickens subjected to different ambient temperatures. *British Poultry Science*, *39*(1), 133-138.

Youssef, A., Exadaktylos, V., & Berckmans, D. A. (2015). Towards real-time control of chicken activity in a ventilated chamber. *Biosystems Engineering*, *135*, 31-43.

Zhang, M., Dunshea, F. R., Warner, R. D., DiGiacomo, K., Osei-Amponsah, R., & Chauhan, S. S. (2020). Impacts of heat stress on meat quality and strategies for amelioration: A review. *International journal of biometeorology*, *64*(9), 1613-1628.

Zhou, H. J., Kong, L. L., Zhu, L. X., Hu, X. Y., Busye, J., & Song, Z. G. (2021). Effects of cold stress on growth performance, serum biochemistry, intestinal barrier molecules, and adenosine monophosphate-activated protein kinase in broilers. *Animal*, *15*(3), 100138.

Zhou, W. T., Fujita, M., & Yamamoto, S. (1999). Thermoregulatory responses and blood viscosity in dehydrated heat-exposed broilers (*Gallus domesticus*). *Journal of Thermal Biology*, *24*(3), 185192.