To my parents, my wife and my son

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ABDOLLAH AKBARIAN

ALLEVIATING SOME PHYSIOLOGICAL RESPONSES TO HIGH AMBIENT TEMPERATURES IN FINISHING BROILERS BY DIETARY PLANT EXTRACTS RICH IN PHENOLIC COMPOUNDS

Thesis submitted in fulfillment of the requirements

for the degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:

MODULEREN VAN SOMMIGE FYSIOLOGISCHE RESPONSEN OP HOGE OMGEVINGS-TEMPERATUREN BIJ VLEESKIPPEN DOOR PLANTENEXTRACTEN IN DE VOEDING RIJK AAN FENOLISCHE VERBINDINGEN

Persian translation of the title:

بهبود برخی فراسنجه‌های فیزیولوژیکی و ملکولی جوجه گوشتی در شرایط تنش گرمایی و تغذیه شده با عصاره‌های گیاهی سرشار از ترکیبات فنولیکی در دوره پایانی پرورش

This study was conducted as a Joint-PhD research between Ferdowsi University of Mashhad (Iran) and Ghent University (Belgium). Part of this study was financed by Vice President in Research and Technology of Ferdowsi University of Mashhad (grant No. 260/16523/3), and part of the study was supported by the Special Research Fund (BOF) of Ghent University (Belgium).

This research was performed at the Laboratory for Animal Nutrition and Animal Product Quality of the Ghent University (Lanupro, Melle, Belgium) and at the Department of Animal Science of Ferdowsi University of Mashhad (Mashhad, Iran).

To refer to this thesis:

Akbarian, A (2014). Alleviating some physiological responses to high ambient temperatures in finishing broilers by dietary plant extracts rich in phenolic compounds. PhD research, Ghent University, Belgium, 235 p.

ISBN 978-90-5989-701-4

LIST OF ABBREVIATIONS

3HADH	3-hydroxyacyl CoA dehydrogenase
a*	meat redness
ALT	alanine aminotransferase
ANT	A nucleotide translocator
AP-1	activator protein 1
AST	aspartate aminotransferase
avUCP	avian uncoupling proteins
B2M	beta-2-microglobulin
BR	*Brahma rassayana*
BVE	*Berberis vulgaris* root extract
BWG	body weight gain
Ca	calcium
CX	*Curcuma xanthorrhiza*
CXEO	*Curcuma xanthorrhiza* essential oil
CAT	catalase
CCO	cytochrome C oxidase
CFU	colony forming units (log_{10}/mL or g)
Cl^-	chloride
COX	cyclooxygenase
CPK	creatine phosphokinase
CS	citrate synthase
DCAB	dietary cation-anion balance (Na + K - Cl)
DL-M	DL-methionine
DL-HMTBA	DL-2-hydroxy-4-methythiobutanoic acid
DPLM	dry powdered leaves of mint
DsbA-L	disulfide-bond A oxidoreductase-like protein
EGCG	epigallocatechin-3-gallate
ELISA	enzyme-linked immunosorbent assay
EO	essential oil
FAME	fatty acid methyl esters

FBS	fasting blood sugar
FCCP	carbonyl cyanide p-trifluoromethoxyphenyl hydrazone
FCR	feed conversion ratio
FI	feed intake
FRAP	ferric reducing ability of plasma
FSE	*Forsythia suspense* extract
GH	growth hormone
GIT	gastro-intestinal tract
GN	genistein
GR	glutathione reductase
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
GSSG	oxidized glutathione
GST	glutathione S-transferase
H_2O_2	hydrogen peroxide
HDL	high density lipoprotein
HS	heat stress
HSP	heat shock protein
IFNγ	interferon gamma
IL-1β	interleukin 1 beta
LPE	lemon peel extract
K^+	potassium
Keap	Kelch-like ECH-associated protein 1
LDL	low density lipoprotein
LDH	lactate dehydrogenase
LL	*Ligustrum lucidum*
ME	metabolizable energy
MDA	malondialdehyde
MOS	mannan-oligosaccharide
MRS Agar	de man rogosa sharpe agar
Na^+	sodium
NCCR	nicotinamide adenine dinucleotide (NADPH) cytochrome c reductase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	nuclear factor erythroid 2-related factor 2
OC	*Origanum compactum*

OCEO	*Origanum compactum* essential oil
OPE	orange peel extract
OS	oxidative stress
P	phosphorus
PBS	phosphate buffered saline
PC	phenolic compounds
PCA	plate count agar
PCR	polymerase chain reaction
PM	probiotic mixture
PTSCE	polyphenols from tamarind seed coat extract
RBC	red blood cell
RH	relative humidity
ROS	reactive oxygen species
RSDBS	reduced sterile dilustion blank solution
SCCR	succinate cytochrome C reductase
SOD	superoxide dismutase
SYN	synbiotic
T_3	3,5,3′-triiodothyronine
T_4	thyroxine
TAOC	total antioxidant capacity
TBARS	thiobarbituric reactive substances
TBP	TATA box binding protein
TNF	tumor necrosis factor
TP	total protein
UA	uric acid
UCP	uncoupling protein
VRB Agar	violet red bile agar
WHC	water holding capacity

TABLE OF CONTENTS

Heat stress

The ambient temperature and relative humidity to which farm animals are exposed, are one of the most important environmental factors for animal productivity and welfare. The "thermoneutral zone" or comfort zone for finishing broilers is defined between 16°C to 25°C (Sahin et al., 2001). It is the range of temperatures in which an animal is able to behave and perform normally and does not have to actively regulate body temperature. Within the thermoneutral zone, body temperature is maintained by the thermal equation (i.e., heat production=heat loss). In fact, in the thermoneutral zone birds do not show signs of discomfort (Ahmad and Sarwar, 2006).

When broilers are exposed to ambient temperatures that are higher than the comfort zone, they will experience heat stress (HS), which is associated with a number of physiological and behavioral adaptations. It should be mentioned that heat stress not only depends on ambient temperature but also on relative humidity. Figure 1.1 illustrates the effect of increasing temperature on poultry behaviour.

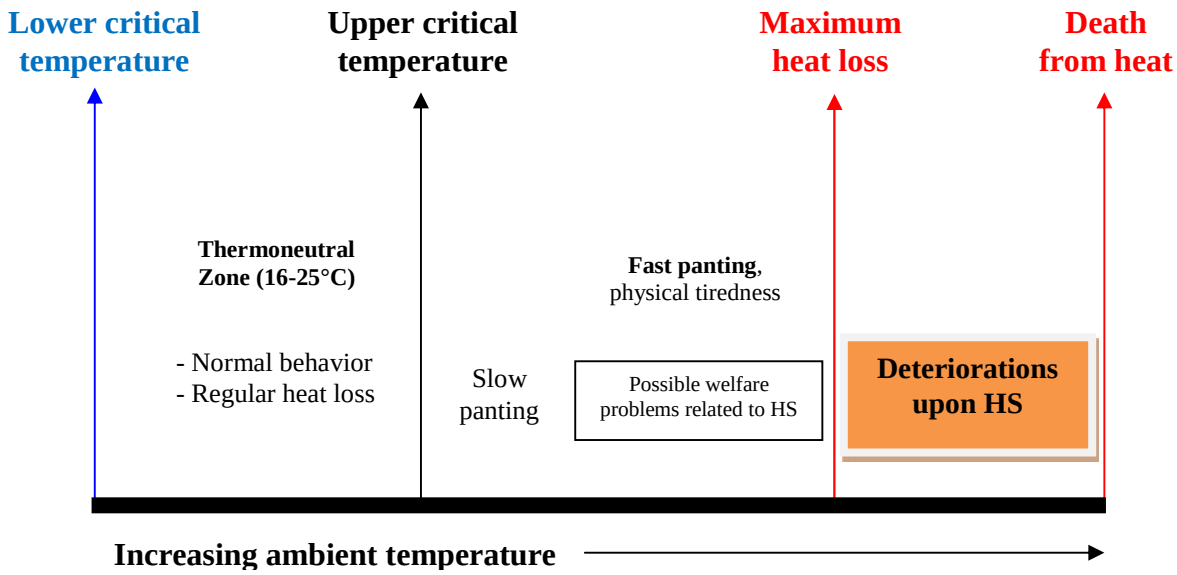


Figure 1.1. A schematic pattern of the reaction of poultry to elevated ambient temperature.

In some regions, like south of Iran, maximum daily temperatures of 30°C to 45°C during the months April – September are common and this affects broiler performance considerably in a negative manner. This leads to huge economic losses for the poultry industry every year, which is the main meat supplier in the country, due to the fact that broiler production is regarded as one of the most efficient systems of animal production in Iran. Therefore, finding proper solutions for heat stress has been a great concern for the poultry industryin Iran thus far.

Consequences of heat stress on broilers

In brief, negative effects on broilers following HS can be categorized as qualitative, quantitative and physiological impairments. Qualitative and quantitative losses such as reduced economic values, e.g., pale, soft, exudative poultry meat (Tankson et al., 2001), poor breast and leg meat yield, increased mortality, decreased weight gain; physiological impairments such as increased core temperature, depressed immunity, alteration in the electrolyte balance and blood pH (Ahmad et al., 2008), impairment in endocrine functions (Rozenboim et al., 2007), decreased energy availability to cells, alteration in the digestibility and metabolism of various nutrients, disruption in the structure and function of intestinal epithelium (Burkholder et al., 2008), and alteration of the normal and protective microbiota (Bailey et al., 2004).

Importantly, high ambient temperature also results in impaired antioxidant status and induces oxidative stress (OS) in poultry (Lin et al., 2006a). Oxidative stress is defined as the presence of reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity, i.e., an imbalance between oxidants and reductants (antioxidants). Gu et al. (2012) claimed that HS may induce OS through creating a redox imbalance in favor of pro-oxidants and inactivating/reducing cellular antioxidant capacity. HS has been shown to produce ROS excessively (Mujahid et al., 2006), and increase inflammation and OS biomarkers such as lipid peroxides in blood and tissues (Sahin et al., 2010). Morever, and depending on duration and intensity of HS, some other effects such as changes in the activities of catalase (CAT), gluthatione peroxidase (GSH-Px) and superoxide

dismutase (SOD) (Onderci et al., 2004; Sahin et al., 2013), and induction of heat shock proteins (HSP) synthesis have been reported as plausible consequences of HS on the oxidative status of broilers (Gu et al., 2012).

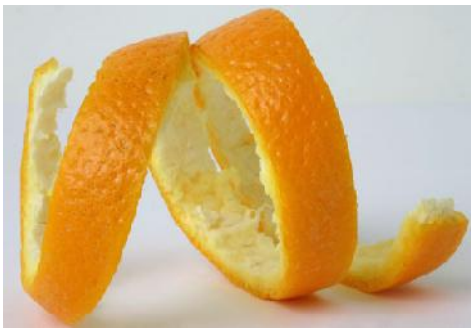
Overcoming the heat stress consequences: interest of phenolic compounds

A couple of strategies have been proposed for improving the broilers welfare and performance under hot conditions such as environmental management (e.g., using fans and fogging in house), altering feeding behaviour by adjusting lighting schemes and interrupting feed supply, reducing the stocking density, breeding (developing lines more resistant to heat), early exposure to high ambient temperature (heat acclimatization), providing cold water, feed restriction, wet feeding, changing the dietary composition (more fat in the diet to lower heat increment) and other dietary interventions, e.g., supplementation with antioxidants such as vitamin C and E, electrolytes and acids, and certain phytogenics with beneficial biological activities (Lin et al., 2006; Yahav, 2009; Lara and Rostagno., 2013). The use of phytogenics has been receiving growing interest owing to the practicality of this approach, the supposed beneficial effects (Bravo, 1998; Hikosaka et al., 2007; Hu, 2011; Rodrigo et al., 2011) and importantly the availability of these compounds with low price in the region of the study. Therefore, the approach in this PhD research was to examine theuse of rather cheap and commonly available plants extracts to counteract some adverse physiological responses to increased ambient temperatures.

In nature, phenolic compounds are found in herbs, spices and their extracts and provideseveral functions to the plant, e.g., pigmentation, growth, reproduction, resistance to pathogens, etc. (Balasundram et al., 2006). These components have been shown to exert anti-bacterial, anti-cancer, anti-inflammatory, and anti-oxidant effects *in vitro* and *in vivo* (reviewed by Brenes and Roura, 2010 and Lee et al., 2004). These ingredients are found in many plants such as fruits and vegetables. Studies have shown that fruits of the Citrus family (particularly orange and lemon), and herbs of the *Zingiberaceae* family (particularly turmeric) and mint family (*Lamiaceae;* particularly *Origanum),*

amongst many others, are rich in phenolic compounds. Orange *(Citrus aurantium)* and lemon *(Citrus limon)* peel are common by-products of the food and juice extraction industry and the most widely consumed citrus in the world (Ghasemi et al., 2009). During citrus juice processing, a considerable quantity of wastes or by-products are generated. Though large quantities of citrus pulps are diverted to animal (ruminant) feeds, the majority of the processing residue are thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications of these by-products. They are also available at low cost in most seasons in countries like Iran. The major phenolic compounds present in lemon peel extract (LPE) and orange peel extract (OPE) obtained in this PhD research are protocatechuic acid and catechol, respectively. *Curcuma xanthorrhiza* (commonly known as temulawak or Javanese turmeric in Indonesia), grows in Southeast Asia and is found both wild and cultivated in Indonesia (Singh, 2011). It is traditionally used for medicinal purposes. *Origanum compactum*, a member of the genus *Origanum* belonging to the mint family (*Lamiaceae*) is native to warm-temperate and Southwestern Eurasia and the Mediterranean region (Singh, 2011). Figure 1.2 illustrates the products that were used in this PhD research.

Lemon*(Citrus limon)* peel



Orange *(Citrus aurantium)* peel

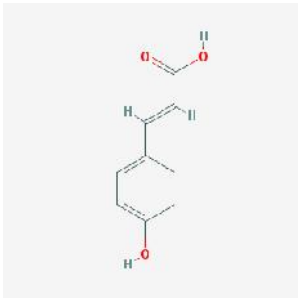
The herb of Origanum

The root of Curcuma xanthorrhiza

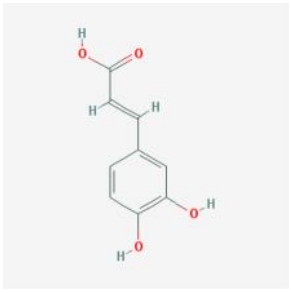
Figure 1.2. Plants and parts from which the extracts used in this PhD were obtained.

The essentials oils of *Origanum compactum* (OCEO) and *Curcuma xanthorrhiza* (CXEO) contain high amounts of simple phenols, and their antioxidant activity is well documented (Sinurat et al., 2009; Luna et al., 2010). The antioxidant activity of OCEO is mainly attributed to its main components carvacrol and thymol, and for CXEO mainly refers to ar-curcumene, β-curcumene, and xanthorrizhol. Carvacrol, thymol, and xanthorrizol are simple phenolic compounds bearing different aliphatic side chains on the aromatic ring. The main bioactive compounds in the experimental plant extracts used in the current study are given in figures 1.3 and 1.4. It should be mentioned that the composition of individual compounds in plant-derived extracts is variable. These differences may be due to a number of reasons, e.g., variation in the agricultural soil profile and time of harvest. Fu et al.

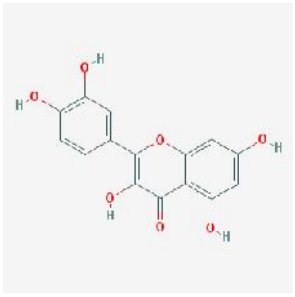
(2005) reported that similar samples produced in different countries could have a different amount of bioactive compounds. In addition, the storage conditions of the raw material and the extraction method also have a large impact on the yield of bioactive compounds, depending on the solvent type and concentration, time, temperature etc. (Lia et al., 2006; Garau et al., 2007).



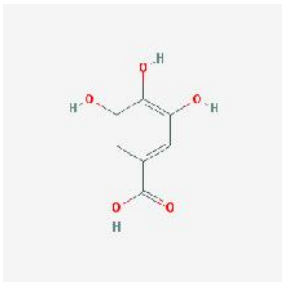
Coumaric acid



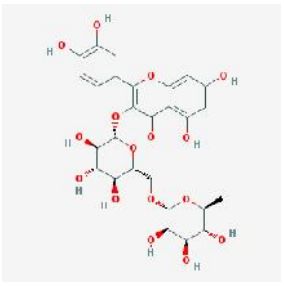
Caffeic acid



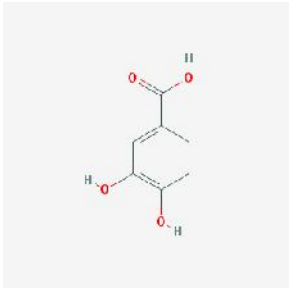
Quercetin



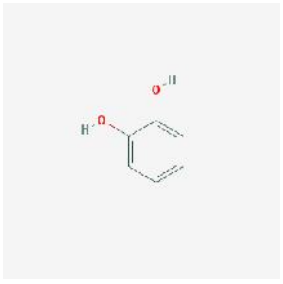
Gallic acid



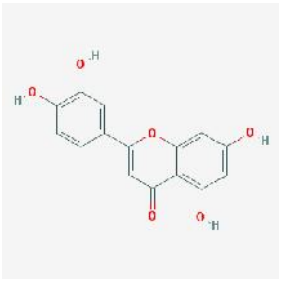
Rutin



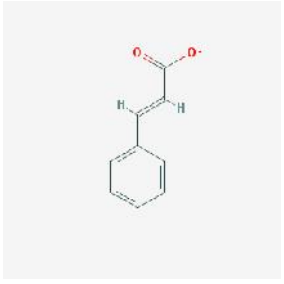
Protocatechuic acid



Catechol

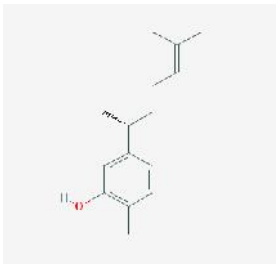


Luteolin

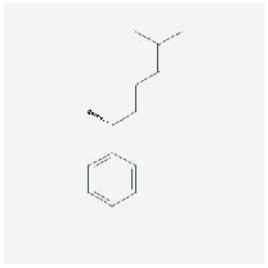


Cinnamic acid

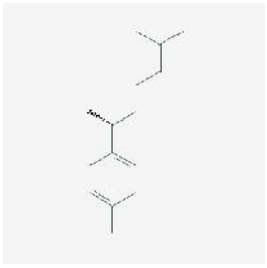
Figure 1.3. Common bioactive compounds in lemon peel and orange peel extracts.



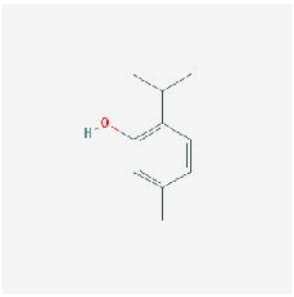
Xanthorrhizol



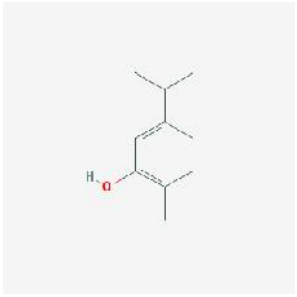
***ar*-curcumene**



β-curcumene



Thymol



Carvacrol

Figure 1.4. The main bioactive compounds in *Curcuma xanthorrhiza* and *Origanum compactum* essential oils.

This PhD research was designed to test whether these components are able to exert positive effects on broilers when submitted to cyclic chronic HS. These compounds were included in the diet during the finishing phase of the rearing period. This period was chosen because broilers at this phase are very susceptible to high temperatures and any extreme increment in barn temperatures leads to lower productivity and increases the time to reach market weight and hence results in economic losses. In order to prevent any acclimatization of broilers to high temperatures, before the commencement of the experiment, i.e., from d 0 to d 25 of age, broilers were kept in a temperature-controlled room. The initial house temperature was 32°C and was gradually decreased to reach 22°C at d 21 of age. A model of cyclic HS was chosen because the same phenomenon does happen in south of Iran in practice and the poultry industry has greatly been suffering from this kind of HS.

CHAPTER 1

A LITERATURE REVIEW OF HEAT STRESS, OXIDATIVE STRESS AND THE POTENTIAL OF ANTIOXIDANTS TO ALLEVIATE HEAT STRESS

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INTRODUCTION

Although a large number of scientific papers have been published thus far indicating deleterious effects of high ambient temperatures on poultry performance, still our knowledge of the mechanisms behind heat stress (HS) is incomplete and inconsistent (Lara and Rostagno, 2013; Mack et al., 2013). Therefore, and in light of the harmful effects of HS on poultry production efficacy in many regions of the world, investigating the mode of action of HS is essential.

Oxidative stress (OS) is regarded as a factor responsible for the deleterious effects of HS on poultry health and welfare. There has been growing evidence suggesting that HS gives rise to OS in poultry tissues. To the best of our knowledge, a compilation covering all different models of HS and its association with OS is not available so far. To this end, a literature survey on published works with special attention to HS and OS was performed to review the plausible mechanisms involved in OS induction upon HS.

Understanding the mechanisms through which reactive oxygen species (ROS; responsible for inducing OS) disturb the cellular function is necessary to take an appropriate practical measure, e.g., dietary intervention, to overcome and mitigate negative consequences of HS. The current review tries to first cover the negative consequences of high ambient temperature on poultry, thereafter, addressing the possible mechanisms involved in OS induction upon high ambient temperatures, and

then practical ways to offset this phenomenon through antioxidant compounds will be offered. The emphasis is being placed on phytogenics having antioxidant properties.

Effects of heat stress on poultry: placing the emphasis on oxidative stress related deteriorations

Definition and expansion of heat (di)stress

Poultry species have a relatively higher body temperature than other species and are less heat tolerant than mammalian species owing to the lack of sweat glands and having firm feathers which can limit their heat dissipation capacity (Yahav et al., 2004). The thermoneutral zone for finishing broilers is defined between 16°C to 25°C. Generally, when the threshold level of high ambient temperatureis crossed it results in heat distress after a cascade of thermoregulatory events. In other words, when the thermal requirements of chickens are not satisfied, heat stress may occur (Reviewed by Lin et al., 2006b) and can be lethal if birds cannot maintain the core temperature within a certain range (Yahav et al., 2005).

Importantly, attention should also be paid to inappropriate levels of relative humidity (RH) and air velocity by which birds can suffer greater than having normal RH (Veldkamp et al., 2002). Generally, it is believed that the onset of heat distress happens at a lower ambient temperature with increasing RH. As indicated in the literature, the higher RH accompanied by high temperature leads to higher respiration rate, thereupon producing negative consequences on blood pH as shown by respiratory alkalosis (Ahmad et al., 2008).

In some regions, a temperature humidity index (THI) is used to describe the conditions of heat stress. The THI is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. This index has been developed as a weather safety index to monitor and reduce heat-stress-related losses (Vale et al., 2010). Different animal species have different sensitivities to ambient temperature and the amount of moisture in the air. When the mean daily temperature falls outside of the animal's comfort zone, the amount of moisture in the air becomes a significant element in maintaining homeostasis of the animal. Because of the differences

in sensitivity to ambient temperature and amount of moisture in the air among species, a range of equations for calculation of THI with different weightings of dry bulb temperature (Tdb) and air moisture have been proposed (St-Pierre et al., 2003). The THI proposed by Tao and Xin (2003) is calculated based on dry and wet bulb temperatures, weighted as 85% and 15%, respectively, of total THI in degrees Celsius. In broilers between 31 and 40 days of age, maximum THI above 30.6 ºC and maximum temperature above 34.4ºC cause high broiler mortality (Vale et al. 2010). Another equation has also been proposed for the THI index, which equals 15 plus 0.4 times the sum of simultaneous readings of the dry- and wet-bulb temperatures. Thus, if the dry-bulb temperature is 90° F (32°C) and the wet-bulb temperature is 50° F (10° C), the discomfort index is 15 + 0.4 (140), or 71. St-Pierre et al. (2003) reported that the THI threshold for heat stress in broiler chickens is 78. Severe heat stress occurs when this index is above 90. In most cases (and depending on the levels of relative humidity) the threshold level for temperature above which finishing broilers start suffering is 30°C.

Heat stress has been a great concern among scientists and producers particularly in arid (dry, hot all year) countries as well as in the tropical (wet, hot all year) regions of the world. In these regions, the environmental temperature during the greater part of the year remains well beyond the upper limit of the bird's thermoneutral zone. However, the latter does not mean that other climates (temperate and Mediterranean having mild and hot summers, respectively) can be denied because of unexpected surge in temperature during spring and summer months. In fact, the above-mentioned regions should be treated similarly and they all need very close attention.

The negative impacts of HS on poultry can be briefly described as follows: quantitative and qualitativelosses includedepressed feed intake, which is the major reason for decreased weight gain,increased mortality, poor meat and egg production, reduced fertility and hatchability, reduced meat quality like pale, soft, exudative poultry meat (Tankson et al., 2001) and reduce egg quality, breast and leg meat yield, increased excretion of trace elements. Physiological impairments include increased core temperature, depressed immunity, impairment in endocrine and reproductive functions (Rozenboim et al., 2007), decreased energy availability to cells, alteration in the digestibility and

metabolism of various nutrients, disruption in the structure and function of intestinal epithelium (Burkholder et al., 2008), alteration of the normal and protective microbiota (Bailey et al., 2004), alteration of the electrolyte balance and blood pH (Ahmad et al., 2008), and increased circulatory cortisol and corticosterone levels.

When considering the effects of heat stress on poultry, the heat production of birds is also an important factor. The heat production of birds varies depending on size of the bird, sex, age, feed energy intake, growth rate, egg production, house temperature, air movement, etc (Hillman et al., 1985). Generally, metabolic heat production increases as environmental temperature declines to provide energy to maintain body temperature, and increases above the zone of minimum metabolism to provide energy for panting. Evaporative heat loss is minimal at low ambient temperatures and increases rapidly as soon as thermoregulation is required to alleviate an increase in ambient temperature. Sensible heat loss, which is heat transfer from the body by radiation, convection, and conduction, is negative when the environment is colder than the body temperature of the bird and positive when the body temperature of the bird is lower than the environment.

However, as commercial breeding continues to increase the growth rate of modern broilers, the rate of their heat production also increases because they consume more feed per time unit. Sandercock et al. (1995) noted that the reduction in metabolic heat production under high ambient temperature is more pronounced in fast-growing broilers than in slow-growing ones. It has been reported that high growth rates are associated with increased fasting heat production which may reduce the birds' resistance to high ambient temperatures (Keller, 1980). It is therefore inferred that genetic selection for rapid growth rate may increase the susceptibility of broilers to heat stress due to an inappropriate increase in metabolic heat production during heat stress. In other words, under heat stress conditions the thermoregulatory effort is maximal and heat loss mechanisms are insufficient to dissipate the thermal load. Usually birds adjust their metabolic heat production through thyroid hormones. In fact, the basal metabolic rate is determined by plasma T_3 concentrations in birds, and therefore a drop in T_3 level would reduce metabolic heat production to alleviate heat stress.

The sensible heat production for broilers is around 4 W/kg body weight × hour and for layers around 2.6 W/kg body weight × hour (Chepete and Xin, 2001). Hence, for example, the total daily heat production in a barn with 10 000 finishing broilers weighing on average 2.2 kg corresponds to 2 112 kW.

Different models of heat stress: Acute *v.* chronic heat stress in poultry and animal responses

Acute and chronic heat stress

In order to understand the specific effects of HS on poultry and take appropriate action, 2 major categories of HS, i.e., 'acute HS' and 'chronic HS' should be considered. Acute HS refers to a short and sudden elevated temperature which rises rapidly with a quick course, whereas chronic HS refers to a persistent high temperature over a long period of time. Another intermediate term, namely'subacute HS' should also be taken into account which is defined as the transition phase between acute and chronic HS (reviewed by Gonzales-Esquerra and Leeson, 2006). In addition, chronic HS should itself be classified as 'cyclic chronic HS' which is referred to a special time of heat exposure followed by comfortable temperature for the rest of the day, and 'constant chronic HS' which is continuously confronted by high ambient temperature.

From another standpoint, the rate of increase in ambient temperature is also an important factor. For instance, Boone (1968) noted that the body temperature of chickens began to rise when the ambient temperature increased above 30°C, if the rate of increment of ambient temperature was rapid (increasing the ambient temperature at the rate of 0.55°C in 2 min). On the other side, it has been argued that if the ambient temperature increases slowly (from 30.6°C to 40.6°C in 5 h), birds can maintain their normal body temperature until the temperature reaches 33°C (Boone and Haughes, 1971).

In another study, Nillpour and Melog (1999) reported that when the environmental temperature increased above 30ºC, approximately a 10-fold increase in respiration rate was observed from a normal rate of 25 breathes per minute. In this situation, non-evaporative cooling mechanisms

(conduction, convection and radiation) fail to dissipate heat and body temperature begins to rise. On the other hand King and Farner (1961) reported that deep body temperature at which panting (hyperventilation) was initiated varied from 41 to 43.5ºC in different species of birds.

Poultry responses to high ambient temperatures

In general, all types and ages of poultry are susceptible to HS. It is worth mentioning that continuous genetic selection for fast growing broilers, despite its positive influence on production capacity, has been associated with less tolerant and increased susceptibility of broilers to HS (Lin et al., 2006b). The harmfulness of HS is strongly linked with the lifespan development, in other words the age of birds; as it can exert more negative effects on older birds as compared to younger. This can be explained by the susceptibility of birds to high ambient temperature during the finisher phase, because as they aged, they also feathered and produce more heat in their body due to greater metabolic activity. In this respect, Mujahid et al. (2007c) showed that chicks (16-day-old) are better adapted to HS as compared with cockerels (87-day-old). The authors showed that exposing cockerels to HS stimulates substrate-independent mitochondrial superoxide production, presumably through down-regulation of avian uncoupling proteins (avUCP), while heat-exposed chicks had no difference in superoxide production.

In an early study, it was shown that poorly feathered fowl are better able to withstand higher ambient temperature, compared to those having normal feather cover. The authors concluded that higher relative humidity has less effect on body temperature and heat generation in poorly feathered fowl (Romijn and Lokhorst, 1961).

Cockerels are more resistant to high temperatures than hens of the same strain, possibly due to their relatively larger surface areas of comb and wattles helping to dissipate heat to a larger extent. Another contributing factor to this difference is the presence of an active ovary in hens and thereupon a higher metabolic activity for egg production, leading to higher heat production. It has also been

shown that laying hens have greater fasting metabolic rate (approximately 25% higher) than that of a cockerel of the same strain (Balnave, 1974).

Broilers are more susceptible than layers and in particular male broilers are more susceptible than female ones. Mujahid et al. (2005) showed broilers had a higher production of ROS in their skeletal muscle mitochondria than those of laying-type.

In general, heavier birds are more susceptible than lighter ones. At the time of HS, if they previously experienced heat shock or other stressors (e.g., feed restriction), birds will tolerate HS easier than non-experienced birds (Lin et al., 2006b). These authors stated that feed restriction at an early age has a beneficial effect on alleviating the subsequent response to heat stress. The enhanced expression of HSP70 (that persists until the later ages) is suggested to be partially responsible for the advantageous effect.

Considering the above-mentioned differences and the higher susceptibility of broilers at the finisher period than in the early life stage, closer attention should be paid to finishing broilers. During the finishing phase, the thermoneutral zone is between 16°C and 25°C (Sahin et al., 2001) and exceeding the barn temperature above this could lead to heat distress dependent on its intensity. Taking these differences into consideration it can be concluded that having a proper house temperature and climate during the finishing phase has a vital role in order to have maximum production efficacy in flocks.

Oxidative stress, definition and its relation to heat stress

Oxidative stress is defined as the presence of ROS (and also other reactive species) in excess of the available antioxidant buffering capacity, in other words, an imbalance between oxidants and reductants (antioxidants). Gu et al. (2012) claimed that HS induces OS through creating a redox imbalance in favor of pro-oxidants and inactivating/reducing cellular antioxidant capacity.

Tables 1.1 and 1.2 summarize the results of research works conducted to investigate the effects of acute and chronic models of HS on oxidative status of poultry, respectively. From Table 1.1, it is clear that the production of ROS, concentration of lipid and protein oxidation products such as MDA

and protein carbonyls, and antioxidant enzymes activities (CAT, GSH-Px and SOD) are inceased after acute HS. Moreover, avUCP mRNA levels are decreased following acute HS. Regarding chronic HS (Table 1.2), different and inconsistent results have been observed. i.e., depending ontissue, duration and severity of HS, the activity of antioxidant enzymes are increased or decreased. However, in all cases (if measured) the concentration of MDA showed a trend to increase and levels of vitamins and minerals involved in the antioxidant defense system to decrease (due to fact that upon increasing the temperature their excretion is increased) indifferent models of chronic HS. Other factors such as changes in acid-base balance, and poor absorption of vitamins, in addition to the reduced availability of vitamin in feed premixes under high temperature could also reduce vitamin utilisation in tissues (Gorman and Balnave, 1994; Belay and Teeter., 1996). Therefore, adequate vitamin and mineral supplementation are needed to improve growth, immunity, egg production, and other biological functions under heat stress conditions.

Depending on the severity and duration of HS, the antioxidant system and associated enzymes behave differently (Tables 1.1 and 1.2). To develop this further, after a short acute HS, the activity of antioxidant enzymes is increased to protect cells against surplus superoxide formation. On the other hand, in case of chronic HS, inconsistent results have been reported thus far. Increased, decreased, or unchanged activities of antioxidant enzymes have been reported. In this respect, Pamok et al. (2009) reported that exposing 28-day-old broilers for 21d to a constant chronic HS at 38±2°C, leads to increased GSH-Px activity until 11 d of HS. Sahin et al. (2013) showed that CAT activity of 21-day-old Japanese quails was decreased in response to 21d of cyclic chronic HS at 34°C for 9 h/d. It should be noticed that these studies did not have a similar protocol.

The levels of lipid peroxidation products after chronic and acute HS have been shown to be different. In other words, both models lead to an increase in blood MDA concentrations but not to the same extent,i.e., a 1.2- to 1.5-fold increase in MDA in chronic HS *v.* 4-fold increase in acute HS.

Wang et al. (2009) reported that the products of protein oxidation (protein carbonyl in breast muscle) are increased upon acute HS (30-d-old broilers were exposed for 5 h to an acute HS at 40°C).The authors showed that oxidative stress was induced after 2 h of acute HS.

Tan et al. (2010) and Yang et al. (2010) reported that the activities of enzymes involved in the respiratory chain (nicotinamide adenine dinucleotide (NADPH) cytochrome c reductase; succinate cytochrome C reductase, and cytochrome C oxidase) are decreased following acute HS (exposing broilers at d 42 and 49 to 38°C and 35°C for 3 h, respectively). This is considered a disturbance of mitochondria upon acute HS.

Gursu et al. (2004) reported that HS (10-day-old Japanese quails were exposed for 30d to a cyclic chronic HS at 34°C for 8 h/d) reduces the activity of arylesterase and paraoxonase in serum and antioxidant supplementation (vitamin C and folic acid) inverts this reduction. Recently, roles for arylesterase and paraoxonase in a number of processes have been studied, including lipid and lipoprotein metabolism, as well as oxidative stress. Gursu et al. (2004) noted that these enzymes show free radical scavenging activity. Oxidative stress was reported to negatively affect the expression and activities of arylesterase and paraoxonase. Reduced activity of these enzymes following shorter time of HS has also been reported (22-day-old Hubbard broilers for 20d exposed to a cyclic chronic HS at 35±1°C for 8 h/d) by Sohail et al. (2011).

Excessive production of ROS upon HS oxidizes and destroys cellular biological molecules, leading to a variety of impairments to intestinal tissues, hence, decreasing nutrient digestibility resulting in poor performance.

It has been shown that ROS affect the calcium release channel or Ca^{2+}-ATPase activity leading to overloading Ca^{2+} in muscles (Kaminishi et al., 1989). In turn, Ca^{2+} overload enhances glycolysis through activating adenosine monophosphate-activated protein kinase,ending with increased lactate production (Lounsbury et al., 2000). After slaughter, the anaerobic muscle metabolism results in accumulation of lactate and a decline in muscle pH until onset of rigor mortis. In case of peri-slaughter stress, which is mostly associated with elevated body temperatures, the rate of post-mortem

metabolism pH fall may be fastened (12-14 hours post-mortem) resulting in increased protein denaturation, with the meat having ultimate pale, soft and exudative characteristics. Therefore, it can be assumed that high temperature exposure, leading to overproduction of ROS and overloaded intracellular Ca^{2+} levels, may also cause deleterious effects on meat quality.

Table 1.1. Effects of acute heat stress (HS) on oxidative status of poultry (the birds were sampled at the end of HS, unless otherwise stated)

Poultry species & Treatments	Significant results (HS *v.* Cont.)	Reference
Cobb and Ross broilers At d 35 and 36 for 3 h were exposed to: 1. Cont. at 20±2°C 2. HS at 38±1°C, (RH>50%)	In both breeds: **Whole blood:** CAT, GSH-Px, and SOD activities and MDA ↑	Altan *et al.* 2003
Cobb meat and laying-type chickens At d 16 for 18 h were exposed to: 1. Cont. at 25°C 2. HS at 34°C	**Pectoralis superficialis muscle of meat type chickens:** ROS ↑	Mujahid *et al.* 2005
Cobb broilers At d 21 and d 35 for 18 h were exposed to: 1. Cont. at 25°C 2. HS at 34°C	**Pectoralis superficialis mitochondria:** at d 35: ROS ↑ **Pectoralis superficialis muscle:** at d 21: avUCP mRNA levels ↓ **Pectoralis superficialis mitochondria:** at d 21: avUCP mRNA levels ↓	Mujahid *et al.* 2006
Cobb broilers At d 35 for 6 h were exposed to: 1. Cont. at 21°C, (RH=45%) 2. HS at 32°C, (RH=40%) At 3 and 6 h after starting HS were sampled.	**Plasma:** MDA ↑ (after 3 and 6 h); FRAP ↓ (after 3 h) **Liver:** MDA ↑ (after 3 and 6 h); FRAP ↑ (after 6 h) **Heart:** SOD activity ↑ (after 6 h)	Lin *et al.* 2006
Cobb broilers At d 32 for 18 h were exposed to: 1. Cont. at 25°C 2. HS at 34°C, (RH=55±5%)	**Plasma, Pectoralis superficialis muscle:** MDA ↑ **Pectoralis superficialis muscle:** protein carbonyl ↑	Mujahid *et al.* 2007
Julia-Leghorn laying type male chickens At d 16 and d 87 for 18 h were exposed to: 1. Cont. at 25°C and 21°C 2. Chicks (d 16): HS at 34°C 3. Cockerels (d 87): HS at 34°C	**Pectoralis superficialis mitochondria:** ROS ↑ in cockerels **Pectoralis superficialis muscle:** avUCP mRNA levels ↓ in cockerels	Mujahid *et al.* 2007

Cobb broilers At d 16 for 18 h were exposed to: 1. Cont. at 25°C 2. HS at 34°C, (RH=55±5%) At 6, 12, 18 h after starting HS were sampled.	**Pectoralis superficialis mitochondria:** ROS ↑ (after all times); avUCP mRNA levels ↓ (after all times); 3HADH and CS activities ↑ (after 6 h) **Sarcoplasma:** avUCP protein levels ↓ (after all times)	Mujahid *et al.* 2007b
Arbor Acres broilers At d 30 for 5 h were exposed to: 1. Cont. at 25±1°C 2. HS at 40±1°C, (RH=53-60%) At 1, 2, 3, 5 h after starting HS were sampled.	**Pectoralis major muscle:** MDA ↑ (after 2, 3, 5 h) **Sarcoplasma:** protein carbonyl ↑ (after 2, 3, 5 h) **Myofibril:** protein carbonyl ↑ (after all times)	Wang *et al.* 2009
Cobb broilers At d 16 for 12 h were exposed to: 1. Cont. at 25°C 2. HS at 34°C, (RH=55±5%)	**Pectoralis superficialis muscle:** avUCP mRNA levels ↓ **Pectoralis superficialis mitochondria:** avUCP protein band integrity ↓ **Pectoralis superficialis muscle and mitochondria:** MDA ↑	Mujahid *et al.* 2009
Arbor Acres broilers At d 42 for 3 h were exposed to: 1. Cont. at 25°C 2. HS at 32°C 3. HS at 35°C 4. HS at 38°C	All 3 treatments *v.* Cont.: **Serum, liver:** SOD, GSH-Px, CAT activities, and MDA ↑ **Liver:** protein carbonyl ↑; NCCR and SCCR activities ↓	Tan *et al.* 2010
Arbor Acres male broilers At d 49 for 3 h were exposed to: 1. Cont. at 25°C 2. HS at 35°C, (RH=70±5%) At 0, 1, 2, 4, 8, 12 h after the end of HS were sampled.	**Serum:** GSH-Px activity and MDA levels ↑ (all times); CAT activity ↑ (at 0, 1, 2 h of HS) **Liver:** ROS, SOD activity, and MDA ↑, NCCR and CCO activities ↓ (all times); CAT activity ↑ (at 0, 1, 2, 4 h of HS); GSH-Px activity ↑ (after 0, 1, 2, 4, 8, 12 h of HS)	Yang *et al.* 2010
Cobb broilers At d 21 for 12 h were exposed to: 1. Cont. at 24°C 2. HS at 34°C, (RH=55±5%)	**Pectoralis superficialis muscle:** MDA, protein carbonyl, H_2O_2 production ↑	Kikusato & Toyomizu 2013

RH, relative humidity; CAT, catalase; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species; avUCP, avian uncoupling proteins; FRAP, ferric reducing ability of plasma; 3HADH, 3-hydroxylacyl CoA dehydrogenase; CS, citrate synthase; NCCR, nicotinamide adenine dinucleotide (NADPH) cytochrome c reductase; SCCR, succinate cytochrome C reductase; CCO, cytochrome C oxidase

Table 1.2. Effects of cyclic chronic heat stress (CyCHS) and constant chronic heat stress (CoCHS) on oxidative status of poultry (the birds were sampled at the end of HS, unless otherwise stated)

Poultry species & Treatment	Significant results (HS *v.* Cont.)	Reference
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver, heart, kidney:** MDA ↑ **Serum:** homocysteine ↑; vitamins C, E, A, Folic acid, B_{12} ↓; retention of Zn, Cu, Fe, Cr ↓	Sahin *et al.* 2003
Laying Japanese quails At 13 wk for 3 wk were exposed to: 1. Cont. at 22°C 2. CoCHS at 34°C, (RH=42%)	**Serum, liver:** MDA ↑; **Serum:** vitamins C, E, Zn ↓	Sahin & Kucuk, 2003
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d, (RH=44%)	**Serum, liver:** MDA ↑ **Serum:** homocysteine ↑; vitamins C, E, A ↓	Onderci *et al.* 2004
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver, heart, kidney:** MDA ↑ **Serum:** homocysteine ↑; vitamins C, E, A, Fe, Zn, Cu, Cr, basal paraoxonase, NaCl-stimulated paraoxonase, arylesterase ↓	Sahin *et al.* 2004
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver:** MDA ↑; vitamins C, E, and A ↓ Excretion of Ash, Ca, P, Mg, Zn, Fe, Cr ↑	Sahin *et al.* 2004
Japanese quails At d 10 for 30 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum:** basal paraoxonase, NaCl-stimulated paraoxonase, arylesterase, Albumin ↓	Gursu *et al.* 2004

Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver:** MDA ↑ **Serum:** vitamins E, C, A ↓	Sahin *et al.* 2005
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver, thigh meat:** MDA ↑	Sahin *et al.* 2005
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C, (RH=57%) 2. CyCHS at 34°C for 8 h/d (RH=42%)	**Serum, liver, heart:** MDA ↑ **Serum:** homocysteine ↑; vitamins C, E, A ↓	Sahin *et al.* 2006
Japanese quails At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver:** MDA ↑ **Serum:** Zn, vitamins C, E ↓	Sahin *et al.* 2006
Male Gramapriya egg type domestic chickens (India) At d 28 for 5 and 10 d were exposed to: 1. Cont. at 30°C,(RH=65%) 2. CyCHS at 40±1°C for 5 d for 4 h/d, (RH=80±5%) 3. CyCHS at 40±1°C for 10 d for 4 h/d, (RH=80±5%)	**Serum, liver:** MDA ↑ at d 5 and 10 **Serum:** CAT activity ↑ at d 5 and 10; SOD activity↓ at d 5 and 10 **Liver:** GSH content ↑ at d 5 and 10; CAT, SOD activities ↑ at d 5 and 10; GSH-Px activity ↑ only at d 5; GR activity↓ at d 5 and 10	Ramnath *et al.* 2008
Arbor Acres broilers At wk 4 for 3 wk were exposed to: 1. Cont. at 22°C 2. CyCHS 28°C to 34°C; time not mentioned	**Liver mitochondria:** H_2O_2 production ↑ **Liver, breast:** MDA ↑	Feng *et al.* 2008
Japanese quails	**Serum, liver:** MDA ↑	Tuzcu *et al.* 2008

At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum:** vitamins C, E, A ↓	
Japanese quails At d 55 for 90 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Serum, liver, egg yolk:** MDA ↑ **Serum, egg white, egg yolk:** Se ↓	Sahin *et al.* 2008
ISA JV 15 broilers At d 28 for 10 d were exposed to: 1. Cont. at 22°C 2. CoCHS at 32°C	**Leg muscle:** UCP rRNA levels ↑	Dridi *et al.* 2008
Broilers At d 28 for 21 d were exposed to: 1. Cont. at 26±2°C 1. CoCHS at 38±2°C for 1, 4, 7, 11, 21 d	**Serum:** MDA ↑ at d 1, 4, 7, 11 **RBC:** GSH-Px activity ↑ at d 1, 4, 7, 11	Pamok *et al.* 2009
Ross broilers At d 0 for 41 d were exposed to: 1. Cont. at 21°C 2. CoCHS at 34°C	**Plasma:** SOD activity ↑ **Plasma, liver, muscle:** MDA ↑ **Blood, liver, kidney, heart:** CAT activity ↑ **Liver, muscle, kidney, heart:** GSH content ↑ **Blood, liver, kidney, heart:** GSH-Px activity ↓	Seven *et al.* 2009
Ross broilers At d 14 for 14 d were exposed to: 1. Cont. at 24°C 2. CyCHS ranging from 32°C to 24°C to 32°C (32°C for 8 h/d) 3. CoCHT at 32°C 4. CoCHS at 34°C	**Plasma:** uric acid ↑ (in constant 34) **Pectorial superficialis muscle:** 3HADH activity ↓ (in cyclic and constant 34); CS activity ↓, Cu/Zn SOD activity ↑ (in constant 34); MDA ↑ (in both constant); avUCP rRNA levels ↑ (in all groups)	Azad *et al.* 2010
Ross broilers At d 19 for 14 d were exposed to: 1. Cont. at 24°C 1. CoCHS at 34°C for 1, 3, 5, 9, 14 d and were	**Pectorial superficialis mitochondria:** H_2O_2 production ↑ at d 5 and 9; membrane potential in state 4 ↑ at d 5; oxygen consumption in state 3 ↑ at d 5; 3HADH activity , FCCP-stimulated oxygen consumption ↑ at d 9; CS activity ↓ at d 14; SOD, CAT activities ↑ at d 14	Azad *et al.* 2010

sampled after each time, (RH=55±5%)		
Female Japanese quails At d 35 for 12 wk were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Liver:** NF-κB expression, MDA ↑; SOD, GSH-Px, CAT activities, Nrf2 expression ↓	Sahin *et al.* 2010
1. Japanese quails AT d 21 for 21 d were exposed to: 1. Cont. at 24°C 2. CyCHS at 34°C for 9 h/d	**Pectorial superficialis muscle:** SOD activity, MDA ↑; GSH content, CAT activity ↓	Halici *et al.* 2012
Ross male broilers At d 14 for 4 wk were exposed to: 1. Cont. decreased from 25.5°C to 18°C 2. CoCHS at 32°C On wk 2, 4 after starting HS were sampled.	**Plasma:** uric acid ↑ after 2 and 4 week **Liver:** total GSH, GSSG ↑; reduced GSH: total GSH, and reduced GSH: GSSG ↓ after 4 week	Willemsen *et al.* 2011
Hubbard broilers At d 22 for 20 d were exposed to: 1. Cont. at 26.7°C, (RH=65±5%) 2. CyCHS at 35±1.1°C for 8 h/d, (RH=75±5%)	**Serum:** total oxidants (μm H_2O_2 equivalent) and total antioxidants (m*M* equivalent of vitamin C), ceruloplasmin ↑; paraoxonase, arylesterase ↓	Sohail *et al.* 2011
Female Japanese quails At d 35 for 12 wk were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d	**Liver:** HSP70 levels, MDA ↑; CAT, SOD, GSH-Px activities ↓	Sahin *et al.* 2013

MDA, malondialdehyde; RH, relative humidity; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; GSH-Px, glutathione peroxidase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; 3HADH, 3-hydroxylacyl CoA dehydrogenase; CCO, cytochrome C oxidase; CS, citrate synthase; avUCP, avian uncoupling proteins; FCCP, carbonyl cyanide p-trifluoromethoxyphenyl hydrazone (an uncoupler for repiratory chain); Nrf2, nuclear factor erythroid 2–related factor 2; GSSG, oxidized glutathione; HSP, heat shock protein

Poor performance of poultry under HS/OS conditions can also be driven from the higher production and release of corticosteroids, primarily corticosterone, which is the physiological response of birds to stress. Several studies have documented that corticosterone levels are enhanced upon HS/OS (e.g., Willemsen et al., 2011). These substances have catabolic effects and high levels of glucocorticoids decrease the protein synthesis rate and elevate proteolysis in muscle leading to muscle wasting and growth retardation (Onderci et al., 2004).

Another negative consequence of HS associated with OS is an increased flux of ROS in intestinal epithelial cells exposed to acute HS (Flanagan et al., 1998). The latter increases the permeability of intestinal epithelium, which in turn helps facilitating and promoting the translocation of harmful bacteria such as *Salmonella Enteritidis* from the intestinal tract. It has been reported that free radicals show the ability of cytoskeleton disruption through promoting excessive actin polymerization in intestinal monolayers (Banan et al., 2001). Such modification could negatively affect the intestinal barrier function and absorption of nutrients, thereupon impairment in growth rate of poultry.

POTENTIAL OF ANTIOXIDANT MECHANISMS TOWARDS PREVENTING/REDUCING OXIDATIVE STRESS

Endogenous system

Living cells possess their own antioxidant system comprising the enzymes CAT, GSH-Px and SOD and non-enzymatic substances such as GSH, vitamins C and E. Different mechanisms have been suggested as mode of action of antioxidants, e.g.,GSH has a hydrogen attached to its thiol cysteine, which transforms in oxidized glutathione (GSSG) after release of the H atom. GSH is also a co-factor for the enzyme GSH-Px, whose antioxidant activity proceeds towards transforming hydroperoxides into readily eliminated alcohols. The supply of sufficient cysteine, which in turn is synthesized from methionine, guarantees appropriate synthesis of GSH. Therefore, in order to have an efficient antioxidant system, sufficient dietary sulfur amino acids are needed.

The mechanism through which antioxidant enzymes are activated is related to response elements present in the promoter region of genes coding for these enzymes (Zhou et al., 2001). AP-1 and NF-κB are these elements that in oxidative conditions combine together and with other redox-sensitive transcription factors activate certain antioxidant enzymes to deal with produced ROS. AP-1 and NF-κB are present in cytosol in an inactive status and are activated by ROS (Khassaf et al., 2003).

It is known that 'mild' uncoupling via UCP leads to less generation of ROS, through increasing the proton leakage that would lead to a reduced membrane potential in cell mitochondria. In fact, 'mild' uncoupling of mitochondrial respiration acts as a natural antioxidant mechanism. Knocking out UCPs, resulted in a strong prooxidative effect, evidenced by overproduction of ROS (Vidal-Puig et al., 2000). In chickens, an increased mitochondrial ROS production was associated with low avUCP expression in the muscle of heat-stressed chickens (Mujahid et al., 2006). The mechanism by which UCP would reduce OS has been defined by their transporting effect. UCP3 was suggested to translocate fatty acid peroxides that are formed in the inner membrane of mitochondria following HS from the inner to the outer membrane leaflet (Goglia and Skulachev, 2003). Other authors have suggested a role of uncoupling proteins in transporting C4 metabolites out of mitochondria, regulating the balance between oxidative and glycolytic metabolism (Vozza et al., 2014).

Besides the abovementioned ability, the avUCP has been shown to be involved in thermogenesis in chickens (Collin et al., 2003a,b), in the limitation of ROS generation in chickens (Abe et al., 2006), in insulin secretion, utilisation of lipids as fuel substrates and in energy metabolism determining the resting metabolic rate (Cahn et al., 1999; Schrauwen et al., 1999).

Besides the 3 main classic antioxidant enzymes (i.e., CAT, GSH-Px and SOD), some other endogenous antioxidantsareceruloplasmin, vitamin C, uric acid, arylesterase and paroxanase.

Ceruloplasmin is an acute phase protein and antioxidant. It is the major copper-carrier in the blood and acts as ferroxidase and SOD (Koh et al., 1996). Uric acid has also been considered as a potent scavenger of ROS. The contribution of uric acid to total antioxidant potential of plasma in broilers is

around 50%. There has been shown a highly positive correlation between plasma uric acid and total antioxidant power of plasma (Benzie and Strain, 1999).

Exogenous antioxidants as spare mechanism for the endogenous antioxidant system

Besides having its own antioxidant system, the body needs exogenous antioxidants in stressful conditions to cope with excessive production of ROS, because HS has been shown to deplete reserves of antioxidants (Tuzcu et al., 2008). It is well known that tocopherols (vitamin E) and ascorbic acid (vitamin C) are among the most potent and natural antioxidants . In fact, HS impairs the absorption of vitamins C and E and increases the dietary requirement of these vitamins.

Vitamin E possesses its antioxidant effect by removing free radical initiators and propagators. In this way, vitamin E transfers an H atom to stabilize free radicals and becomes itself a low reactivity free radical (Fellenberg and Speisky, 2006). An extensive review has been recently published on the effect of ascorbic in heat stressed poultry (Khan et al., 2012). Vitamin C is a potent antioxidant which is normally synthesized in the kidney of chickens. Two pathways have been proposed for the antioxidant activity of vitamin C: 1) it readily oxidizes to dehydroascorbic acid in a reversible reaction and also forms ascorbate radical and destroys hydroxyl, singlet oxygen and superoxide radicals (Lin et al., 2006b)- with having this ability, vitamin C is able to act synergistically with antioxidant enzymes (e.g., CAT, GSH-Px and SOD); and 2) vitamin C helps vitamin E to neutralize free radicals and also helps through converting the oxidized form of α-tocopherol back to α-tocopherol (Khan et al., 2012). Figure 2.1 illustrates the interaction between vitamin C, E and antioxidant tiols GSH and GSSG.

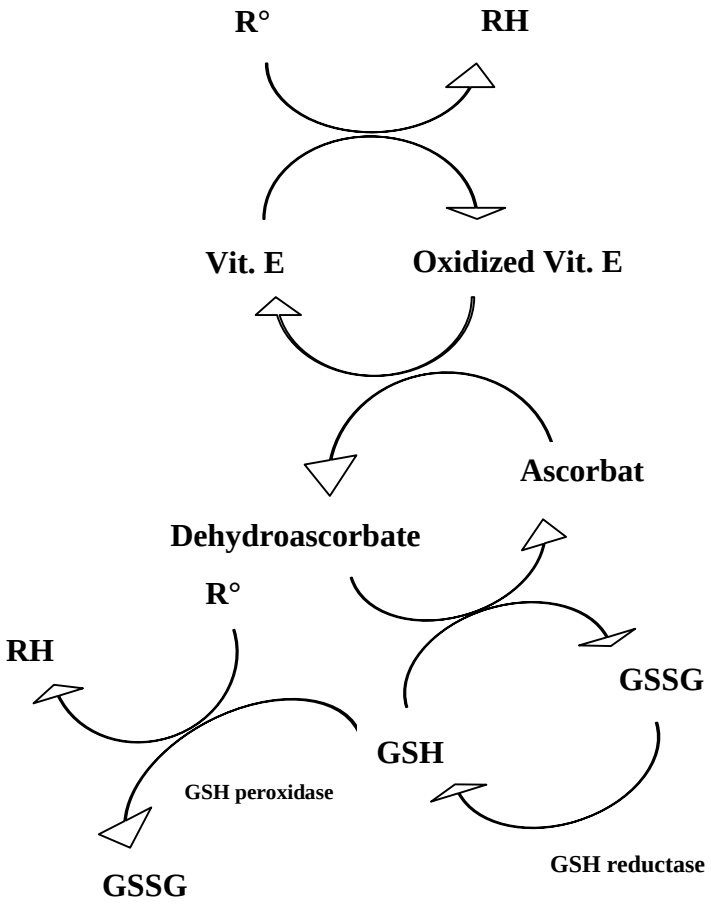

Fig. 2.1. Pathway depicting the interaction between Vit. C, Vit. E, and thiol antioxidants. Vit. E during the process of breaking the lipid peroxidation chain gets converted to the oxidized form (tocopheroxyl) that is converted back to the reduced form by ascorbic acid (Vit. C), which in turn gets converted to the oxidized or dehydroascorbate form. The regeneration of the reduced form takes place by reduced glutathione (GSH), which gets oxidized during this process and is converted back to the reduced form by glutathione reductase using NADPH as the hydrogen donor. Free radicals can also be quenched by glutathione peroxidase that requires the presence of reduced GSH (adapted from Packer et al., 2001).

Table 1.3 summarizes the results of dietary supplements on the oxidative status of poultry under acute and chronic HS. All experiments have been conducted in chronic HS models except one case. Antioxidant supplementations (either natural antioxidants like vitamin C or phytogenics with antioxidants potential) showed a positive effect on lipid peroxidation by decreasing the concentration of MDA in different tissues and reducing ROS generation and they could also increase the activity of antioxidant enzymes.

Supplementation of quails diets with epigallocatechin-3-gallate (a major polyphenol component in green tea) has been shown to enhance the activity of antioxidant enzymes (CAT, GSH-Px and SOD) under cyclic chronic HS conditions (Sahin et al., 2010). Anacardic acid, a bioactive phenolic compound of cashew nut shell oil, has been shown to exert a mild-uncoupling effect in mitochondria and also attenuated TNFα/IFNγ-stimulated ROS production in chickens (Suzuki et al., 2009).

Some results also show that these compounds are able to reduce the excretion of vitamins and minerals under HS conditions, thereby increasing their levels in serum.

Onderci et al. (2004) reported that adding an extract of genistein positively improved the oxidative status as shown by reducing levels of MDA in serum and liver and increasing concentrations of vitamins E, C, and A in serumof 10-d-old Japanese quails when they were exposed to a cyclic chronic HS at 34°C for 32 d and 8 h/d.

Positive effects of phytogenics in laying hens has also been reported. Ma et al. (2005) showed that supplementation of 2 different extracts including *Schisandra chinensis* and *Ligustrum lucidum to* Hi-Line laying hens had been successful to diminish the lipid peroxidation products in serum, liver and egg yolk when exposed at wk 60 to a constant chronic HS at 32°C for 28 d. It should be mentioned that all birds were exposed to HS and a group without HS had not been installed. These authors also reported that the activity of glutathione reductase was increased in different tissues of laying hens when they received plant extracts with antioxidant properties. This in turn helps to increase the levels of reduced glutathione in tissues.

Homocysteine is a non-protein amino acid. A high level of homocysteine makes an organism more prone to inflammation, which in turn can result in oxidative injury. Sahin et al. (2006) reported that including lycopene (extracted from tomato with high antioxidant activity) in 10-d-old Japanese quails resulted in lower concentrations of serum homocysteine. The author exposed the Japanese quails to a cyclic chronic HS at 34°C for 8 h/d for 32 d. Levels of MDA in serum, liver, and heart were lower in supplemented quails with lycopen compared to those of control birds.

ADDRESSING THE EFFECTIVENESS OF PHYTOGENICS TO ATTENUATE THE CONSEQUENCES OF HEAT STRESS

Antioxidant and favourable properties of phytogenics

Generally, the most synthetic antioxidants are derived from phenolic structures, e.g., butylated hydroxytoluene (BHT), butylated hydroxyanisode (BHA) and *tert*-butylhydroxiquinone (TBHQ). But synthetic antioxidants are under significant public scrutiny and have limitations in their use. Therefore, there is currently a great interest in exploring the effects of natural compounds.

Natural compounds such as phenolic compounds (e.g., thymol, carvacrol, xanthorrhizol, proantocyanidins, catechin, epicatechin, etc.) are classified as putative antioxidants with free-radical scavenging capacity. Recently, our study showed that phenolic compounds have beneficial effects on poultry owing to their antioxidant, antiinflammatory and antimicrobial activities (Akbarian et al., 2011).

It has been proposed that phenolic compounds supplemented to the diet exert their potential health profits to support the antioxidant system possibly through: induction and activation of antioxidant enzymes, direct scavenging of ROS produced after stress conditions and/or preventing the formation of ROS by inhibiting enzymes or chelating trace metals (Thring et al., 2011), inhibition of pro-oxidant enzymes such as NADPH-oxidase and increase in uric acid levels (Schewe et al., 2008). Effects of polyphenols in the gut may occur when they come into direct contact with epithelial cells before they are metabolized (Surai et al. 2004).

It is known that Fe and Cu possess a prooxidant effect by generation of ROSvia Haber-Weiss and/or Fenton reactions in muscle (Letelier et al., 2010). It has been suggested that polyphenols impose chelating properties and therefore change the content of free Fe and Cu in the muscle leading to lower lipid peroxidation and reduced MDA concentration (Surai, 2014).

In a recent review, Siriwardhana et al. (2013) noted that phenolic compounds might indirectly combat OS through inhibitory actions on pro-inflammatory pathways. These bioactive compounds

have been shown to suppress hepatic NF-κB activation and IκB degradation, resulting in reduced levels of pro-inflammatory cytokines such as TNFα,interleukin-1β andinterleukin 6in different tissues and inhibit the expression of some other parameters of OS such as nitric oxide synthase (Gonzales and Orlando, 2008). Other indirect mechanisms towards improving the oxidative status upon consuming phenolic compounds involves the disulfide-bond A oxidoreductase-like protein (DsbA-L) synthesis by which levels of adiponectin (a 30-KDa protein with antioxidant properties) in tissues is increased (Siriwardhana et al., 2013).

Bakker et al. (2010) reported that feed components comprising phenolic compounds are able to increase the expression of 2,3,3-trihydroxybutanoic acid, which in turn helps to regenerate vitamin C. Recently, it has been found that fruits and vegetables including phenolic compounds, noticeably increased the concentration of pro-vitamins and vitamins (β-carotene, vitamin C and E) in human serum, followed by reduced OS biomarkers (Esfahani et al., 2011).

Recently, Surai (2014) argued that some natural polyphenol compounds such as flavonoids (the largest and the most important single groups of polyphenols) are not well absorbed in the tissues of chicken/animals and their availability (mostly lower than 1 μM in plasma of healthy subjects) in target tissues is lower than a sufficient level for acting as an effective antioxidant. Therefore, it was proposed by the author to conduct more research to throw more light on their molecular mechanisms in biological systems.

Table 1.3. Effects of dietary supplements on oxidative status of poultry under cyclic chronic heat stress (CyCHS), constant chronic heat stress (CoCHS), and acute heat stress (AHS) (the birds were sampled at the end of HS, unless otherwise stated)

Heat stress model	Poultry species & diets	Significant results	Reference
At wk 13 for 3 wk were exposed to: 1. Cont. at 22°C 2. CoCHS at 34°C, (RH=42%) A 2*3 factorial design	Laying Japanese quails 1. Cont., T1; 2. Cont. + Zn at 30 mg/kg, T2; 3. Cont. + Zn at 60 mg/kg, T3	**Serum, liver:** MDA in HS groups ↓ by T2 and T3 *v.* T1 **Serum:** vitamins C, E, Zn ↑ by T2 and T3 *v.* T1	Sahin & kucuk 2003
At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d, (RH = 44%) A 2*4 factorial design	Japanese quails 1. Cont.; 2. Cont. + GN at 200 mg/kg; 3. Cont. + GN at 400 mg/kg; 4. Cont. + GN at 800 mg/kg	Under both conditions: **Serum, liver:** MDA ↓ by GN **Serum:** homocysteine ↓ by GN; vitamins C, E, A ↑ by GN	Onderci *et al.* 2004
At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d A 2*4 factorial design	Japanese quails 1. Cont.; 2. Cont. + vitamin C at 250 mg/kg 3. Cont. + melatonin at 40 mg/kg; 4. Cont. + combination	All dietary treatments *v.* Cont.: **Serum, liver:** MDA ↓ **Serum:** vitamins C, E, A ↑ Excretion of Ash, Ca, P, Mg, Zn, Fe, Cr ↓	Sahin *et al.* 2004
At d 10 for 30 d all groups were exposed to CyCHS at 34°C for 8 h/d followed by 22°C	Japanese quails 1. Cont.; 2. Cont. + vitamin C at 250 mg/kg; 3. Cont. + Folic acid at 1 mg/kg; 4. Cont. + combination	All dietary treatments *v.* Cont.: **Serum:** basal paraoxonase, NaCl-stimulated paraoxonase, arylesterase, Albumin ↑	Gursu *et al.* 2004
At wk 60 for 28 d all groups were exposed to CoCHS at 32°C	Hy-Line laying hens 1. Cont.; 2. Cont. + SC at 1%; 3. Cont. + LL at 1%	**Serum, liver, heart,egg yolk**: MDA ↓ by SC and LL **Serum, kidney, liver:** GR activity ↑ by SC and LL **Heart:** GR activity ↑ by LL	Ma *et al.* 2005
At d 10 for 32 d were exposed to: 1. Cont. at 22°C, (RH=57%) 2. CyCHS at 34°C for 8 h/d, (RH=42%) A 2*4 factorial design	Japanese quails 1. Cont.; 2. Cont. + lycopene at 50 mg/kg; 3. Cont. + lycopene at 100 mg/kg; 4. Cont. + lycopene at 200 mg/kg	Under both conditions: **Serum, liver, heart:** MDA ↓ by lycopene **Serum:**homocysteine ↓ by lycopene; vitamins C, E, A ↑ by lycopene	Sahin *et al.* 2006

At d 1 for 48 d all groups were exposed to CoCHS at 38.63±1.30°C, (RH=64±6.0%) On wk 3, 5 after starting HS were sampled.	Cobb broilers 1. Cont., T1; 2. Cont. + vitamin E at 200 mg/kg, T2; 3. Cont. + DPLM at 10 g/kg, T3; 4. Cont. + DPLM at 30 g/kg, T4; 5. Cont. + mixed Amla-Electrolyte at 1 g/kg, T5	**RBC:** MDA ↓ by all dietary treatments at 3rd and 5th week; GSH content ↑ by T3, T4, T5 *v.* T1; CAT, SOD, GR activity ↑ by all dietary treatments **Heart, liver, brain cortex:** MDA ↓ by all dietary treatments at d 49; SOD, GR activities ↑ by all dietary treatments	Maini *et al.* 2007
At d 3 for 39 d all groups were exposed to CoCHS at 32°C, (RH=44±6%)	Male Arbor Acres broilers 1. Cont., T1; 2. Cont. + vitamin C at 200 mg/kg, T2; 3. Cont. + FSE at 100 mg/kg, T3	**Serum:** TAOC ↑ by T2 and T3 at d 21 and d 42; MDA ↓ by T2 and T3 at d 21 and 42; SOD activity ↑ by T3 at d 21. **Liver:** MDA ↓ by T3 at d 42; SOD activity ↑ by T2 and T3 at 21 and by T3 at d 42. **Muscle:** SOD activity ↑ by T3 at d 42; MDA ↓ by T2 and T3 at d 42	Wang *et al.* 2008b
At d 28 for 10 d were exposed to: 1. Cont. at 30°C, (RH=65%) 2. CyCHS at 40±1°C for 5 d for 4 h/d, (RH=80±5%) 3. CyCHS at 40±1°C for 10 d for 4 h/d, (RH=80±5%)	Male Gramapriya egg type domestic chickens (India) 1. Cont.; 2. Cont. + BR at 2 g/kg	**Serum:** CAT and SOD activities ↑ by BR **Serum, liver:** MDA ↓ by BR **Liver:** CAT, SOD, GSH-Px, GR activities ↑ by BR	Ramnath *et al.* 2008
At d 10 for 32 d were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d A 2*3 factorial design	Japanese quails 1. Cont., T1; 2. Cont. + EGCG at 200 mg/kg, T2; 3. Cont. + EGCG at 400 mg/kg, T3	Under both conditions: **Serum, liver:** MDA ↓ by both T2 and T3 **Serum:** vitamins C, E, A ↑ by both T2 and T3	Tuzcu *et al.* 2008
At d 16 for 12 h were exposed to: 1. Cont. at 25°C 2. AHS at 34°C, (RH=55±5%) A 2*2 factorial design	Cobb male broilers 1. Cont., T1; 2. Cont. + olive oil (6.7 parts in 100 parts of basal diet supplemented at d 8 for 8 d, T2)	**Pectorial superficialis mitochondria:** H_2O_2 production ↓ in T2; Ratio of avUCP mRNA to 18s rRNA ↑ T2; avUCP protein band integrity ↑ in T2; MDA ↑ in HS group and ↓ in T2 **Pectorial superficialis muscle:** MDA ↑ in HS group and ↓ in T2	Mujahid *et al.* 2009
At d 0 for 41 d were exposed to: 1. Cont. at 22°C 2. CoCHS at34°C	Ross broilers 1. Positive Cont. (no HS, T1); 2. Negative Cont. (HS, T2); 3. Cont. + vitamin C at 250 mg/kg, T3; 4. Cont. + perspolis at 0.5 g/kg, T4; 5. Cont. +	All results only in HS groups: **Plasma:** SOD activity ↑ by T2 *v.* all dietary treatments; MDA ↓ in T3 and T6 *v.* T2 **Liver, muscle:** MDA ↓ by T6 *v.* T2	Seven *et al.* 2009

	perspolis at 1 g/kg, T5; 6. Cont. + perspolis at 3 g/kg, T6	**Blood, liver:** CAT activity ↑ by T2 *v.* T6 **Blood, liver, kidney, heart:** CAT activity ↑ by T2 *v.* T6; GSH-Px activity ↑ by T6 *v.* T2 **Muscle:** GSH content ↑ by T2 *v.* T3, T5, T6	
At d 18 for 27 d were exposed to: 1. Cont. at 26±2°C 2. CyCHS at 38±2°C for 6 h/d. On d 1, 7, 14, 21 after starting HS were sampled.	Male broilers 1. Cont., T1; 2. Cont. + PTSCE at 100 mg/kg, T2; 3. Cont. + PTSCE at 200 mg/kg, T3; 4. Cont. + PTSCE at 300 mg/kg, T4; 5. Cont. + PTSCE at 400 mg/kg, T5; 6. Cont. + PTSCE at 500 mg/kg, T6	**Serum:** after 1 d: MDA ↓ by T5 *v.* T1 and T2; after 7 d MDA ↓ by T1, T3, T5, T6 *v.* T2 **Serum (in average):** MDA ↓ by T1, T4, T5, T6 *v.* T2	Aengwanich & Suttajit 2010
At d 0 for 42 d all groups were exposed to CoCHS at 32.86±0.68°C.	Cobb broilers 1. Cont.; 2. Cont. + polyherbal mix of vitamin C and bioflavonoids at 1 kg/ton; 3. Cont. + vitamin C at 100 g/ton	**Serum:** SOD and GR activities ↑ in both dietary treatments	Sujatha *et al.* 2010
At d 35 for 12 wk were exposed to: 1. Cont. at 22°C 2. CyCHS at 34°C for 8 h/d A 2*3 factorial design	Female Japanese quails 1. Cont., T1; 2. Cont. + EGCG at 200 mg/kg, T2 3. Cont. + EGCG at 400 mg/kg, T3	**Liver:** MDA ↓ by T2 and T3 *v.* T1; CAT, SOD, GSH-Px activities ↑ by T2 and T3 *v.* T1; NF-κB expression ↓, Nrf2 expression ↑ by T2 and T3 *v.* T1	Sahin *et al.* 2010
At d 14 for 4 wk were exposed to: 1. Cont. decreased from 25.5°C to 18°C 2. CoCHS at 32°C A 2*5 factorial design	Ross male broilers 1. Cont.; 2. Cont. + DL-M at 1 g/kg; 3. Contr. + DL-M at 1.2 g/kg; 4. Cont. + DL-HMTBA at 1 g/kg; 5. Cont. + DL-HMTBA at 1.2 g/kg	**Plasma:** DL-M at HS *v.* DL-M at cont.: MDA ↑ **Liver:** DL-HMTBA *v.* DL-M: Reduced GSH:total GSH, Reduced GSH:GSSG ↑	Willemsen *et al.* 2011
At d 22 for 20 d were exposed to: 1. Cont. at 26.7°C, (RH=65±5%) 2. CyCHS at 35±1.1°C for 8 h/d, (RH=75±5%)	Hubbard broilers 1. Cont.; 2. Cont. + MOS at 0.5%; 3. Cont. + PM at 0.1%; 4. Cont. + SYN (MOS + PM)	All results only in HS groups: **Serum:** total oxidants (μm H_2O_2 equivalent) ↓ by PM and SYN; total antioxidant (m*M* equivalent of vitamin C) ↓ by all dietary treatments; Zn and Cu ↑ by MOS and SYN; Mg ↑ by MOS	Sohail *et al.* 2011
At d 35 for 12 wk were exposed to: 1. Cont. at 22°C	Female Japanese quails 1. Cont.; 2. Cont. + BVE at 200 mg/kg; 3. Cont. +	Under both conditions: **Liver:** MDA ↓ and CAT, SOD, GSH-Px activities ↑ by both dietary treatments	Sahin *et al.* 2013

2. CyCHS at 34°C for 8 h/d A 2*3 factorial design	BVE at 400 mg/kg		
At d 1 for 41 d all groups were exposed to CoCHS at 39±2°C. On d 14, 28, 42 after starting HS were sampled.	Ross 308 broilers 1. Cont., T1; 2. Cont. + vitamin C at 1 g/kg, T2); 3. Cont. + Zn bacitracin at 100 mg/kg, T3); 4. Cont. + combination, T4	**Serum:** vitamin C ↑ in T2 *v.* T1; CAT activity ↑ in T2 at d 28 *v.* T1 - GST activity ↑ in T2 and T3 at d 28 *v.* T1; SOD activity ↑in T2 at d 28, and T3 at d 14, 28, and 42, and T4 at d 14, 28, 42 *v.* T1; MDA ↓ in T2, T3, and T4 *v.* T1 at d 42	Ismail *et al.* 2013
At d 28 for 14 d all groups were exposed to CyCHS at 34°C for 5 h/d followed by 22°C, (RH=50-60%) On d 3 and 14 after starting HS were samples.	Ross 308 broilers 1. Cont., T1; 2. Cont. + CXEO at 200 mg/kg, T2; 3. Cont. + CXEO at 400 mg/kg, T3; 4. Cont. + OCEO at 200 mg/kg, T4; 5. Cont. + OCEO at 400 mg/kg, T5	**Plasma:** MDA ↓ in T3 *v.* T1 (after 3 d); ↓ in T2, T3, and T4 *v.* T1 (after 14 d) **RBC:** GSH content ↑ all dietary treatments (after 14 d) **Liver:** CAT activity ↑ in T2 and T5 *v.* T1 (after 14 d); GSH-Px activity ↑ in T2 *v.* T1 (after 14 d); SOD activity ↑ in T3 *v.* T1 (after 3 d); HSP70 mRNA levels ↓ in T5 *v.* T1 (after 3 d) **Kidney:** SOD activity ↑ in T3 and T5 *v.* T1; SOD mRNA levels ↑ in T5 *v.* T1; HSP70 mRNA levels ↓ in T5 *v.* T1 (all after 3 d) **Heart:** CAT activity ↑ in T3 and T5 *v.* T1; GSH-Px activity ↑ in T2, T3, and T5 *v.* T1; SOD activity ↑ in T2 and T3 *v.* T1; CAT mRNA levels ↑ in T3 and T5 *v.* T1; SOD mRNA levels ↑ in T3 *v.* T1 (all after 3 d); HSP70 mRNA levels ↓ in T3 *v.* T1 (after 14 d)	Akbarian *et al.*2014b
At d 28 for 10 d all groups were exposed to CyCHS at 34°C for 5 h/d followed by 22°C, (RH=50%)	Ross 308 broilers 1. Cont., T1; 2. Cont. + CXEO at 200 mg/kg, T2; 3. Cont. + CXEO at 400 mg/kg, T3; 4. Cont. + LPE at 200 mg/kg, T4; 5. Cont. + LPE at 400 mg/kg, T5; 2. Cont. + OPE at 200 mg/kg, T6; 3. Cont. + OPE at 400 mg/kg, T7	**RBC:** GSH-Px activity ↑ in T2, T3, and T7 *v.* T1; SOD activity ↑ in T3 *v.* T1	Akbarian *et al.*2014a

RH, relative humidity; MDA, malondialdehyde; GN, genistein; SC, *schisandra chinensis*; LL, *ligustrum lucidum*; GR, glutathione reductase; DPLM, dry powdered leaves of mint; RBC, red blood cell; GSH, glutathione; CAT, catalase; SOD, superoxide dismutase; FSE, *forsythia suspense* extract; TAOC, total antioxidant capacity; BR, *brahma rassayana* (made by the mixed of extracts from plants); EGCG, epigallocatechin-3-gallate; H_2O_2, hydrogen peroxide; avUCP, avian uncoupling proteins; GSH-Px, glutathione peroxidase; PTSCE, polyphenols from tamarind seed coat extract; Nrf2, nuclear factor erythroid 2–related factor 2; DL-M, DL-methionine; DL-HMTBA, DL-2-hydroxy-4-methylthiobutanoic acid; GSSG, oxidized glutathione; MOS, mannan-oligosaccharide; PM, probiotic mixture; SYN, synbiotic (combination of probiotic and MOS); BVE, *berberis vulgaris* root extract; GST, glutathione-S-transferase; CXEO, *curcuma xanthorrhiza* essential oil; OCEO, *origanum compactum*essential oil; HSP, heat shock protein; LPE, lemon peel extract; OPE, orange peel extract

Prooxidant properties of phytogenics and its association with oxidative stress

Besides their antioxidant activity, prooxidant properties of polyphenols have also been reported, which depends on the concentration and structure of these compounds in the biological cells, chemical instability, depletion of cellular glutathione and mobilization of cellular ions (Hu, 2011). However, their limited prooxidant property can also be beneficial, i.e., by their auto-oxidation they can produce ROS and hydrogen peroxide (H_2O_2), hence, mild OS. Thereupon, the antioxidant defence is induced and enhanced, leading to higher cell protection (Rodrigo et al., 2011).

CONCLUSIONS

In summary, it is obvious that both acutely and chronically exposing poultry to HS increases ROS formation, but the important issue is whether these higher levels of ROS cause OS or not. These 2 models do not generate ROS and lipid peroxidation products in the same extent. Studies have shown that acute HS induces OS via down-regulating avUCP, higher proton gradient and increasing the membrane potential. Data regarding the activity of antioxidant enzymes upon acute HS are consistent, i.e., there is a surge in their activities but this is insufficient. In other words, increased activity of the antioxidant system does not eliminate excessively produced ROS properly and sufficiently, thus resulting in oxidative damage.

Considering chronic HS, the literature does not draw a consistent picture, i.e., unchanged, increased, or decreased activity of antioxidant enzymes have been reported. Constant chronic HS has been shown to reduce metabolic oxidation capacity, and both constant and cyclic models of chronic HS have been shown to up-regulate the avUCP in poultry. Higher production of ROS via enhanced respiratory chain activity in mitochondria of broilers exposed to constant chronic HS was lasted only for 1 week, and this overproduction was not evident after 2 weeks of heat exposure. Lipid peroxidation products as OS biomarkers have also been reported to increase but not to the same extent as acute HS. One important factor that may contribute to this difference is that in cyclic

chronic HS, poultry have few hours of comfortable temperature which is very helpful for them to recover their weakened/impaired system following hot hours of the day. The latter indicate that in prolonged cyclic heat exposure, a higher potential for acclimatization does exist; however, antioxidant supplementations should be considered for both models.

OBJECTIVES AND OUTLINE OF THE PhD RESEARCH

Based on the literature overview in this introduction, the following **specific objectives** of the present study were formulated:

[1] to investigate the effects of plant extracts on performance and meat quality in broilers;

[2] to evaluate the effects of plant extracts on gut health parameters in broilers;

[3] to examine their effects on oxidative stress parameters and antioxidant gene expression, and the association with the gene expression of heat shock protein 70.

The outcomes of this research should allow the poultry industry to improve the broilers tolerance to high ambient temperature through dietary manipulation during the finishing phase of rearing period.

This study aimed at focusing on responses of Ross 308 broiler chickens to cyclic chronically increased daily temperature in the finishing phase (from d 28 to d 48 of age) to dietary supplements, i.e., dietary plant extracts rich in phenolic compounds. To this end, lemon peel extract (LPE), orange peel extract (OPE), and their combination, *Curcuma xanthorrhiza* essential oil (CXEO), and *Origanum compactum* essential oil (OCEO) were obtained and were included in the diet at levels of 200 and 400 mg/kg of diet.

Chapter 1 (this chapter) describes different models of heat stress, their effects on poultry parameters with emphasis on the oxidative status, and also the potential of antioxidant additives to alleviate heat stress consequences are addresssed.

In **Chapters** 2 to 5, the results of the own experimental works are presented. To test the objectives, several animal experiments were set-up with broilers in controlled HS conditions. Laboratory analyses were performed on samples taken from the birds after euthanasia following a HS period. The first and second experiments was carried out in Iran and the third experiment was conducted in Belgium at LANUPRO.

In **Chapter 2** (first experiment), a blend of LPE and OPE at different inclusion levels were tested on broiler performance, serum components and intestinal morphology under cyclic chronic HS (increasing the room temperature form 22°C to 34°C for 5 h/d during finishing phase at d 28 to d 38 of age). Firstly, an extract of lemon peel and orange peel was obtained and then 2 levels of OPE (0 and 200 mg/kg) were combined with 3 levels of LPE (0, 200, and 400 mg/kg) (6 experimental diets). This was done to test their possible synergistic effect on different parameters.

Chapter 3 describes part of the results of asecond experiment. In this experiment, LPE, OPE and CXEO at 2 levels each (200 and 400 mg/kg) were included giving 6 dietary treatments next to a control treatment. The experimental protocol was comparable to the first experiment. Performance parameters, intestinal microbiology and morphology were characterized in this experiment.

Chapter 4 describes another part of the results of the second experiment, i.e. the effect of aforementioned extracts on antioxidant enzymes, immune system parameters, plasma hormones and serum metabolites of finishing broiler chickens.

The molecular mechanisms through which essential oils may mediate oxidative stress in broilers under high ambient temperatures were investigated in a third experiment (**Chapter 5**). For this purpose, CXEO and OCEO at 2 levels each (200 and 400 mg/kg) were added to the diet giving 4 diets next to a control diet. Oxidative status parameters incuding the concentration of MDA, FRAP, GSH, GSSH/GSH, and the activities of CAT, GSH-Px, SOD and the levels of heat shock protein 70 (HSP70) were characterized. Meat quality variables were also measured in this experiment. In this experiment, birds were also sampled at 3 d after starting the exposure to elevated ambient temperature as a model for acute HS, as well as at the end of the rearing period for evaluating the effects on cyclic chronic HS like in the experiments 1 and 2.

Finally, a general discussion regarding the effect of HS on broilers, with particular attention to the potential of plant derived compounds in relieving HS and also some future prospects aredelivered in **Chapter 6**.

CHAPTER 2

EFFECT OF FEEDING CITRUS PEEL EXTRACTS ON GROWTH PERFORMANCE, SERUM COMPONENTS, AND INTESTINAL MORPHOLOGY OF BROILERS EXPOSED TO HIGH AMBIENT TEMPERATURE DURING THE FINISHER PHASE

Redrafted after

Akbarian, A., Golian, A. Gilani, A., Kermanshahi, H., Zhaleh, S., Akhavan, A., De Smet, S., Michiels, J. (2013). Effect of feeding citrus peel extracts of growth performance, serum components, and intestinal morphology of broilers exposed to high ambient temperature during the finisher phase. Livestock Science, **157**, 490-497.

CHAPTER 2

EFFECT OF FEEDING CITRUS PEEL EXTRACTS ON GROWTH PERFORMANCE, SERUM COMPONENTS, AND INTESTINAL MORPHOLOGY OF BROILERS EXPOSED TO HIGH AMBIENT TEMPERATURE DURING THE FINISHER PHASE

ABSTRACT

The main aim of the current experiment was to assess the potential of dietary orange peel extract (OPE) and lemon peel extract (LPE) as promoters of broiler resistance to high ambient temperature. The experiment was conducted as a 2 × 3 factorial arrangement of treatments with 2 levels of OPE (0 and 200 mg/kg feed) and 3 levels of LPE (0, 200, and 400 mg/kg feed). At d 25, a total of 288 Ross 308 broilers were randomly assigned to 6 dietary treatments with 4 replicates of 12 broiler chickens each. The temperature was increased to 34°C with 50% relative humidity for 5 h daily starting from d 28 until d 38. At the end of the trial (d 38), 4 male broiler chickens per pen were sampled for determination of serum components and variables of intestinal morphology. Dietary OPE and LPE did not affect weight gain, feed intake, and feed conversion ratio of broiler chickens. The inclusion of 200 mg/kg OPE increased serum total protein, but reduced serum lactate dehydrogenase and creatine phosphokinase activity in broiler chickens ($P< 0.05$). Lemon peel extract supplementation decreased the activity of lactate dehydrogenase quadratically ($P = 0.039$) and creatine phosphokinase linearly ($P = 0.037$). No differences in the other blood characteristics and intestinal traits were observed with the exception of muscularis thickness of duodenum, which was reduced when LPE was added to the diet (linear, $P = 0.011$). These results indicate that OPE, LPE, and their combination might modify

some blood components and the proximal intestinal morphology, but without beneficial effect on growth performance of broiler chickens under hot conditions.

INTRODUCTION

The deleterious effects of high ambient temperature during some months of the year on poultry production have been of great concern in many countries. It has been well documented that exposing broiler chickens to continuously high ambient temperatures, especially during the finisher phase, leads to chronic heat stress by increasing the body temperature and peroxidation products in blood and tissues (Ahmad et al., 2008; Sahin et al., 2003), and could exert profound effects on performance, health, and overall physiology of birds (Borges et al., 2004; Campo and Davila, 2004; Han et al., 2010; Melsse et al., 2011; Olanrewaju et al., 2007; Prieto and Campo, 2010; Quinterio-Filho et al., 2010). Elevated ambient temperature also causes a disruption in the structure and function of intestinal epithelium, including reduced regeneration (Burkholder et al., 2008) and integrity (Meddings and Swain, 2000; Saunders et al., 1994; Soderholm et al., 2002) of the intestinal epithelium. However, good intestinal health in broilers is of utmost importance to achieve target growth rates and feed efficiency (Montagne et al., 2003).

Several management approaches have been used to minimize the deleterious impacts of elevated temperatures. Undoubtedly, diet manipulation can be a viable option to resolve this issue (Sahin et al., 2002, 2003; Dai et al., 2011). It has previously been reported that adding plant extracts to broiler chickens' diets under optimal environmental conditions has either a positive or no effect on their growth performance (Botsoglou et al., 2002; Lee et al., 2003a). Lee et al. (2003a; 2003c; 2003d) reported that dietary plant extracts could stimulate growth performance in broilers fed a suboptimal diet. Plant extracts may stimulate crypt cell proliferation and subsequent tissue turnover and, thus, result in a healthier gut (Brenes and Roura, 2010; Garcia et al., 2007; Incharoen et al., 2010; Jamroz et al., 2006).

Studies have shown that phenolic compounds (PC) may exert antimicrobial and antioxidant effects when fed to poultry (Viveros et al., 2011). These compounds are found in many plants such as fruits and vegetables. Orange *(Citrus aurantium)* and lemon *(Citrus limon)* peel are common by-products

of the food and juice extraction industry and the most widely consumed citrus in the world (Ghasemi et al., 2009). The peel from citrus fruit represents approximately one-fourth of whole fruit mass and is obtained after the extraction of juice and removing the remaining pulp inside mechanically (Braddock, 1999). These by-products are available at low cost in most seasons in some countries like Iran. During citrus juice processing, a considerable quantity of wastes or by-products is generated. Though large quantities of citrus pulps are used for animal (ruminant) feeds, the majority of the processing residue is thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications of these by-products. Currently, almost no information is available on feeding orange and lemon peel or extracts to broiler chickens under hot conditions (e.g., Akbarian et al., 2013).

Brenes and Roura (2010) indicated that botanical interactions need to be investigated because of the complexity in terms of the number and the variability of bioactive compounds, and their possible synergistic effects. Therefore, the hypothesis was tested whether the dietary inclusion of 2 citrus peel extracts could relieve some of the metabolic and digestive disturbances induced by elevated environmental temperature. The possible interaction or synergism of the 2 extracts or both were investigated.

MATERIALS AND METHODS

Citrus peel extracts

Fruit peels of orange *(C. aurantium)* and lemon *(C. limon)* were obtained from a commercial source (Nader company of Agro-industry, Mashhad, Iran) in October 2010. The products were dried in a forced air oven (IndiaMart, Gopal Niwas, Maharashtra, India) at 40ºC for 12 h. The dried samples were ground into 3 to 5 mm particles using a laboratory mill (Model 2001DL; Braun GmbH, Kronberg, Germany), then packed in polyethylene bags and stored at –20ºC until use. Fifty grams of each ground sample (orange peel and lemon peel) were extracted 3 times with 500 mL of ethanol

solution (50%) using a Teflon-coated magnetic stir bar and stir plate (Ikaflon 155; IKA, Staufen, Germany) for 6 h at room temperature. Extracts were filtrated through Whatman No. 1 filter paper (Sigma-Aldrich, Munich, Germany). The combined filtrates from the 3 extractions were concentrated in a rotary evaporator (Bio-Equip RE-52-3-5; Shanghai Qingpu Huxi Instruments Factory, Shanghai, China) at 40°C to a final volume of 100 mL crude extract and stored at –20°C until use. These extracts were denominated as orange peel extract (OPE) and lemon peel extract (LPE), and used as such in the feeding experiment.

Total phenolic compounds were determined with Folin–Ciocalteu reagent using tannic acid as a standard according to the method described by Taga et al. (1984). Results are expressed as milligram tannic acid equivalents/gram extract. Separation and quantification of phenolic compounds was established as described by Ricardo-da-Silva et al. (1993) using HPLC [Consta Metric 4100 pump and ODC-2 column (3 μm; 150 mm × 4.6 mm i.d.); Thermo Separation Products, Evisa, Riviera Beach, FL, USA), a fluorescence detector (FL 3000,Excitation wavelength: 250 nm – Emission wavelength: 400 nm (2475 Multi λ, Waters Corporation, Manchester, UK)], and interfaced with computer equipped with a software (PC 1000 Chromatography Software,Version 3.5; Dionex, California, USA). Methanol:ammonium acetate (12:88 vol/vol) was used as the mobile phase with a flow rate of 1 mL/min.

To test for the presence of flavonoid compounds in the samples, a modified colorimetric aluminum chloride method was used (Woisky and Salatino, 1998). Absorbance was measured at 415 nm using a spectrophotometer (UV-160A; Shimadzu Co., Kyoto, Japan). Results are expressed as milligram quercetin equivalents/gram extract. Separation and quantification of flavonoids was performed using HPLC (Thermo Separation Products) according to the method described by Baldi et al. (1995). The standards were obtained commercially (Sigma-Aldrich, Munich, Germany).

Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of Ferdowsi University of Mashhad (Mashhad, Khorasan Razavi, Iran). A total of 288 unsexed Ross 308 broiler chickens was obtained from a commercial hatchery (Seamorgh Co., Quchan, Mashhad, Iran) and raised for 25 d before the commencement of the study, i.e., the feeding of the experimental diets. Broiler chickens were vaccinated for infectious bronchitis on d 1, Newcastle disease and avian influenza on d 7, and infectious bursal disease on d 14 of age. At d 25 of age, broiler chickens were randomly allotted to 24 floor pens with 12 birds each. Each pen (1 m^2) was equipped with a manual feeder and 2 nipple drinkers, and the floor was covered with clean wood shavings. The ventilation rate was 0.12 m/s during the whole period. Light was made available around the clock with an intensity of approximately 20 lux (23 h light:1 h dark). The initial house temperature was 32°C and was gradually decreased to reach 22°C at 21 d of age. The birds were given a finisher diet from d 25 to 38. The basal diet was formulated to meet the nutrient requirements with slightly lower nutrient specifications than those recommended by Ross 308 broiler management guide (Aviagen, 2009). Ingredient and chemical composition of the basal finisher diet are shown in Table 2.1.

A 2 × 3 factorial experiment with 2 levels of dietary OPE (0 and 200 mg/kg) and 3 levels of LPE (0, 200, and 400 mg/kg) was used in a completely randomized design with 4 pen replicates per treatment. The extracts were first mixed very well with the associated corn oil and then gradually added to the basal diet. Feed and water were offered *ad libitum*. The feeding experiment period lasted 13 d (25 to 38 d of age). To accustom to the experimental diets, a 3-d adaptation period was included before increasing house temperature. From d 28, a different temperature regimen was followed as reported by Aksit et al. (2006). The basal temperature was 22°C. Between 08:30 and 10:00, the temperature was gradually increased to 34°C and this high temperature was then maintained for 5 h (until 15:00). After that, the temperature was gradually decreased to the basal level by 16:30. Temperature control was executed by heating elements, air conditioner, and dynamic ventilation

(Toyuransanat Pri. Co., Mashhad, Iran). Average relative humidity was kept at 50% during both the experimental period and the rest of the day.

Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined during the experiment. Therefore, average pen weight was recorded by commencing the experiment (d 28) and at the end of the trial (d 38). Feed intake was determined from the difference between supplied at d 28 and residual feed at d 38 in each pen and was adjusted for mortality. The FCR was calculated as the ratio between feed intake and weight gain.

Table 2.1. Ingredient and calculated nutrient composition of the basal finisher diet fed from 25 to 38 d of age.

Item	Content
Ingredients, g/kg	
Corn	607.8
Soybean meal	319.7
Vegetable oil	36.8
Limestone	10.4
Dicalcium phosphate	14.2
Common salt	4.3
DL-Methionine	1.0
L-Lysine HCL	0.8
Vitamin and mineral premix[a]	5.0
Calculated composition	
ME (MJ/kg)	12.76
CP (g/kg)	193.0
Ca (g/kg)	8.1
Available P (g/kg)	4.0
Ileal digestible lysine (g/kg)	10.5
Ileal digestible methionine (g/kg)	4.0
Ileal digestible Met + Cys (g/kg)	7.2
DCAB(mEq/kg)[b]	211.0

[a] vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 9,790 IU; vitamin E, 121 IU; vitamin B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg; sodium selenite, 0.3 mg; potassium iodide, 1 mg; copper(II) sulfate, 10 mg; and iron sulfate, 50 mg.

[b] DCAB = dietary cation-anion balance (Na + K – Cl).

Blood sampling and determination of serum components

At the end of the experiment (38 d of age and after the 5 h of high ambient temperature), 4 male birds from each pen (16 per treatment) were randomly selected and weighed individually after a 6-h fasting

period. Blood samples were collected from the wing vein with a 25 G needle (Zhejiang Oujian Medical Apparatus Co., Ltd., Zhejiang, China). Serum was obtained by centrifugation of the coagulated blood (839 x g for 10 min at 4°C) within 30 min after sampling to measure serum chemical components. Following variables were analyzed: total protein (TP), fasting blood sugar (FBS), and albumin (Tietz, 1995), total and direct bilirubin, activity of lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) (Young, 1995).These were analyzed by an automatic analyzer (Bio Systems S.A., Barcelona,

Histomorphology of the small intestine

Following blood sampling the selected broiler chickens were killed by cervical dislocation. Intestinal tissues were obtained immediately after slaughter. Intestinal segments were defined as follows: 1) duodenum as the intestine from the gizzard (pylorus) to pancreatic and bile ducts, 2) jejunum as the portion of intestine extending from the bile duct entrance to Meckel's diverticulum, and 3) ileum as the region from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. Three-centimeter tissue samples were taken from the midpoint of the aforementioned sections of the intestine, then rinsed with saline and immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3 µm), stained by hematoxyline-eosin, and analyzed under a light microscope (Olympus, Shinjuku, Japan) to determine morphometric indices using image-analysis software (QWinPlus v. 3.1.0; Lieca Ltd., Cambridge, UK). The morphometric variables (all expressed in micrometer) measured included villus height, crypt depth, and villus width at the top and the base, thickness of the mucosal and submucosal layer, and muscularis thickness. The 10 longest and straightest villi and associated crypts were measured from each segment. Measurements for the villi height were taken from the tip of the villus to the villus-crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villi and the villus width was measured at the top and bottom of villi. Muscularis thickness was determined from the

submucosa to the external layer of the intestine. The mean from 10 measurements per sample was used as the average value for further analysis.

Statistical analyses

All data were analyzed using the General Linear Model procedure of the SAS (SAS, 2004) using a model that included the fixed effects of OPE (2 levels) and LPE (3 levels). Orthogonal polynomial contrasts were used to determine linear and quadratic effects of LPE. All statements of significance were based on probability of $P< 0.05$.

RESULTS AND DISCUSSION

Composition of citrus peel extracts

The total content of phenolic compounds was 34.9 and 33.7 mg tannic acid equivalents/g extract for OPE and LPE respectively, with protocatchic accounting for approximately 80% in both extracts, catechol for 6.4 and 10% respectively and the other compounds each less than 5% relative to the total content of phenolic compounds (Table 2.2). The total flavonoid contents were 3.68 and 4.52 mg quercetin/gram OPE and LPE, respectively. The phenolic and flavonoid contents were lower than data reported by other authors, e.g.,using similar analytical methods. Ghasemi et al. (2009) reported 131 mg gallic acid equivalent and 16.2 mg quercetin equivalent per gram extract powder (following methanolic extraction) for total phenolic and flavonoids content respectively in lemon peel.

As discussed by Akbarian et al. (2013a), the content of bioactive compounds in plants depends on several factors.

Table 2.2. Content of some bioactive compounds of orange peel extract (OPE) and lemon peel extract (LPE).

Item	OPE	LPE
Total phenolic compounds (mg tannic acid equivalents/g extract)	34.92	33.71
Composition of phenolic compounds (%)		
Protocatchic	80.7	78.9
Catechol	6.4	10.0
Cinnamic	4.5	2.4
Caffein	3.3	1.7
Vanillic	2.9	2.1
Syringic	1.2	0.8
Chrisin	0.5	0.4
Coumarin	0.1	0.6
Total flavonoid compounds (mg quercetin/g extract)	3.68	4.52
Composition of flavonoid compounds (%)		
Quercetin	48.1	37.5
Luteolin	0.0	21.8
Rutin	18.5	15.6
Hesperetin	3.3	4.1
Unknown Compounds	30.1	21.0

Performance and blood components

The effects of dietary extracts on BWG, FI and FCR are given in Table 2.3. Mortality data were not subjected to statistical analysis because just 1 case of mortality in the control treatment was observed throughout the trial. Dietary OPE and LPE did not affect BWG, FI, and FCR of the broiler chickens. Although many studies have been conducted with plant extracts rich in phenolic compounds on broiler performance, the data obtained from these studies are controversial. It has been shown that using high doses of propolis rich in phenolics and vitamin C could partially overcome the depression in growth and carcass quality caused by high temperature in broiler chickens (Seven et al., 2008). In agreement with our results, Reisinger et al. (2011) found that supplementation of broiler chicken diets with a mixture of phytogenic feed additive containing oregano, anise, and citrus peel oils did not affect FI or FCR. The same result was found in a study of Lee et al. (2003b)who reported no differences in FI, BWG and FCR of broiler chickens fed thymol, cinnamaldehyde and a commercial mixture of essential oil components. Elsewhere, incorporation of a blend of plant oils derived from oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel into laying hens diet during

the summer season, did not exert a pronounced effect on FI but an improved FCR for supplemented groups compared to their control was observed (Çabuk et al., 2006).

Several reasons might explain these inconsistencies. The efficacy of plant extracts to impart on animal performances depends on several factors, e.g., dose of the plant extract used and concentration and profile of active components present in the extracts, physiological state of the animal, background diet, housing conditions, etc. The plant extract composition is in turn determined by extraction method, storage method, soil and growth conditions of the plants, etc. (Basmacioğlu et al., 2010; Brenes and Roura, 2010; Lee et al., 2003a, 2003b).

The effects of dietary OPE and LPE on serum components of broiler chickens are given in Table 2.4. The inclusion of 200 mg/kg OPE increased total protein, and reduced the activity of LDH and CPK ($P< 0.05$). Also, dietary LPE decreased LDH activity ($P< 0.05$). Lemon peel extract supplementation decreased the activity of lactate dehydrogenase quadratically ($P = 0.039$), and creatine phosphokinase linearly ($P = 0.037$). The interaction term was only significant for the activity of LDH, with the 200 OPE and both 200 and 400 LPE treatments having lower values than the control treatment ($P = 0.036$). The other blood characteristics, i.e.,fasting blood glucose, albumin, total and direct bilirubin were not affected by the treatments.

Table 2.3. Effect of dietary orange peel extract (OPE) and lemon peel extract (LPE) on performance of broilers exposed to high ambient temperature during 28 to 38 d of age.

Item	OPE (mg/kg)		SEM [a]	*P*-value	LPE (mg/kg)			SEM [a]	*P*-value
	0	200			0	200	400		
Daily feed intake (g)	155	148	2.9	0.053	154	152	147	3.6	0.468
Daily body weight gain (g)	88	87	1.4	0.617	87	89	87	1.7	0.775
Feed conversion ratio (g/g)	1.76	1.70	0.04	0.220	1.77	1.71	1.69	0.05	0.520

[a] Standard error of mean.

Table 2.4. Effect of dietary orange peel extract (OPE) and lemon peel extract (LPE) on concentration of some serum components in broilers exposed to high ambient temperature during 28 to 38 d of age.

Item	OPE (mg/kg)		SEM [a]	*P*-value	LPE (mg/kg)			SEM	*P*-value
	0	200			0	200	400		
TP (g/dL)	3.50	3.92	0.10	0.009	3.67	3.86	3.59	0.12	0.325
Albumin (g/dL)	1.72	1.72	0.05	0.987	1.70	1.70	1.76	0.06	0.776
FBS (mg/dL)	278	280	2.8	0.987	279	277	282	3.5	0.776
Total Bilirubin (mg/dL)	0.27	0.39	0.07	0.273	0.28	0.46	0.25	0.08	0.227
Direct Bilirubin (mg/dL)	0.15	0.24	0.05	0.277	0.17	0.27	0.13	0.07	0.385
LDH (IU/L)[b,c]	4,468	3,120	166	<0.001	4,225	3,423	3,735	204	0.038
CPK (IU/L)[d]	7,551	6,181	343	0.011	7,738	6,460	6,400	420	0.063

[a] Standard error of mean.
[b] Quadratic effect of LPE, $P = 0.039$.
[c] OPE × LPE interaction ($P = 0.036$). Mean values: 0 OPE and 0 LPE = 4,787, 0 OPE and 200 LPE = 4,550, 0 OPE and 400 LPE = 4,067,200 OPE and 0 LPE = 3,662, 200 OPE and 200 LPE = 2,296, and 200 OPE and 400 LPE = 3,402.
[d] Linear effect of LPE, $P = 0.037$.

A number of enzymes are used in the clinical biochemistry as tools for differential diagnosis, such as CPK and LDH. Since the bulk of each is located in different tissues, their abnormal appearance in the blood can give a hint to specific muscle or organ damage (Pech-Waffenschmidt, 1992). In broiler chickens, CPK is released into the circulation following changes in the permeability of the sarcolemma in response to various pathologies and exposure to environmental stressors (Mitchell and Carlisle, 1992; Mitchell and Sandercock, 1995). In addition, overt muscle damage in birds is associated with an increase in the plasma activity of the intracellular muscle isoenzyme CPK (Eraslanet al., 2007). Publications about the effect of high environmental temperature on the CPK activity are not consistent. Even in broiler chickens of the same age, size, and breed, large variations were observed in their responses to elevated temperature, as evaluated by blood composition and

behavior. In this connection, Sandercock (2001) and Bogin et al. (1996) observed a significant increase in CPK activity in the plasma of broiler chickens exposed to high ambient temperature reflecting heat stress-induced myopathy. Similarly, Yalçin et al. (2009) reported an increase in plasma CPK activity on broiler chickens exposed to a daily cyclic heat treatment. According to the results obtained by Bogin et al. (1996) heat exposure increased the activities of CPK in different organs like brain, breast muscle, and heart. They also found a significant increase in the LDH activity in the heart muscle. This was also supported by previous findings of Melesse et al. (2011), who reported that the activity of CPK in plasma was increased by long term high temperature in the laying hen. On the other hand, according to the results obtained by Pech-Waffenschmidt et al. (1995), heat exposure did not change the enzyme activities in the broiler chicken's serum. This was also supported by findings of Ward and Peterson (1973), who reported that the activity of CPK was not influenced even by acute heat exposure. Hence, the decreased LDH and CPK activity found in our study, supports that LPE and OPE extracts may act to decrease the harmful effects of high temperature in broilers which might be related to the antioxidant capacity of the PC present in the extracts (e.g.,Farrellet al., 1966). The highest reduction of LDH activity was obtained when both extracts were included in the diet. Clearly, a synergistic effect might be assumed in the case of treatment 200 OPE and 200 LPE.

The metabolic changes induced in broiler chickens by high temperature include impairment of endocrine functions (Sinurat et al., 1987) and reduced serum protein concentrations (Khan et al., 2012). Regarding total protein, consistent with our results, Eraslan et al. (2007) observed an increase in total protein in the serum of rats fed propolis, which is rich in flavonoid and phenolic compounds. One mechanism through which OPE may exert its hyperproteinemia action is via transamination through its phenolic compounds. The phenol and its derivatives can alter protein metabolism by altering the transamination rate of amino acids by enhancing the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Abdel-Hamid, 2007).

Some studies have shown that feeding broilers with phytogenics (e.g., rosemary leaves, *Moringa stenopetala* extract, which are rich in phenolics) increases the levels of total protein in the blood serum. This may reflect a more intensive protein metabolism and enhanced nutrient supply and transport as suggested by Sirvydis et al. (2006). In fact, the increased nutrient supply for growth is reflected in enhanced nutrient transport in the blood. Therefore, we could expect to see a positive effect of plant extracts on broiler performance, but this was not the case. This can be explained by the HS condition that might have elicited some pathological and physiological changes in the animals, leading to poor absorption or poor utilization of protein in the diets (Bonnet et al., 1997).

Fasting blood glucose, albumin, total and direct bilirubin in blood were not influenced by the treatments. Related to the results for glucose levels, this is not surprising since Bogin et al. (1981) and Arad et al. (1983) showed that glucose levels were not influenced by elevation in house temperature in broiler chickens and laying hens. Also, Seven et al. (2008) reported that using propolis rich in flavonoid and phenolic acids, had no influence on biochemical parameters of blood including glucose and albumin of broiler chickens exposed to an increased temperature. In another study and in contrast to our results, Geraert et al. (1996) found that exposing broilers to high ambient temperatures leads to hyperglycaemia. These authors reported that high temperatures increase the secretion of glucocorticoids, which in turn increases gluconeogenesis, hence increasing blood glucose levels. Several reports have shown that using PC had no influence on serum glucose of broiler and fish (Biavatti et al., 2003; Roche and Boge, 2000).

Intestinal morphology

The effects of dietary OPE and LPE on duodenal morphological characteristics are presented in Table 2.5. The treatments did not have any effect on the duodenal traits with an exception for muscularis thickness which was reduced when LPE was added to the diet (linear, $P = 0.011$). The interaction term was only significant for villus height:crypt depth, with the control and mixture of

200 LPE and 200 OPE treatments having higher values than the 0 OPE and 200 LPE treatment (*P* = 0.042). Plant extracts did not have effect on jejunum and ileum measured criteria (data not shown). Several observations support the hypothesis that herbal feed additives may favorably affect gut functions (e.g., enzyme activity, microbial eubiosis) *in vitro*(Liu et al., 2011). Phytogenic compounds enhanced mucus production and thickness in the stomach and jejunum suggesting a potential protective against colonization by gut pathogens (Jamroz et al., 2006). It is likely that changes in cell proliferation would be observed first in the stem cells of the crypt rather than the villus because of the high proliferative activity of the crypt (Yamauchi et al., 1995). Garcia et al. (2007) indicated that addition of 200 mg/kg plant extract comprising a blend of oregano, cinnamon, and pepper essential oil increased villus height.

Table 2.5. Effect of dietary orange peel extract (OPE) and lemon peel extract (LPE) on morphological characteristics of the duodenum (µm) in broilers exposed to high ambient temperature during 28 to 38 d of age.

Item	OPE (mg/kg)		SEM [a]	*P*-value	LPE (mg/kg)			SEM	*P*-value
	0	200			0	200	400		
Villus height	1,325	1,493	64	0.090	1,416	1,426	1,387	78	0.943
Villus width	151	170	9.8	0.208	178	146	157	12	0.214
Crypt depth	264	274	14	0.635	255	267	285	17	0.502
Mucosal layer	1,620	1,758	67	0.176	1,645	1,723	1,698	83	0.800
Submucosal layer	48	51	2.6	0.422	49	52	48	3.2	0.715
Muscularis thickness[b]	313	309	19	0.894	344	338	251	24	0.034
Villus height:crypt depth[c]	5.10	5.56	0.32	0.327	5.60	5.41	4.98	0.39	0.542

[a] Standard error of mean.
[b] Linear effect of LPE, *P* = 0.011.
[c] OPE × LPE interaction (*P* = 0.042). Mean values: 0 OPE and 0 LPE = 5.99,0 OPE and 200 LPE = 4.25,0 OPE and 400 LPE = 5.05, 200 OPE and 0 LPE = 5.21, 200 OPE and 200 LPE = 6.57, and 200 OPE and 400 LPE = 4.91.

It has been reported that birds subjected to 30°C for 24 h had reduced crypt depth compared with birds at 23°C, but villus height and the villus height:crypt depth were unchanged in birds exposed to 30°C (Burkholder et al., 2008). It has been shown that mucus content of small intestine during a short-term feed withdrawal of broiler chickens as a stressor was reduced (Thompson and Applegate, 2006). In this study, it was observed that dietary LPE modified the muscularis thickness of duodenum

(linear, P = 0.011). An increased temperature could result in intestinal irritation and increase muscular thickness. It is suggested that the balance between tissue irritation and beneficial effects of intestinal hygiene may determine the overall impact of plant products on gut morphology. In the present study, feeding with LPE at the highest level (400 mg/kg) apparently improved some histomorphological criteria that are negatively affected by increased temperature.

There were no differences between treatments in the jejunum and ileum parts. These results are consistent with the findings of Yamauchi et al. (1996; 1995) who indicated that morphological changes in response to fasting occur more rapidly in the proximal part of the small intestine than in the distal. There are several possible reasons why middle and distal intestine characteristics were unchanged in our study in response to elevated temperature, including the short duration of the heat shock and the resistance of these parts to structural change compared with the proximal part of the small intestine.

CONCLUSION

A combination of lemon and orange peel extracts supplemented to the finisher diet might modify some blood components and proximal intestinal muscularis thickness, but without beneficial effects on performance of broiler chickens under hot conditions. Further studies are required to fully explore dose-response effects on broiler chickens performance and the animal’s physiology.

CHAPTER 3

GROWTH PERFORMANCE AND GUT HEALTH PARAMETERS OF FINISHING BROILERS SUPPLEMENTED WITH PLANT EXTRACTS AND EXPOSED TO DAILY INCREASED TEMPERATURE

Redrafted after:

Akbarian, A., Golian, A., Kermanshahi, H., Farhoosh, R., Raji, A.R., De Smet, S., Michiels, J. (2013). Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature. Spanish Journal of Agricultural Research. **11** (1), 109-119.

CHAPTER 3

GROWTH PERFORMANCE AND GUT HEALTH PARAMETERS OF FINISHING BROILERS SUPPLEMENTED WITH PLANT EXTRACTS AND EXPOSED TO DAILY INCREASED TEMPERATURE

ABSTRACT

The effects of three plant extracts, *i.e.*,lemon peel extract (LPE), orange peel extract (OPE) and *Curcuma xanthorrhiza* essential oil (CXEO), on the performance and gut health parameters of broilers exposed to high temperature was investigated. A total of 336 unsexed Ross 308 broilers were distributed to seven dietary treatments, a control diet and 6 diets containing 200 or 400 mg kg^{-1} feed of one of the three products between d 25-38 (12 chicks per pen, four replicates). To induce chronic heat stress, the temperature was increased to 34°C with 50% relative humidity for 5 h daily starting from d 28 until d 38. At d 38, four animals per pen were sampled for morphological characteristics (duodenum, jejunum and ileum) and microbial counts (ileo-cecal contents). Plant extracts did not affect the bird performance. The bursa weight of the control birds was lower (p<0.05) comparing to those fed 400 mg kg^{-1} OPE and 200 and 400 mg kg^{-1} CXEO diets. Feeding 400 mg kg^{-1} of LPE decreased the duodenal villus:crypt ratio compared to control and 200 mg kg^{-1} OPE fed birds. Plant extracts did not have effect on ileal histo-morphology. Feeding with 400 mg kg^{-1} of LPE and CXEO caused a decrease in coliform counts in ileum and feeding of 400 mg kg^{-1} CXEO diet decreased coliform counts in caecum compared to control birds (p<0.05). These results elucidate that CXEO, OPE and LPE might modify some microbial and intestinal traits, but without beneficial effect on performance of broilers under heat stress.

INTRODUCTION

The effect of high ambient temperature during some months of the year on poultry production has been of great concern in many countries. In south of Iran, maximum air temperatures of 35 to 45°C during the months of April to September is normal, and performance of birds is reduced drastically. During the finishing phase, the suitable ambient temperature for poultry is between 16 and 25°C(Sahin et al., 2001). It has been well documented that exposing broiler chickens to continuously high temperature especially during the finisher phase leads to chronic heat stress (Sahin et al., 2003; Ahmad et al., 2008). Heat stress induces profound effect on overall physiology and animal health which can lead to changes in body composition. The gastrointestinal tract is particularly responsive to stressors like heat stress, which modify the normal and protective microbiota (Bailey et al., 2004) and decreased integrity of the intestinal epithelium (Lambert, 2009) which in turn can affect its barrier function and the absorption of nutrients, impairing productive performance of animals (Liu et al., 2009).

Numerous techniques have been proposed as possible therapies to offset the consequences of heat stress. Dietary manipulation could be a feasible option. In this regard, the possibility of using new plant derived additives in animal diets is being researched. Among the natural products, phenolic compounds (PC) seem to be potential candidates. The phenolic compounds show the ability to protect the microvilli, which are responsible for the absorption of nutrients (Sehm et al., 2007). These functions of the intestinal mucosal layer are connected with their intrinsic antioxidative activity at both cell and tissue levels (Rhodes, 1996). In addition, phenolic compounds exhibit considerable antimicrobial activity. Their antimicrobial ability may modulate the gut ecosystem to affect feed efficacy (Si et al., 2006). These ingredients are found in many plants such as fruits and vegetables. Studies showed that fruits of the Citrus family (particularly orange and lemon), and herbs of the *Zingiberaceae* family (particularly turmeric), are rich in phenolic compounds. Orange *(Citrus aurantium)* and lemon *(Citrus limon)* peel are common by-products of the food and juice extraction

industry and the most widely consumed citrus in the world (Ghasemi et al., 2009). They are also available at low cost in most seasons in some countries like Iran, and currently there is no information available about feeding orange and lemon peel extracts to broiler chickens under heat stress conditions. *Curcuma xanthorrhiza* (commonly known as temu lawak or Javanese turmeric in Indonesia), grows in Southeast Asia and is found both wild and cultivated in Indonesia. It is traditionally used for medicinal purposes.

The objective of the current study was to determine the effect of dietary lemon peel extract (LPE), orange peel extract (OPE) and *C. xanthorrhiza* essential oil (CXEO) on performance, body composition traits, intestinal microbiota and morphology of broiler chickens exposed to high ambient temperature. It was expected that selected plant extracts would relieve the deleterious effects of heat stress.

MATERIALS AND METHODS

Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. A total number of 336 unsexed Ross 308 broiler chicks was obtained from a commercial hatchery (Quchan, Mashhad, Iran) and commercially raised for first 25 d before the commencement of the study. Chicks were vaccinated for Infectious Bronchitis on the first d, Newcastle Disease and Avian Influenza on d 7 and Infectious Bursal Disease on d 14. At d 25 of age, the birds were weighed, and randomly allotted to 28 floor pens with 12 birds each. Each pen (1 m^2) was equipped with a manual feeder and two nipple drinkers, and the floor was covered with clean wood shavings. The ventilation rate was 0.12 m s^{-1} during the whole period. Light was made available around the clock with an intensity of approximately 20 lux (23 h light:1 h dark). The initial house temperature was 32°C and then gradually decreased to reach 22°C at 21 d of age. The birds were given a finishing diet from d 25 to 38. The basal diet was formulated to meet or exceed the

nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide (Aviagen, 2011). Ingredient and chemical composition of the basal diet are shown in Table 3.1.

Table 3.1. Ingredient and calculated nutrient composition of the basal finisher diet fed from 25 to 38 d of age.

Item	Content
Ingredients, g/kg	
Corn	607.8
Soybean meal	319.7
Vegetable oil	36.8
Limestone	10.4
Dicalcium phosphate	14.2
Common salt	4.3
DL-Methionine	1.0
L-Lysine HCL	0.8
Vitamin and mineral premix[a]	5.0
Calculated composition	
ME (MJ/kg)	12.76
CP (g/kg)	193.0
Ca (g/kg)	8.1
Available P (g/kg)	4.0
Ileal digestible lysine (g/kg)	10.5
Ileal digestible methionine (g/kg)	4.0
Ileal digestible Met + Cys (g/kg)	7.2
DCAB (mEq/kg)[b]	211.0

[a] vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 9,790 IU; vitamin E, 121 IU; vitamin B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg; sodium selenite, 0.3 mg; potassium iodide, 1 mg; copper(II) sulfate, 10 mg; and iron sulfate, 50 mg.
[b] DCAB = dietary cation-anion balance (Na + K – Cl).

A completely randomized design was used with seven dietary treatments replicated in four pens each. The dietary treatments were: a basal diet (control treatment) and the same diet supplemented with either OPE, LPE or CXEO at two different levels (200 and 400 mg kg^{-1}). The extracts and essential oil were first mixed very well with the associated corn oil and then gradually added to the basal diet which was provided as mash form. Feed and water were offered *ad libitum.* The feeding experiment period lasted 13 days (25-38 d of age). In order to accustom to the experimental diets; a 3-d adaptation period was included before imposing chronic heat stress. To induce chronic heat stress, birds were exposed to an ambient temperature of 34°C with 50% relative humidity for 5 h daily (from 10:00 AM until 15:00 PM) between 28 and 38 d of age as reported by Akşit et al. (2006).

Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured during the trial. Feed intake was determined from the difference between supplied and residual feed in each pen. The FCR was calculated from the ratio between feed intake and weight gain of chick, in each pen and was adjusted for mortality. Dead birds were weighed and recorded daily. At 38 d of age, four chicks per pen (16 chicks per treatment) were randomly selected and killed by cervical dislocation, to determine the body composition traits. The heart, empty proventriculus, empty gizzard, liver, spleen, pancreas, visceral fat, bursa of Fabricius and both carcass sides of the breast and thigh muscles (without skin) of each chick were weighed.

Curcuma xanthorrhiza essential oil and citrus peel extracts

C. xanthorrhiza essential oil was obtained from PT. Phytochemindo Reksa (Bogor, Indonesia). According to the compositional data provided by the supplier the main bioactive compounds were: ar-curcumene (approximately 11.4%), β-curcumene (approximately 8.5%) and xanthorrhizol (hydroxy-ar-curcumene) (approximately 28%). Xanthorrizhol is a sesquiterpenoid compound and the antioxidant properties of *C. xanthorriza* essential oil are ascribed to the phenolic structure of this compound. This essential oil of *C. xanthorriza* was used as such in the feeding experiment.

Fruit peels of orange *(C. aurantium)* and lemon *(C. limon)* were obtained from the fields of Mashhad in Khorasan Razavi province of Iran on October 2010. The products were dried in an air draught oven at 40ºC for 12 h. The dried samples were ground into 3-5 mm particles using a laboratory mill (Braun, Model 2001DL, Germany), then packed in polyethylene bags and stored at –20ºC until use. Fifty grams of each ground sample (orange peel and lemon peel) were extracted three times with 500 mL of ethanol solution (50%) using a Teflon-coated magnetic stir bar and stir plate for 6 h at room temperature. Extracts were filtrated through Whatman No. 1 filter paper. The combined filtrates from the three extractions were concentrated in a rotary evaporator at 40°C to a final volume of 100 mL crude extract and stored at –20°C until use. These extracts were used in the feeding experiment.

Total phenolic compounds were determined with Folin–Ciocalteu reagent using tannic acid as standard according to the method described by Taga et al. (1984). Results are expressed as mg tannic acid equivalents g^{-1} dried extract. Separation of phenolic compounds was carried out as described by Ricardo-da-Silva et al. (1993) using HPLC.

To test for the presence of flavonoid compounds in the samples, a modified colorimetric aluminum chloride method was used as reported by Woisky & Salatino (1998). Separation of flavonoids was performed using HPLC according to the method described by Baldi et al. (1995).

Histomorphology of the small intestine

Intestinal tissues were obtained immediately after slaughter. Segments were removed from the duodenum, jejunum, and ileum as follows: 1) intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum, 2) the jejunum was defined as the portion of intestine extending from the bile duct entrance to Meckel's diverticulum, 3) the ileum was defined as the region from Meckel's diverticulum to a point 40 mm proximal to the ileo-cecal junction. Tissue samples (3 cm) were taken at the midpoint of each section and immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3 µm), stained by hematoxyline-eosin, and analyzed under a light microscope to determine morphometric indices using image-analysis software. The morphometric variables included villus height, crypt depth, villus width, tunica mucosal, tunica submucosal and tunica muscularis. The ten longest and straightest villi and associated crypts were measured from each segment. Measurements for the villi height were taken from the tip of the villus to the villus-crypt junction. The crypt depth was defined as the depth of the invagination between adjacent villi and the villus width was measured at the top and bottom of villi. Tunica mucosal was measured from top of the villus (epithelium) until the lower part of muscularis mucosa. Tunica submucosal was measured from under part of muscularis mucosa until internal muscular layer and tunica muscularis measurement was performed from under part of submucosa until outer part of

external muscularis layer. The mean from 10 measurements per sample was used as the average value for further analysis.

Intestinal microbial populations

Samples of the contents from the ileum and both caeca were immediately collected per chick (four chicks per pen, 16 chicks per treatment) into glass containers, sealed, and put on ice until they were transported to the laboratory for enumeration of microbial populations. One gram of mixed contents was blended into 9 mL of reduced sterile dilution blank solution (RSDBS). Further serial dilutions were made in RSDBS for aerobic bacterial enumeration. The initial dilution in RSDBS was also used as a source for serial dilutions in PBS for enumeration of aerobic bacterial populations. The samples from the ileum and caeca were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. From each dilution, 0.1 mL was inoculated on agar plates for aerobics. Bacteria were enumerated on Plate Count Agar (PCA) (total aerobes), MRS agar (facultative anaerobes *Lactobacillus* spp.), and VRB agar (Coliform) (Merck, Germany). For bacterial growing, all the plates were incubated at 37^{o}C. MRS agar plates were incubated anaerobically for 48 h (Gas-Pack container, AnaeroPack^{TM}, Tokyo, Japan), and other plates were incubated aerobically for 24 h. Total numbers of bacterial colonies were counted at each incubation period and expressed as $\log_{10}$ cfu g^{-1} digesta. The spread plate method for plate count determination was performed in accordance with the procedure recommended by the APHA (1993).

Statistical analysis

Data were analyzed by a linear model with the treatment as fixed effect using the General Linear Model procedure of the SAS Inst Inc (vers. 9.1, Raleigh, NC, USA). Tukey means separation test was used to determine significant differences between treatment mean values (p<0.05).

RESULTS

Composition of citrus peel extracts

The total content of phenolic compounds was 34.9 and 33.7 mg tannic acid equivalents g^{-1} dried extract for OPE and LPE respectively, with protocatchic accounting for approximately 80% in both extracts, catechol for 6.4 and 10% respectively and the other compounds each for less than 5% relative to the total content of phenolic compounds (Table 2.2). The total flavonoid contents were 3.68 and 4.52 mg quercetin g^{-1} dried extract in OPE and LPE, respectively.

Performance

The effects of dietary extracts on BWG, FI and FCR are given in Table 3.2. Throughout the experiment, there was only one case of mortality and it was in the control treatment. Because of this limited number of death cases no statistical analysis was performed. Dietary OPE, LPE and CXEO did not affect BWG, FI and FCR of broiler chickens during 28-38 d of age.

Table 3.2. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and C*urcuma xanthorrhiza* essential oil (CXEO) on performance parameters and mortality rate of broilers during 28-38 d of age.

	Feed intake (gd^{-1})	Body weight gain ($g\ d^{-1}$)	Feed conversion ratio ($g\ g^{-1}$)
Control	177	91	1.96
OPE (mg kg^{-1})			
200	160	91	1.78
400	161	96	1.68
LPE (mg kg^{-1})			
200	168	93	1.80
400	163	94	1.74
CXEO (mg kg^{-1})			
200	163	93	1.74
400	166	94	1.77
p value	0.517	0.927	0.544
SEM[1]	5.84	3.27	0.09

[1] Standard error of mean. Values were taken from four replicates per treatment.

Body composition traits

Effect of dietary supplemental plant extracts on different organ weights are presented in Table 3.3. Relative spleen, pancreas, gizzard, liver, proventriculus, thigh, breast, heart and visceral fat weight were not influenced by the use of plant extracts. The relative bursa weight (g kg^{-1} BW) of the control birds was lower (p<0.05) compared to those fed 400 mg kg^{-1} OPE and 200 and 400 mg kg^{-1} CXEO diets. Also, the birds fed with 400 mg kg^{-1} CXEO had significantly heavier bursa than those fed 200 mg kg^{-1} OPE.

Small intestinal morphology

The effects of dietary OPE, LPE and CXEO on duodenal and jejunal criteria are presented in Tables3.3 and 3.4, respectively. Regarding the duodenal morphology, the inclusion of 200 mg kg^{-1} CXEO in the diet increased crypt depth compared to birds fed 200 and 400 mg kg^{-1} OPE or LPE diets (p<0.05). Feeding 400 mg kg^{-1} of LPE diet decreased villus:crypt ratio compared to control and 200 mg kg^{-1} OPE fed birds (p<0.05). There were significant differences between treatments for villus height of jejunum. In this regard, birds fed 400 mg kg^{-1} of LPE and CXEO diets had longer villi than those fed 200 and 400 mg kg^{-1} OPE diets (p<0.05). Feeding both 200 mg kg^{-1} of CXEO and LPE diets decreased villus:crypt ratio in jejunum as compared to those fed 400 mg kg^{-1} LPE diet (p<0.05). Jejunal muscular thickness was lower in birds fed 400 mg kg^{-1} CXEO diet and was higher in those fed 200 mg kg^{-1} LPE diet (p<0.05). Plant extracts did not have a significant effect on ileum measured criteria (data not shown).

Intestinal microbiology

Effect of dietary supplemental plant extracts on bacterial counts in ileal and cecal digesta of broiler chickens are shown in Table 3.5. In this respect, feeding with both 400 mg kg^{-1} of LPE and CXEO caused a decrease in coliform counts in ileum compared to control group and feeding of 400 mg kg^{-1} CXEO diet decreased the number of coliforms in ceacum compared to those fed control diet

(p<0.05). Counts of *Lactobacillus* spp. and total aerobic counts of ileum and caecum were similar among the treatment groups.

Table 3.3. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on relative weight of body composition traits (g kg^{-1}BW) of broilers at 38 d of age.

Item	Heart	Breast (left andright)	Thigh (left andright)	Proventriculus	Gizzard	Liver	Spleen	Pancreas	Visceral fat	Bursa of Fabricius
Control	8.73	298	278	7.22	31	36	1.99	4.86	15	3.42[c]
OPE (mg kg^{-1})										
200	8.84	298	282	6.80	30	35	1.94	4.94	16	3.79[bc]
400	8.11	303	282	6.56	33	33	2.01	4.56	14	4.06[ab]
LPE (mg kg^{-1})										
200	9.14	291	281	6.84	30	35	1.96	4.69	13	3.81[abc]
400	8.38	290	275	7.31	31	33	2.05	5.01	15	4.02[abc]
CXEO (mg kg^{-1})										
200	8.47	294	274	7.32	33	33	1.84	4.94	14	4.34[ab]
400	8.13	291	293	6.93	32	34	2.00	4.79	13	4.43[a]
p value	0.797	0.928	0.866	0.337	0.176	0.385	0.463	0.731	0.479	0.021
SEM[1]	0.53	8.90	10.09	0.26	0.92	1.16	0.07	0.20	1.13	0.19

[a-c.] Means within a column with different superscripts are significantly different ($p<0.05$).[1] Standard error of mean. Values were taken from four replicates per treatment.

Table 3.4. The effect of feeding orange peel extract (ope), lemon peel extract (lpe) and *Curcuma xanthorrhiza* essential oil (cxeo) on morphological criteria of the duodenum and jejunum of broilers at 38 d of age.

Intestinal criteria (µm)	Villus height		Villus width		Crypt depth		Tunica mucosal		Tunica submucosal		Tunica muscularis		Villus:crypt ration	
	duodenum	jejunum	Duodenum	jejunum	duodenum	jejunum	duodenum	jejunum	duodenum	jejunum	duodenum	jejunum	duodenum	jejunum
Control	1119	604^{abc}	139	89	333^{ab}	294	1506	727	47	48	275	162	4.06^{a}	2.16^{ab}
OPE (mg kg^{-1})														
200	1143	570^{c}	139	80	288^{a}	277	1326	688	45	56	276	190^{ab}	4.03^{a}	2.15^{ab}
400	1047	582^{c}	115	106	297^{a}	257	1472	856	50	54	260	207^{ab}	3.66^{ab}	2.28^{ab}
LPE (mg kg^{-1})														
200	1103	606^{abc}	123	96	297^{b}	266	1395	739	50	49	227	215^{a}	3.88^{ab}	1.81^{b}
400	1107	695^{a}	115	99	276^{b}	263	1517	778	51	43	297	190^{ab}	2.98^{b}	2.73^{a}
CXEO (mg kg^{-1})														
200	1052	595^{bc}	139	82	372^{a}	335	1386	832	52	53	256	201^{ab}	3.17^{ab}	1.81^{b}
400	1163	687^{a}	121	100	333^{ab}	326	1439	870	48	53	279	138^{b}	3.49^{ab}	2.47^{ab}
p value	0.663	0.036	0.146	0.371	0.028	0.138	0.760	0.525	0.852	0.582	0.401	0.044	0.046	0.048
SEM[1]	52.08	29.97	8.34	9.28	19.48	23.13	93.58	74.38	3.85	4.79	21.13	21.70	0.32	0.21

[a-c.] Means within a column with different superscripts are significantly different (p<0.05).[1] Standard error of mean. Values were taken from four replicates per treatment.

Table 3.5. The effect of feeding orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on ileal and cecalbacteriain broilers at 38 d of age

Bacterial group (log_{10} $CFUg^{-1}$)	Ileum			Cecum		
	Lactobacilli	Coliforms	Total Aer. Count	Lactobacilli	Coliforms	Total Aer. Count
Control	3.99	3.93[a]	5.01	4.62	4.45[a]	5.62
OPE (mg kg^{-1})						
200	4.15	3.69[ab]	5.46	5.16	4.22[ab]	5.89
400	4.12	3.73[ab]	5.38	5.14	4.42[ab]	5.96
LPE (mg kg^{-1})						
200	4.06	3.71[ab]	5.29	5.15	4.22[ab]	5.82
400	4.11	3.50[b]	5.10	4.92	4.12[ab]	6.01
CXEO (mg kg^{-1})						
200	4.40	3.71[ab]	5.47	5.19	4.14[ab]	5.93
400	4.55	3.42[b]	5.50	5.20	3.96[b]	6.41
p value	0.336	0.049	0.500	0.369	0.043	0.501
SEM[1]	0.18	0.12	0.20	0.20	0.14	0.25

[a-b.] Means within a column with different superscripts are significantly different ($p<0.05$).[1] Standard error of mean. Values were taken from four replicates per treatment.

DISCUSSION

Composition of citrus peels

The phenolic and flavonoid contents were lower than data reported by other authors (Wang et al., 2008a; Ghasemi et al., 2009), *e.g.*, using similar analytical methods. In this regard, Ghasemi et al. (2009) reported 131 mg gallic acid equivalent and 16.2 mg quercetin equivalent g^{-1} extract powder (following methanolic extraction) for total phenolic and flavonoids content respectively in lemon peel. Corresponding values for orange peel were223 mg gallic acid equivalent and 7.7 mg quercetin equivalent g^{-1} extract powder. Wang et al. (2008a) reported 32.7 mg rutin equivalents g^{-1} dried lemon

peel (after methanolic extraction also) for total flavonoid contents, with hesperidin being the major flavonoid. These differences may be due to a number of reasons, *e.g.*, variation in the agricultural soil profile and time of harvest. Fu et al. (2005) reported that similar samples produced in different countries could have different amount of bioactive compounds. However, the condition of the raw material and the extraction method has also a large impact on the yield of bioactive compounds, depending on the solvent type and concentration, time, temperature etc. (Lia et al., 2006; Garau et al., 2007).

Performance

Reported effects of dietary supplementation with plant extracts rich in phenolic compounds are inconsistent. Seven et al. (2008) found that using high doses of propolis rich in phenolics and vitamin C could partially overcome the depression in growth and carcass quality caused by heat stress in broilers. Reisinger et al. (2011)conducted an experiment with broiler chickens fed a phytogenic feed additive containing a blend of essential oils from oregano, anise, and citrus peel. Parallel to our results, there was no difference for feed intake and feed conversion ratio among the treatments. Sinurat et al. (2009) used *Curcuma longa* and *C. xanthorrhiza* powder as a feed additive for broiler chickens. In agreement with our results, these authors reported that supplementation with these plant products did not affect feed intake or feed conversion ratio. Lee et al. (2003) fed thymol, cinnamaldehyde and a commercial mixture of essential oil components to female broilers, and observed no differences in feed intake, weight gain and feed conversion ratio. Çabuk et al. (2006) used a blend of plant oils derived from oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel for laying hens during the summer season. They did not find a significant effect of plant oils on the feed intake but they found an improved feed conversion ratio for supplemented groups compared to their control. The non-significant effects of plant extracts observed in the current study can be due to several reasons, consisting of either inappropriate doses used or short duration of heat stress exposure in both hours and days. Also, as reviewed by Brenes & Roura (2010), the extraction

methods of plants, storing method and conservation duration of plant products could affect the results coming out from the plant extracts. Using higher levels of these plant extract could be taken into the account to see the positive effects on the broilers performance during hot weather of the year.

Body composition traits

In agreement with our results, Khaligh et al. (2011) indicated that supplementation of broiler diets with medicinal plant blends did not alter liver, gizzard and abdominal fat weight. On the other hand, Debersac et al. (2001) indicated that a herbal extract from rosemary, containing rosmarinic acid, flavones, and monoterpenes, enhanced hepatic metabolism and increased liver weight in rats.

The bursa of Fabricius, known as central or primary lymphoid organ, plays a crucial role in enzymatic maturation and acquisition of immunological competence of T- and B-lymphocytes (Rudrappa and Humphrey, 2007). Any disturbances in the development of bursa of Fabricius caused by stressors might result in significant deficiencies of the immune system functions in chicken (Oznurlu et al., 2010). Quinteiro-Filho et al. (2010) also observed that heat stress caused a decrease in the weight of the bursa of Fabricius. Zulkifli et al. (2002) reported that heat stress reduced antibody production in young chickens. A glucocorticoid-dependent mechanism during stress was reported to induce lymphoid organ involution (Shini et al., 2008). The increased relative weight of bursa of Fabricius found in our study supports the assumption that dietary OPE and CXEO may act to decrease heat stress in broiler chickens.

Small intestinal morphology

Heat stress leads to generation of free radicals, which can induce lipid peroxidation and thereby damage cell structures (Altan et al., 2003). Maintenance of normal morphology and structural integrity of the small intestine are imperative for preventing bacterial translocation from the intestinal tract. Heat stress could exert deleterious effects on the absorptive epithelium of the intestine, resulting in reduction in villus height and crypt depth (Yamauchi et al., 2006). Burkholder et

al.(2008) reported that birds subjected to 30°C for 24 h had reduced crypt depth compared with birds at 23°C. Smith et al. (1990) reported that villus height was reduced by 18.8% in heat stressed birds.

It has been suggested that some components of feeds can affect the mucosa thickness and villus height and intestinal brush border (Jamroz et al., 2006). Concerning phytogenic feed additives literature does not draw a consistent picture. Based on literature, feeding broiler chickens and pigs with phytogenic products could cause to increased, unchanged as well as reduced villus length and crypt depth in gut (Namkung et al., 2004; Nofrarías et al., 2006; Oetting et al., 2006; García et al., 2007). In our study, there were no significant differences between control and treated groups, with exception for villus:crypt ratio in the duodenum part that was reduced when chickens were fed with 400 mg kg^{-1} LPE diet as compared to control birds and those fed with 200 mg kg^{-1} OPE. It is hypothesized that the overall impact of plant products on gut morphology depends on the balance between tissue irritation and beneficial effects on intestinal hygiene. On the other hand, heat stress is associated with intestinal irritation. Results found in this study could be explained in this way that selected plant extracts at the present doses were not able to improve histomorphological criteria that might be negatively affected by heat stress and exert positive effect on gut morphology.

There was not any significant difference between the treatments in the ileum part. There are several possible reasons why ileal structure was unchanged in this experiment, including the resistance of the ileum to structural changes compared with other regions of the small intestine and possibly, the imposed high temperature stress was not severe enough so that plant extracts could exert a substantial effect. Yamauchi et al. (1996) indicated that morphological changes in response to stressors occur more rapidly in the proximal two-thirds of the small intestine than in the ileum.

Our findings on intestinal morphology indicate that feeding plant extracts which are high in phenolic compounds showed low activity and may not exert a proper effect on gut structure of broiler chickens under heat stress condition; therefore more studies with different doses and/or combination of selected extracts for inducing synergistic effects could be taken into account.

Intestinal microbiology

The indigenous gut microbiota is a complex ecosystem that can benefit the host by serving as a barrier to pathogen colonization (Van der Waaij, 1989). Alteration of this protective barrier may leave the host more susceptible to colonization by enteric pathogens (Durant et al., 1999). Neurohormones associated with stress can increase growth and virulence factor expression in pathogenic bacteria within the lumen (Lyte and Bailey, 1997). Hinton et al. (2000) showed an increase in intestinal *Enterobacteriaceae* and cecal aerobes with a concurrent decrease in lactic acid bacteria in broilers subjected to a 24-h feed withdrawal. Burkholder et al. (2008) noted that heat stress significantly decreased the intestinal bacterial populations of birds.

For many years, herbs and their extracts have been used as pharmaceuticals as a natural therapy, their antimicrobial ability may modulate the gut ecosystem to affect digestibility of feeds (Hernández et al., 2004). In total, there is clear experimental evidence for an overall antimicrobial efficacy of phytogenic feed additives whether arising directly from an antimicrobial action or indirectly mediated by phytogenics to affect the microbiota: 1) The direct effect of plant oils is due to their lipophilic activities, adhesion and pass into the bacterial membrane which prevents activation of bacterial enzymes. Liu et al. (2008) concluded that phenolic compounds due to their hydrophobicity are able to disintegrate the outer membrane of gram-negative bacteria, and disturbing the bacterial structure. Likewise, Michiels et al. (2007) concluded that phenolic compounds can especially be used to reduce the bacterial population in the proximal and more acidic parts of the gastrointestinal tract. 2) Indirect effect of plant extracts have been reported due to reducing ileal pH value (which, unfortunately has not been executed in this experiment), while increasing the number of lactic acid bacteria and decreasing the coliform counts in the ileum and caecal contents of broiler chickens (Vidanarachchi et al., 2006). In addition, it is mentioned that enhancement of activities of digestive enzymes by plant extracts could also increase nutrient digestibility and improve the regulation and stabilization of the gut microbiota. In this study, there was a significant reduction for coliform counts with no effect on other bacteria. In accordance with our results, Si et al. (2006) reported that it is

possible to select plant bioactive compounds with a strong antimicrobial action against gut pathogens whilst not harming beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*.

Based on our results, reducing pathogenic bacteria such as coliforms by 400 mg kg^{-1} LPE and CXEO, could contribute to a balanced gut microbiota, thus improving the ability to preserve intestinal integrity.

Under the conditions of this study, even though dietary CXEO, OPE and LPE did not have a significant effect on chickens performance and gut morphology, it can be stated that dietary CXEO and LPE at 400 mg kg^{-1} feed could be an option to use in broiler chicken diet during the finisher phase to prevent or diminish the stress-induced alteration of the intestinal microbiota and immunological functions.

Further studies are required to fully explore dose-response effects on the broiler performances and gut histomorphology.

CHAPTER 4

ANTIOXIDANT ENZYMES ACTIVITIES, PLASMA HORMONE LEVELS, AND SERUM METABOLITES OF FINISHING BROILER CHICKENS REARED UNDER HIGH AMBIENT TEMPERATURE AND FED LEMON AND ORANGE PEEL EXTRACTS AND *CURCUMA XANTHORRHIZA* ESSENTIAL OILS

Redrafted after:

Akbarian, A., Golian, A., Kermanshahi, H., De Smet, S., Michiels, J. (2014) Antioxidant enzymes activities, plasma hormone levels, and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and *Curcuma xanthorrhiza* essential oil. Journal of Animal Physiology and Animal Nutrition.DOI: 10.1111/jpn.12188.

CHAPTER 4

ANTIOXIDANT ENZYMES ACTIVITIES, PLASMA HORMONE LEVELS, AND SERUM METABOLITES OF FINISHING BROILER CHICKENS REARED UNDER HIGH AMBIENT TEMPERATURE AND FED LEMON AND ORANGE PEEL EXTRACTS AND *CURCUMA XANTHORRHIZA* ESSENTIAL OILS

ABSTRACT

The negative effects of high ambient temperature during some months of the year on poultry production has been of great concern in many countries. Dietary modifications are among the most practical ways to alleviate the effects of high temperature. Possible effects of dietary supplementation with 200 or 400 mg/kg feed of lemon peel extract (LPE), orange peel extract (OPE) and *Curcuma xanthorrhiza* essential oil (CXEO) under hot conditions (34°C with 50% relative humidity for 5 h daily starting from d 28 until d 38 of age), on blood antioxidant enzyme activities, biochemical parameters and antibody titers of broiler chickens were investigated. All extracts are rich in phenolic compounds and highly available. Compared to control, supplementation with OPE at 400 mg/kg and CXEO significantly increased erythrocyte glutathione peroxidase and superoxide dismutase activity, plasma growth hormone concentrations and serum phosphorus, total protein and chloride concentrations and decreasedserum LDL-cholesterol and total cholesterol concentrations in chickens at 38 d of age. Regarding antibody titers, CXEO supplementation at 400 mg/kg caused a significant increase in Bronchitis antibody titers. Supplementation with LPE and OPE gave more inconsistent

results. Most interesting, 400 mg/kg LPE significantly increased 3,5,3′-triiodothyronine concentration as compared to the control. In conclusion, the herbal extracts tested in this study, in particular CXEO at 400 mg/kg, may relieve some of the changes in blood composition induced by elevated ambient temperatures.

INTRODUCTION

Chronic heat and high humidity, occurring in temperate countries as well as in the tropical world, exert deleterious effects on performance and mortality of broiler chickens. In south of Iran, the environmental temperature during the greater part of the year remains well beyond the upper limit of the bird's thermoneutral zone, and the adverse effects of high temperature can make poultry production difficult and uneconomical (Ahmad et al., 2006). It has been well documented that exposing broiler chickens to continuously high temperature, especially during the finisher phase leads to chronic heat stress (Sahin et al., 2003; Ahmad et al., 2006). The metabolic changes induced in chickens by chronic heat exposure include impairment in endocrine functions. Decreased serum concentrations of T_3 and T_4, important growth promoters in animals, and suppressed immune system function during high ambient temperature have been reported (Yahav and McMurtry, 2001). Heat stress increases plasma corticosterone, glucose and cholesterol concentrations, reduces serum protein concentrations, depletes potassium and other minerals in the body, elevates lipid peroxidation products in blood and tissues and alters the delicate electrolyte balance in the body. Alteration in electrolyte balance due to increased temperature is probably an indirect result of panting or hemodilution following increased water consumption. Importantly, high ambient temperature also results in impaired antioxidant status and induces oxidative stress in poultry (Lin et al., 2006a; Mujahid et al., 2007).

The broiler industry employs mixing fans, tunnel ventilation, and evaporative cooling to reduce heat stress. When outside temperatures are above 30^{o}C, increasing air circulation in the broiler house may not be the best solution, and misting increases humidity levels. High humidity and high temperature are conducive to the growth of pathogenic microorganisms and spread of disease. In this respect, dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature on poultry performance. Studies have shown that under stressful conditions, the requirements for antioxidants are thought to be increased to protect tissues from lipid

peroxidation, and antioxidant nutrient supplementation, could be used to attenuate the negative effects of environmental stress (Sahin et al., 2002). It has been shown that vitamin A, vitamin E and vitamin C as antioxidants when increased or included in the diet of broiler chickens can counteract heat induced effects (Lin et al., 2006b). Because of the fluctuating availability and prices of some of these nutrients and the growing interest in substances derived from natural sources the potential of some plant extracts was investigated.

Recently, studies have shown that phenolic compounds (PC) have beneficial effects related to their antioxidant, antiinflammatory, and antimicrobial activities (Akbarian et al., 2011; Viveros et al., 2011). These ingredients are found in many plants such as fruits and vegetables. Fruits of the citrus family (particularly orange and lemon), and herbs of the *Zingiberaceae* (particularly turmeric) and *Lamiacae* family (e.g., rosemary) have been extensively studied for their antioxidant activity. Orange *(Citrus aurantium)* and lemon *(Citrus limon)* peel are common by-products of the food and juice extraction industry and the most widely consumed citrus in the world (Ghasemi et al., 2009). In fact, the production of orange and lemon reached nearly 1, 723 and 472 metric thousand tons in 2011, respectively in Iran (FAO, 2012). The peel from citrus fruit represents approximately one-fourth of whole fruit mass and is obtained after the extraction of juice and removing the remaining pulp inside mechanically (Braddock, 1999). Citrus peels are available at low cost in most seasons of the year in Iran. During citrus juice processing, a considerable quantity of wastes or by-products is generated. Though large quantities of citrus pulps are diverted to animal (ruminant) feeds, the majority of the processing residue are thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications of these by-products. Currently, almost no information is available about feeding orange and lemon peel or extracts hereof to broiler chickens under hotconditions (e.g., Akbarian et al., 2013). *Curcuma xanthorrhiza* (commonly known as temulawak or Javanese turmeric in Indonesia), grows in Southeast Asia and is found both wild and cultivated in Indonesia. It is traditionally used for medicinal purposes. The rhizome and root of this plant contain beneficial constituents that have been used in the treatment of acne and skin

inflammations. More recently, studies have demonstrated that phenolic compounds derived from *C. xanthorrhiza* showed antioxidative and anti-inflammatory characteristics *in vitro* (Ozaki, 1990; Masuda et al., 1992; Rukayadi et al., 2006).

The objective of the present study was to test the effects of different plant extracts at various inclusion levels on the antioxidant function, biochemical changes and immune responses of broilers chickens when they are subjected to a high ambient temperature in the finishing phase. With that goal, all groups were exposed to high ambient temperature throughout the experiment and a control group without receiving any of plant extracts was included.

MATERIALS AND METHODS

C. xanthorrhiza essential oil and citrus peel extracts

C. xanthorrhiza essential oil was obtained from PT. PHYTOCHEMINDO REKSA (Bogor, Indonesia). According to the compositional data provided by the supplier the main bioactive compounds were: ar-curcumene (app. 11.4%), β-curcumene (app 8.5%) and xanthorrhizol (hydroxy-ar-curcumene) (app. 28%). Xanthorrizhol is a sesquiterpenoid compound and the antioxidant properties of *C. xanthorriza* essential oil are ascribed to the phenolic structure of this compound. This essential oil of *C. xanthorriza* was denominated as CXEO, and used as such in the feeding experiment.

Fruit peels of orange *(C. aurantium)* and lemon *(C. limon)* were obtained from a citrus processing industry of Mashhad in KhorasanRazavi province of Iran on October 2010. The products were dried in an air draught oven at 40ºC for 12 h. The dried samples were ground using a laboratory mill (Braun, Model 2001DL, Germany), then packed in polyethylene bags and stored at –20ºC until use. Fifty grams of each ground sample (orange peel and lemon peel) were extracted three times with 500 ml of ethanol solution (50%) using a Teflon-coated magnetic stir bar and stir plate for 6 h at room temperature. Extracts were filtrated through Whatman No. 1 filter paper. The combined filtrates from

the three extractions were concentrated in a rotary evaporator at 40°C to a final volume of 100 ml crude extract and stored at –20°C until use. These extracts were denominated as OPE (orange peel extract) and LPE (lemon peel extract), and used as such in the feeding experiment.

Total phenolic compounds were determined with Folin–Ciocalteu reagent using tannic acid as standard according to the method described by Taga et al. (1984) with some modifications. Extract samples and standard were prepared in 70:30 acidified ethanol/water (0.3% HCl). Hundred µL of the test solutions (extracts or standard) was mixed with 0.2 mL of saturated sodium carbonate (Na_2CO_3). After 2 min at room temperature, 100 mL of 50% Folin-Ciocalteu reagent wasadded. The mixture was then allowed to stand at room temperature for 25 min. The absorbance was measured at 750 nm on a spectrophotometer (UV-160A; Shimadzu Co., Kyoto, Japan). All samples were analyzed in triplicate. Results are expressed as mg tannic acid equivalents/g extract. Separation and quantification of phenolic compounds was established as described by Ricardo-da-Silva et al. (1993) using HPLC [Consta Metric 4100 pump and ODC-2 column (3 µm; 150 mm × 4.6 mm i.d.); Thermo Separation Products, Evisa, Riviera Beach, FL, USA), a fluorescence detector (FL 3000,Excitation wavelength: 250 nm – Emission wavelength: 400 nm (2475 Multi λ, Waters Corporation, Manchester, UK)], and interfaced with a computer equipped with software (PC 1000 Chromatography Software,Version 3.5; Dionex, California, USA). Methanol:ammonium acetate (12:88 vol/vol) was used as the mobile phase with a flow rate of 1 mL/min.

To test for the presence of flavonoid compounds in the samples, a modified colorimetric aluminum chloride method was used as reported by Woisky and Salatino (1998). Separation of flavonoids was performed using HPLC according to the method described by Baldi et al. (1995).

Animals, diets and experimental design

The experimental protocol was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. A total number of 336 unsexed Ross 308 broiler chicks was obtained from a commercial hatchery (Quchan, Mashhad, Iran) and commercially raised for 25 days before the

commencement of the study. Chicks were vaccinated for Infectious Bronchitis (Nobilis IB 4/91, Merck Animal Health, Boxmeer, The Netherlands) on the first day, Avian Influenza (NobilisInfuenza TRT, Merck Animal Health, Boxmeer, The Netherlands)on day 7 and Newcastle Disease and Infectious Bursal Disease (Nobilis G+ND,Merck Animal Health, Boxmeer, The Netherlands) on day 14 of age. At d 25 of age, the birds were randomly allotted to 28 floor pens with 12 birds each. Each pen (1 m^2) was equipped with a manual feeder and two nipple drinkers, and the floor was covered with clean wood shavings. The ventilation rate was 0.12 m/s during the whole period. Light was made available around the clock with an intensity of approximately 20 lux (23 h light:1 h dark). The initial house temperature was 32°C and then gradually decreased to reach 22°C at 21 d of age. The birds were given a finisher diet from d 25 to 38. The basal diet was formulated to meet the nutrient requirements of the broiler chickens as recommended by Ross 308 broiler management guide (Aviagen; available at http://en.aviagen.com/ross-308/). Ingredient and chemical composition of the basal finisher diet are shown in Table 4.1.

A completely randomized design was used with seven dietary treatments replicated in 4 pens each. The dietary treatments were: a basal diet (control treatment) and the same diet supplemented with either OPE, LPE or CXEO at two different levels (200 and 400 mg/kg). The extracts and essential oil were first mixed very well with the associated corn oil and then gradually added to the basal diet. Feed and water were offered *ad libitum.* The feeding experiment period lasted 13 days (25-38 d of age). In order to accustom to the experimental diets; a three day adaptation period was included before increasing house temperature. From d 28, a different temperature regime was followed as reported by Aksit et al. (2006). The basal temperature was 22°C. Between 8:30 and 10:00 AM the temperature was gradually increased to 34°C and this high temperature was then maintained for five hours (until 15:00 PM). After that, the temperature was gradually decreased to the basal level by 16:30 PM. Temperature control was executed by heating elements, air conditioner and dynamic ventilation. Average relative humidity was kept 50% during both the experimental period and the rest

of the day. Body weight gain (BWG) and feed intake (FI) were measured and feed conversion ratio (FCR) was calculated during the experiment.

Table 4.1. Ingredient and calculated nutrient composition of the basal finisher diet fed from 25 to 38 d of age.

Item	Content
Ingredients, g/kg	
Corn	607.8
Soybean meal	319.7
Vegetable oil	36.8
Limestone	10.4
Dicalcium phosphate	14.2
Common salt	4.3
DL-Methionine	1.0
L-Lysine HCL	0.8
Vitamin and mineral premix[a]	5.0
Calculated composition	
ME (MJ/kg)	12.76
CP (g/kg)	193.0
Ca (g/kg)	8.1
Available P (g/kg)	4.0
Ileal digestibe lysine (g/kg)	10.5
Ileal digestible methionine (g/kg)	4.0
Ileal digestible Met + Cys (g/kg)	7.2
DCAB(mEq/kg)[b]	211.0

[a] vitamin and mineral premix supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 9,790 IU; vitamin E, 121 IU; vitamin B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg; sodium selenite, 0.3 mg; potassium iodide, 1 mg; copper(II) sulfate, 10 mg; and iron sulfate, 50 mg.
[b] DCAB = dietary cation-anion balance (Na + K – Cl).

Blood sampling

Four chicks from each pen (16 per treatment) were randomly selected and weighed individually after a 6-h fasting period at the end of the experiment (38 d of age, sampling starting after the 5 hours of high ambient temperature). Blood samples were collected from the wing vein with a 25 G needle. Three blood samples were obtained for subsequent determination of serum, plasma and red blood cells chemical constituents. The first sample was collected for plasma 3,5,3′-triiodothyronine (T_3), thyroxine (T_4) and growth hormone (GH) measurements and placed into heparinized tubes. The tubes were centrifuged at 839 g for 15 min to obtain plasma, which was stored at -20^{o}C pending analysis.

The birds were handled with care and the blood was drawn within 2 min to minimize artifact on hormone responses due to handling. The second sample was collected in a heparinized graduated centrifuge tube up to the marked level to obtain hemolysate. Plasma was separated and erythrocytes were washed and centrifuged (839 g, 15 min) thrice with normal saline solution, then distilled water was added to erythrocyte pellet slowly with constant stirring up to the marked level which was stored in aliquots at -20°C until further analysis. A third coagulated blood sample was centrifuged (839 g, 10 min) within 30 min after sampling to obtain serum. Serum samples were divided in two aliquots and kept at -20°C until analyzed.

Chemical analysis of blood components

Plasma 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) concentrations were determined by double-antibody RIA using commercially available RIA kits (China Institute of Atomic Energy, Beijing, China) as described by (Darras et al., 1992).The blood antioxidant status was evaluated by measuring the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity in hemolysate. GSH-Px activity was determined using a commercially available enzyme kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). SOD activity was determined using the commercially available enzyme kit (Ransod, RANDOX/SD-125 supplied by Randox Laboratories, Crumlin, UK). The reported procedures were used to measure calcium (Ca), phosphorous (P), electrolytes, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Burtis and Ashwood, 1998), total protein (TP), glucose, albumin, total & direct bilirubin and uric acid (Tietz, 1995), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerol and total cholesterol (Nauck et al., 2002). Second serum samples were tested using HI test (Newcastle) according to Xu et al. (1997) and indirect antibody enzyme-linked immunosorbent assay (ELISA) kit (Influenza, Bronchitis and Gamboro) according to the manufacturer's (Svanova Biotech, Uppsala, Sweden) instruction (Looraine, 1982). To avoid inter-assay variability, all samples were run in the same assay.

Statistical analysis

Data were analyzed by a linear model with the fixed effect of treatment using the General Linear Model procedure of the Statistical Analysis System (SAS, 2004). Tukey's multiple range test was used to compare the means. Prior to analysis, the antibody titers were normalized using arcsin transformation. All statements of significance are based on probability $P<0.05$.

RESULTS

Composition of citrus peel extracts

The total content of phenolic compounds was 34.9 and 33.7 mg tannic acid equivalents per g extract for OPE and LPE respectively, with protocatchic accounting for approximately 80% in both extracts, catechol for 6.4 and 10.0% respectively and the other compounds each for less than 5% relative to the total content of phenolic compounds (Table 2.2). The total flavonoid contents were 3.68 and 4.52 mg quercetinper g extract in OPE and LPE, respectively. Notable differences were only seen for the luteolin content.

Performance

Animal performances were satisfactory, however lower when compared with target performances as outlined by Aviagen for Ross 308 broilers (Broiler Performances Objectives 2012; Aviagen, available at http://en.aviagen.com/ross-308/). Dietary OPE, LPE and CXEO did not significantly affect BWG, FI, and FCR of chickens during the experiment. The results are reported and discussed by Akbarian et al. (2013).

Erythrocytes antioxidant status

Our findings show that OPE and CXEO promote antioxidant enzyme responses in broiler chickens, with GSH-Px being the most sensitive (Table 4.2). Birds fed supplemented diets (400 mg/kg OPE, 200 and 400 mg/kg CXEO) showed a significant increase in GSH-Px activity as compared to those fed control diet ($P<0.05$), whereas birds fed 400 mg/kg CXEO diet showed an increase in SOD activity ($P<0.05$).

Thyroid plasma hormones

The plasma T_4 concentrations did not significantly change when birds were supplemented with LPE, OPE or CXEO, whereas the addition of 400 mg/kg LPE significantly increased plasma T_3 concentration compared to those fed control diet.

Table 4.2. The effect of dietary orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on antioxidant status in erythrocytes and metabolic hormone concentrations in plasma of broilers at d 38 when exposed to chronic heat stress during 28-38 d of age

	Control	OPE(mg/kg)		LPE(mg/kg)		CXEO(mg/kg)		SEM[1]	*P value*
		200	400	200	400	200	400		
GSH-Px[2] (U/mL)	30146^{d}	31161^{cd}	33365^{bc}	32225^{cd}	32215^{cd}	34613^{ab}	36488^{a}	702.3	0.001
SOD[3] (U/mL)	177^{b}	188^{ab}	188^{ab}	194^{ab}	195^{ab}	198^{ab}	208^{a}	6.6	0.047
T_3[4] (ng/100 mL)	119^{b}	125^{b}	139^{ab}	132^{b}	159^{a}	123^{b}	128^{b}	7.9	0.029
T_4[5] (mg/100 mL)	1.48	1.33	1.45	1.50	1.45	1.28	1.28	0.086	0.313

[a-c.] Means within a row with no common superscript are significantly different (P<0.05).
[1]Standard error of mean; [2] Glutathione peroxidase; [3]Superoxide dismutase; [4]3,5,3′-triiodothyronine; [5]Thyroxine

Serum biochemical parameters

The calcium, albumin, total and direct bilirubin, uric acid, creatinine concentrations and ALT activity were similar in birds fed diets with/without LPE, OPE or CXEO supplementation (Table 4.3). Feeding 400 mg/kg CXEO to birds caused an increase in serum phosphorus compared to control and other ones. Serum glucose concentrations tended to be affected (P=0.072) by treatment whereby the supplementation with CXEO at 400 mg/kg resulted in the lowest serum glucose concentrations. The inclusion of 200 and 400 mg/kg OPE and 400 mg/kg CXEO significantly increased serum total protein levels compared to control group. A tendency was found (P=0.073) for the effect on AST activity in serum. All supplemented groups showed reduced AST activities in serum with 400 mg/kg CXEO being the lowest.

The effects of plant extracts on serum lipid profiles following administration of broiler chickens is shown in Table 4.4. Even though dietary supplementation with OPE, LPE and CXEO did not influence serum HDL and triacylglycerol levels, feeding 200 mg/kg OPE and 400 mg/kg CXEO diets significantly decreased serum LDL cholesterol, and 400 mg/kg CXEO diet decreased total cholesterol level of serum in heat exposed broiler chickens. The serum levels of sodium and potassium were not significantly influenced by the extracts (Table 4.4). Serum level of chloride was significantly higher in all OPE, LPE and CXEO supplemented birds compared to control ones.

Table 4.3. Effect of orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on theserum concentration of biochemical parameters in broilers at d 38 when exposed to chronic heat stress during 28-38 d of age

	Control	OPE(mg/kg)		LPE(mg/kg)		CXEO(mg/kg)		SEM[1]	*P value*
		200	400	200	400	200	400		
AST[2] (IU/L)	246	239	234	217	222	227	201	9.8	0.073
ALT[3] (IU/L)	12	11	11	11	12	11	12	0.6	0.985
Calcium (mg/100 mL)	7.73	7.85	8.20	8.20	8.60	8.32	8.72	0.382	0.526
Phosphorus (mg/100 mL)	6.35^{b}	6.90^{b}	7.13^{ab}	6.53^{b}	7.07^{ab}	7.16^{ab}	7.87^{a}	0.261	0.013
Total Protein(g/100 mL)	3.59^{b}	4.17^{a}	4.20^{a}	3.66^{b}	3.91^{ab}	3.72^{b}	4.05^{a}	0.102	0.009
Glucose (mg/100 mL)	293	274	291	280	284	285	268	6.1	0.072
Albumin (g/100 mL)	1.87	1.77	1.75	1.65	1.86	1.85	1.65	0.082	0.264
Total Bilirubin (mg/100 mL)	0.30	0.30	0.23	0.30	0.28	0.28	0.23	0.034	0.630
Direct Bilirubin (mg/100 mL)	0.18	0.15	0.18	0.18	0.18	0.18	0.15	0.034	0.991
Uric Acid (mg/100 mL)	9.75	8.25	9.75	10.00	9.50	8.75	9.75	0.697	0.545
Creatinine (mg/100 mL)	0.42	0.44	0.42	0.39	0.40	0.38	0.37	0.027	0.642

[a-c.] Means within a row with no common superscript are significantly different (P<0.05).
[1] Standard error of mean; [2]Aspartate aminotransferase; [3]Alanine aminotransferase

Table 4.4. Effect of orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) onthe serumconcentration of lipids and electrolytes in broilers at d 38 when exposed to chronic heat stress during 28-38 d of age

	Control	OPE(mg/kg)		LPE(mg/kg)		CXEO(mg/kg)		SEM[1]	*P value*
		200	400	200	400	200	400		
LDL[2](mg/100 mL)	27[a]	18[b]	22[ab]	22[ab]	23[ab]	23[ab]	17[b]	2.0	0.048
HDL[3](mg/100 mL)	86	95	96	93	100	101	97	5.3	0.477
Triacylglycerol(mg/100 mL)	93	81	86	93	83	82	81	4.2	0.227
Cholesterol(mg/100 mL)	149[a]	122[ab]	137[ab]	124[ab]	143[ab]	126[ab]	118[b]	8.4	0.019
Sodium(mEq/L)	117	132	131	123	126	131	131	4.5	0.219
Potassium(mEq/L)	5.3	4.9	4.7	4.8	4.9	4.9	5.4	0.32	0.789
Chloride(mEq/L)	32[b]	46[a]	54[a]	50[a]	53[a]	50[a]	54[a]	2.9	0.002

[a-c.] Means within a row with no common superscript are significantly different (P<0.05).
[1]Standard error of mean; [2]Low density lipoprotein; [3] High density lipoprotein

Serum antibody titers

The effects of dietary supplementation with extracts on serum antibody titers are given in Table 4.5. In contrast to antibody titers for Newcastle, Gumburo and Influenza only antibody titers for Bronchitis were affected by treatment (P=0.015; data not given). Feeding birds with 400 mg/kg LPE (1.25 relative value without unit) and 400 mg/kg CXEO (1.27) significantly increased the Bronchitis antibody titer compared to those fed control diet (1.17).

Table 4.5. Effect of orange peel extract (OPE), lemon peel extract (LPE) and *Curcuma xanthorrhiza* essential oil (CXEO) on serum antibody titers[1] inbroilers at d 38 when exposed to chronic heat stress during 28-38 d of age (n=16)

	Control	OPE (mg/kg)		LPE (mg/kg)		CXEO (mg/kg)		SEM[2]	*P value*
		200	400	200	400	200	400		
Newcastle	1.37	1.34	1.31	1.33	1.38	1.38	1.36	0.025	0.511
Bronchitis	1.17^{bc}	1.15^{c}	1.15^{c}	1.22^{abc}	1.25^{a}	1.24^{ab}	1.27^{a}	0.025	0.015
Gumbro	1.13	1.18	1.18	1.16	1.15	1.16	1.18	0.035	0.950
Influenza	1.34	1.36	1.35	1.35	1.36	1.35	1.33	0.014	0.791

[a-c.] Means within a row with no common superscript are significantly different (P<0.05).
[1] Arbitrary unit
[2] Standard error of mean

DISCUSSION

The phenolic and flavonoid contents were lower than data reported by other authors (Wang et al., 2008a; Ghasemi et al., 2009), e.g., using similar analytical methods. In this regard, Ghasemi et al. (2009) reported 131 mg gallic acid equivalent and 16.2 mg quercetin equivalent per g extract powder (following methanolic extraction) for total phenolic and flavonoid contents respectively in lemon peel.As discussed by Akbarian et al. (2013), the content of bioactive compounds in plants depends on several factors.

Dietary plant extracts did not affect BWG, FI, FCR, and mortality of chickens. The performances data are sub-sets of results from the same experiment and have been discussed by Akbarian et al. (2013a). Elevated ambient temperature is believed to disturb the balance between the production of reactive oxygen species (ROS) and the antioxidant systems in broiler chickens (Lin et al., 2006a). It is noted that oxidative stress has been associated with not only the elevated production of free radicals but also with changes to the scavenging capacity of antioxidant systems (Fu et al., 2008). Living organisms are able to adapt to oxidative stress by inducing the synthesis of antioxidant

enzymes and damage removal/repair enzymes. The antioxidant systems in the body contain antioxidant enzymes such as SOD and GSH-Px which are employed to protect the body from oxidative stress. In the present study, SOD and GSH-Px activities were used to estimate the responses of enzymatic scavenging systems. For both enzymes significant effects were found; however it appears that mainly CXEO at 200 mg/kg (GSH-Px) and at 400 mg/kg (GSH-Px and SOD) could up-regulate these enzymes. Likely the higher final concentration of phenolic compounds provided by this extract as compared to the citrus peel extracts can explain the differences in effects on the antioxidant system. In this respect, no differences were found between OPE and LPE. Our findings are consistent with those of Seven et al. (2008), who reported that feeding plants extracts rich in phenolic compound resulted in an increase in blood GSH-Px activity of chronic heat exposed broiler chickens. Also, Akbarian et al. (2011) reported that using *Zingiber officinale* root rich in phenolic compounds, caused an increase in plasma GSH-Px activity in laying hens. Vissers et al. (2004) concluded that phenolic compounds are able to increase antioxidant power, either by increase in the activity of antioxidant enzymes or scavenging the reactive oxygen species in the cells. The effect on the antioxidant system of the chicken by CXEO is most likely attributed to the phenolic compounds, i.e.,*xanthorrizol*. In diabetic rats treated with an ethanolic extract of *Aloe vera* leaves, a significant increase in the activity of antioxidant enzymes was observed. The oral administration of an ethanolic extract of *Aloe vera* leaves (containing large amount of phenolic compounds) to mice reduced oxidative stress induced by higher levels of antioxidant enzyme activity in plasma (Rajasekaran et al., 2005). Faix et al. (2009) reported that the GSH-Px activity in blood erythrocytes was affected by *Cinnamomum zeylanicum* essential oil as a potential antioxidant. Thus, it appears that these spices exert antioxidant protection through their ability to up-regulate the antioxidant enzymes.

To expand this further, this effect (up-regulation of antioxidant enzymes via plant antioxidants) can act as a spare mechanism for living cells when they are challenging with heat stress for long time, e.g., the condition of the present study. In other words, long term of heat stress could negatively affect antioxidant defence system by increasing the excretion of minerals such as Zn, Cu, and Se and

vitamins such as vitamin C and E and therefore causing marginal deficiencies of these substances that act as co-factors and co-enzymes for antioxidant enzymes (Sahin and Knuck, 2003; Balnave, 2004; Sahin et al., 2009). Consequently, the activity of antioxidant enzymes will be reduced and including feed antioxidants can reverse the excretion of vitamins and minerals under high ambient temperature, therefore positive effect on antioxidant system of birds (Sahin et al., 2006).

With feeding these phenolic bioactive compounds, this study aimed at reducing oxidative stress, and this effect was expected because the plant extracts were chosen owing to their antioxidant properties. Some general ways have been proposed as strategies of antioxidant system to mitigate oxidative stress. In this regard, it has also been suggested that phenolic compounds supplemented to the diet can support antioxidant system and exert their potential health profits possibly through: direct scavenging of ROS produced after stress conditions and/or prevent the formation of ROS by inhibiting enzymes or chelating trace metals (Thring et al., 2011), activation of antioxidant enzymes, inhibition of pro-oxidant enzymes such as NADPH-oxidase and lipoxygenase, increase in uric acid levels, attenuation of oxidative stress caused by free radicals (Schewe et al., 2008). Given all these potential beneficial effects of phenolic compounds on oxidative stress and consequently overall health of chickens, in our previous works, we have shown that incorporating phenolic compounds into the diet could show some subtle beneficial effects on biochemical and gut health parameters of broiler chickens raised under hot conditions (Akbarian et al., 2013 a,b).

As reviewed by Siriwardhana et al. (2013), phenolic compounds might also indirectly combat oxidative stress through positive effects on inflammation. These bioactive compounds have been shown to suppress hepatic NF-κB activation, and IκB degradation, resulting in reduced levels of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6 in different tissues and inhibit the expression of some other parameters of oxidative stress such as nitric oxide synthase (Gonzales and Orlando, 2008).

It has been shown that feed components consisting of phenolic compounds are able to increase the expression of 2,3,3-trihydroxybutanoic acid. The latter helps to regenerate vitamin C which is known

for its antioxidant activity (Bakker et al., 2010). In a recent work, Esfahani et al. (2011) have reported that fruits and vegetables including phenolic compounds, noticeably increased the concentration of pro-vitamins and vitamins (β-carotene, vitamin C and E) in human serum, followed by reducing oxidative stress markers.

Another indirect mechanism towards improving oxidative status via phenolic bioactive compounds involves the disulfide-bond A oxidoreductase-like protein (DsbA-L) synthesis which in turn increases levels of adiponectin (a 30-KDa protein with antioxidant properties) in tissues (Siriwardhana et al., 2013).

However, it should be stated that in the present study we measured only 2 parameters of oxidative status (GSH-Px and SOD activity) and further measurements such as levels of antioxidants like glutathione, total antioxidant capacity of blood and organs, etc. can be considered to cast more light on the mode of action of specific phenolic compounds on oxidative status of broilers under high ambient temperatures.

The importance of thyroid gland hormones in adaptation to high ambient temperature is related to the central role that thyroid hormones play in the regulation of metabolic rate of birds during growth and egg production (May et al., 1986). The inverse relationship between plasma concentration of T_3 and increased temperature is well known (Sahin and Kucuk, 2003). Chronic heat stress markedly depressed the activity of the thyrotrophic axis in layer hens as reflected by reduced plasma T_3 concentrations resulting in functional hypothyroidism (Mitchell and Carlisle, 1992). Mitchell and Carlisle (1992) and Geraert et al. (1996) found a dramatic decline of plasma T_3 in broiler chickens reared at 35°C and 32°C environmental temperature, respectively. In the current experiment, plasma T_4 levels were not changed, whereas T_3 levels were found to be higher in broilers fed with plant extracts, which may imply an altered peripheral deiodinase system, i.e., alteration of iodide peroxidise ezyme activity in blood. Sahin et al. (2003) conducted an experiment with broiler chickens reared under high ambient temperature and antioxidant supplementation (vitamin C and chromium). They found a significant increase in T_3 levels in chickens fed vitamin C and chromium compared to

their control. This leads to the presumption that antioxidant supplementation may be favorable to counteract the negative effects of heat stress on the thyrotrophic axis. This is in agreement with the current results where the treatments that showed a clear upregulation of GSH-Px and SOD (400 mg/kg CXEO and non-significant 400 mg/kg LPE) also resulted in increased T_3concentrations. Supplementation with these extracts may provide tolerance to stress and/or attenuate the negative responses of high temperature in broiler chickens.

A number of enzymes are used in the clinical biochemistry as tools for differential diagnosis, such as ALT and AST. Since the bulk of each is located in different tissues, their abnormal appearance in the blood plasma can give a hint to specific muscle or organ damages (Pech-Waffenschmidt et al., 1995). The increase in the activities of AST and ALT enzymes in plasma is an indication of liver damage and thus causes alterations in liver function (Kim et al., 2008).

The effect of hot conditioning on the activity of investigated enzymes was found to be inconsistent in relation to age and genotypes. According to the results obtained by Pech-Waffenschmidt, (1995), heat exposure did not significantly change the enzyme activities in the chicken's serum. This was also supported by previous findings of Ward and Peterson, (1973), who reported that the activity of AST was not influenced even by acute heat exposure. In the present study, it was found that AST activity tended to decrease in birds fed with 400 mg/kg CXEO compared to other treatments ($P<0.073$). The result of the present study is in agreement with Kolankaya et al.(2002), who reported that the plasma AST activity was decreased by supplementary phytogenics containing phenolic compounds. Once again, Seven et al. (2008) reported that AST activity of broilers fed vitamin C and phytogenics rich in phenolic compounds were significantly decreased compared when chronically heat exposed. Zaidi et al. (2005) showed that the AST activity in blood significantly increased under stressful conditions. The decreased AST activity found in our study supports the assumption that CXEO, particularly when supplemented at 400 mg/kg, may decrease the high temperature responses in broiler chickens.

The inclusion of 400 mg/kg CXEO diet to birds caused an increase in serum phosphorus compared to control and other treatments. Arad et al. (1983) documented that high temperature exposure caused a decrease in plasma levels of phosphorus and calcium. The present results, may indicate that the CXEO is able to alleviate increases in mineral excretion due to ahigh ambient temperature. Furthermore, the increase in total serum protein in birds exposed to high temperature showed additional beneficial effects of feeding plant extracts containing phenolic compounds. Our results are consistent with the results of Eraslan et al. (2007), these authors observed a significant increase in total protein in serum of rats fed propolis, which is rich in flavonoid and phenolic compounds. One mechanism through which plants extracts rich in phenolic compounds may exert their hyperproteinemia action is via transamination. Phenol and its derivatives can alter protein metabolism by altering transamination rate of amino acids by enhancing the activity of AST and ALT (Nassr-Allah, 2007), However, this study showed a tendency to decrease the AST activity. This discrepancy may be due to differences in the experimental conditions, for instance, exposing chickens to high temperature in our study. In our study, increased total serum protein levels were found for CXEO at 400 mg/kg and for OPE at both inclusion levels. The latter effect is surprising because it differs from the effect of LPE. It remains difficult to relate these discrepancies to differences in the phenolic and/or flavonoid composition of both citrus peel extracts.

It is speculated that the low level of LDL-cholesterol is associated with high serum GSH-Px activities in young adults (Luoma et al., 1990). Attia et al. (2011) noted that chronically elevated temperature significantly increased the value of plasma triacylglycerols, while ascorbic acid supplementation as an antioxidant significantly decreased plasma triacylglycerols.

Lipoproteins are macromolecules of lipid and protein that transport lipids (including cholesterol and triacylglycerols) through the vascular and extravascular body fluids. They are involved in a diversity of processes such as immune reactions, coagulation and tissue repair (McCarthy et al., 1987). It was generally accepted that high LDL-cholesterol levels might be a marker for the metabolic syndrome, an enhanced atherosclerotic disease state that is also associated with hypertriglyceriaemia (Colpo,

2005). An increase of HDL-cholesterol and a reduction of triacylglycerols, total cholesterol and LDL-cholesterol are considered to reduce coronary artery disease (Castelli et al., 2005). Therapeutic strategies aimed at enhancing cholesterol efflux from the arterial wall may be of additional benefit for patients with atherosclerosis. Numerous epidemiological studies have associated decreased cholesterol with an inverse risk for coronary artery disease. In the present study, feeding LPE and CXEO (200 mg/kg and 400 mg/kg diets, respectively) and 400 mg/kg CXEO diet for 13 days resulted in a significant decrease in LDL-cholesterol and total cholesterol, respectively.

Studies have shown that birds respond to increasing ambient temperature by panting (Ahmad et al., 2008). This phenomenon results in loss of carbon dioxide from blood and eventually rises plasma pH (respiratory alkalosis). In this situation, birds struggle to maintain and correct their blood pH, which is done via excretion of negatively charged bicarbonate ions. Excretion of bicarbonate ions through urine is done coupled via positively charged ions such as Na^+ and K^+. That is the reason for reduction in Na^+ and K^+ upon high ambient temperature. Hemodilution following heat stress (because of increased water consumption) has also been proposed as an important factor in reduction of levels of K^+ and Na^+ in blood. However, it should be noted that intensity and duration of thermal stress from which birds had been suffered and time of sampling are key factors that may lead to no change, small change, or even large fall in levels of aforementioned ions (Borges et al., 2004). Acclimatization of birds to high ambient temperature prior to the experiment (with heat stress) can also affect their responses to heat stress. In the present study there was no significant difference between treatments regarding Na^+ level in serum, but still the levels of Na^+ were lower than those reported in the literature (Borges et al., 2004). This can be explained by different models of heat stress and breed of chickens that were used in other studies compared to the current study. Bogin et al. (1996) reported that exposing broilers to prolonged heat stress leads to dramatic physiological changes in different tissues, as is the case in the present study. The author demonstrated that even in chickens having same age, breed, and size, large variation in their responses to high ambient temperature could be observed. There was an increase in levels of Cl^- in all dietary treatments compared to the control

group. Several studies have shown that because of respiratory alkalosis (higher blood pH) arising after heat stress, more Cl^- is needed in order to exert an acidic effect to normalize perturbed blood pH (Borges et al., 2004). Although, increasing the concentrations of Cl^- in the condition of this study (high temperature exposure) has been counted as a positive effect, a surge and large increase in all supplemented groups was found (about 1.4 to 1.7-fold increase compared to those of control) that might be considered as toxic and/or lethal effect. The reason for such increment is not clear. However, it should be stated that in spite of such big changes we did not see toxicity and/or lethal signs on hematological variables of supplemented birds and also no significant difference in their mortality rate of supplemented groups compared to those of control group (Akbarian et al., 2013a).

Despite of non-significant effects of dietary treatments on serum calcium levels, generally low level of this mineral was found in this experiment. On the other hand, high levels of phosphorus in serum were observed (while level of phosphorus in control group was lower than CX400 treatment), which can be linked back to potential degree of renal insufficiency due to heat stress and consequently increasing the levels of inorganic phosphates (which are mined to obtain phosphorus) (Bogin, 1992; Bogin et al., 1996). Several reports have documented that heat stress exposure decreases blood flow to the intestine, perturbs intestinal mucosa Na-K-ATPase activity, and reduces digestive enzymes secretion and digestive tract motility (Gorman and Balnave, 1994; Belay and Teeter, 1996). These negative effects might alter mineral absorption and metabolism, thereupon their balance in blood. However and taking all these possible explanations into consideration, it should be stated that literature regarding effects of high ambient temperatures on various blood minerals does not draw a consistent picture. Kohne and Jones, (1975) found a significant increase in plasma potassium in turkey hens subjected to high temperature. Bogin et al. (1996) reported that heat shock in broiler chickens caused an increase in sodium and potassium levels. In agreement with our results, Seven et al. (2009) found that feeding plant extracts rich in phenolic compounds, under chronic heat stress, did not affect serum sodium and potassium levels. Literature regarding the influence of high ambient temperature on blood chloride status in chickens is conflicting. While Kohne and Jones, (1975)

reported an elevated blood chloride level in response to high temperature, Ait-Boulahsen et al. (1989)noted otherwise.

Bogin et al. (1996) noted that genetic background may contribute to heat resistance and adaptability in chickens as shown by different behaviour of chickens under hot conditions. Therefore, different results between our findings and those of literature may be owing to different strains of chickens used, different models (temperature exposure) of heat stress, composition of diets, time of sampling, and methods by which mineral concentrations were measured.

The positive effect of feeding plant extracts on immune responses is in agreement with literature. Ethanol extracts of *Allium sativum* (garlic), *Glycyrrhiza glabra* (licorice), *Plantago major* (plantain) and *Hippophae rhamnoides* (sea buckthorn), which are containing phenolic compounds, had some beneficial effects on cellular immunity in laying hens (Dorhoi et al., 2006). The phagocytic activity of peripheral blood leucocytes in chickens orally administered sugar cane extracts or a polyphenol-rich fraction of the sugar cane extract (500 mg/kg) for 3 consecutive days increased significantly, when compared with that of saline-administered control chickens (Hikosaka et al., 2007).*Ligustrum lucidum* and *Schisandra chinensis*, which are rich in phenolic compounds, improved antioxidative metabolism and immunity of laying strain male chicks (Ma et al., 2005). Aniseed (*Pimpinella anisum*), a plant seed high in phenolic compounds, used up to 4% inclusion in laying quail diets provided beneficial effects on immune responses (Bayram et al., 2007). Similar effects were found in broiler chicks (Mehmet et al., 2005).

Taken together, the present study provides some evidence that specific dietary plant extracts rich in phenolic compounds can be used under hot conditions in broiler chickens during the finisher phase to enhance antioxidantenzyme activities and to improve metabolic functions, thereby relieving some of the metabolic changes that occur as a consequence of increased environmental temperature. However it should be admitted that the antioxidant system in living organisms comprises a complex system and in order to investigate and identify the mode of action of phenolic compounds on this system and judge accordingly, measuring more parameters of the oxidative status should be addressed in further

research. Clearly, including the essential oil derived from *C. xanthorrhiza* at 400 mg per kg of the diet provides major benefits. In contrast, the effects of citrus peel extracts were rather inconsistent. More research using a negative control (rearing at normal temperature) with or without these plant extracts in a complete factorial design is warranted to determine which compounds are most effective in this respect and at which doses.

CHAPTER 5

GENE EXPRESSION OF HEAT SHOCK PROTEIN 70 AND ANTIOXIDANT ENZYMES, OXIDATIVE STATUS, AND MEAT QUALITY OF CYCLICALLY HEAT CHALLENGED FINISHING BROILERS FED *ORIGANUM COMPACTUM* AND *CURCUMA XANTHORRHIZA* ESSENTIAL OILS

Redrafted after

Akbarian, A., Michiels, J., Golian, A., Buyse, J., Wang, Y., De Smet, S. 2014. Gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, and meat oxidative stability of cyclically heat challenged finishing broilers fed *Origanum compactum* and *Curcuma xanthorrhiza*essential oils. Poultry Science (accepted 7 May 2014).

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GENE EXPRESSION OF HEAT SHOCK PROTEIN 70 AND ANTIOXIDANT ENZYMES, OXIDATIVE STATUS, AND MEAT QUALITY OF CYCLICALLY HEAT CHALLENGED FINISHING BROILERS FED *ORIGANUM COMPACTUM* AND *CURCUMA XANTHORRHIZA* ESSENTIAL OILS

ABSTRACT

Heat stress in poultry is a serious problem in many countries and causes enormous economic losses. Heat stress has been associated with oxidative stress. Hence, nutritional interventions with antioxidants might be beneficial. Therefore, the effects of dietary *Curcuma xanthorrhiza* (CX) and *Origanum compactum* (OC) essential oils; both rich in simple phenolic compounds, on performance, mRNA levels of heat shock protein 70 (HSP70) and antioxidant enzymes, oxidative status and meat quality of heat challenged broilers were studied. Starting on d 25 of age of Ross 308 broilers, dietary treatments were applied, i.e., a control diet and 4 diets containing 200 or 400 mg/kg feed of CX or OC (CX200, CX400, OC200, OC400 diets). Each diet was given to 3 pen replicates of 20 chickens each in a completely randomized design. Commencing on d 28 of age, the temperature was increased from 22°C to 34°C with 50% relative humidity for 5 h daily during 2 wk. Dietary CX or OC did not affect zootechnical performance of the chickens ($P > 0.05$). Compared to control, mRNA levels of HSP70 were reduced at d 31 by feeding CX400 and OC400 in kidney and liver, respectively ($P < 0.05$). The mRNA levels of SOD were increased at d 31 on the OC400 diet in kidney and on the CX400 diet in heart ($P < 0.05$). Feeding both 400 mg/kg of CX and OC increased the mRNA levels of CAT at d 31 ($P < 0.05$). In heart, at d 31, both dietary levels of CX and OC200 resulted in higher GSH-Px activity ($P < 0.05$). Feeding CX400 increased SOD activity in liver, kidney, and heart at d

31 ($P < 0.05$). A higher activity of CAT was observed in the CX200 and OC400groups at d 42 ($P < 0.05$). Feeding CX at both levels decreased the ferric reducing ability in plasma at d 42 ($P < 0.05$). Feeding CX at both levels and OC200 decreased plasma malondialdehyde concentrations at d 42 ($P < 0.05$). There were no differences in breast WHC, pH (0 and 24 hours post-mortem), and the a* value in fresh breast meat ($P > 0.05$), whereas feeding CX400 and both levels of OC increased the a* value in stored breast meat ($P < 0.05$). Feeding OC diets tended to decrease the TBARS values in fresh breast muscle ($P = 0.061$). In conclusion, these results indicate that dietary essential oils rich in simple phenolic compounds offer potential for improving the antioxidant defense against heat stress induced changes.

INTRODUCTION

Broilers can properly perform if they are not subjected to suboptimal environmental conditions. It has been well documented that exposing broilers to continuously high temperatures, especially during the finisher phase leads to chronic heat stress and may reduce performances (Sahin et al., 2003; Ahmad et al., 2006). Ambient temperatures above thethermoneutral zone of birds have been associated with oxidative stress (Lin et al., 2006a), as reflected in elevated lipid peroxidation products in blood and tissues, and causing protein and DNA oxidation (Floyd and Carney, 1992). Besides impairing the oxidative status *in vivo*, it is also documented that elevated temperatures may result in a higher incidence of pale, soft, exudative (PSE) broiler meat which is less color stable and prone to a shorter shelf life (Tankson et al., 2001).

Exposing broilers to a heat shock increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in short term in order to protect cells from the negative consequences of excessive generation of free radicals (Lin et al., 2006). In contrast, after a long term heat challenge, there might be a decrease in their activity in some tissues due to the development of lesions in cells and increased excretion of minerals such as Zn, Cu, and Se that act as co-factors for antioxidant enzymes, and vitamins such as vitamin C and E (Sahin and Kucuk, 2003). Hence, the activities of the antioxidant enzymes might be insufficient to counteract the initiation and propagation of oxidative damage, and using antioxidants in the diet can be considered as a mechanism for cells to increase their protection against damage induced by free radicals in the case of long term heat challenge.

Typically an up-regulation of heat shock proteins is observed upon heat stress (Craig and Gross, 1991). Heat shock proteins are a group of proteins produced in all cells and tissues in order to protect against harmful effects of environmental stressors, especially elevated temperatures. The most important member of these proteins with respect to heat stress is heat shock protein 70 kDa (HSP70) (Yahav et al., 1997).The transcription factors AP-1 and NFκB involved in the stimulation of

antioxidant enzymes and HSP transcription factor (HSF-1) are sensitive to the 'redox' status of the cell (Khassaf et al., 2003). Hence, antioxidants supplied by the diet may interact with the activity of antioxidant enzymes by maintaining the reducing state of cells and thereby interfering with the activation of these transcription factors.

Numerous studies have been performed in poultry on the effects of various dietary antioxidants on the bird oxidative status and the oxidative stability of their meat post-mortem, e.g., reviewed by Brenes et al. (2009) for essential oils, Bou et al. (2009) and Surai (2014). Synthetic antioxidants and some other antioxidant additives are under significant public scrutiny and have limitations in their use, e.g., their inclusion levels in all feeding stuffs of all species of meat animals are regulated in EU countries. Therefore, there is currently a great interest in exploring the effects of natural compounds.

Origanum compactum (OC), a member of the genus *Origanum* belongingto the mint family (*Lamiaceae*) is native to warm-temperate and Southwestern Eurasia and the Mediterranean region. *Curcuma xanthorrhiza* (CX, commonly known as temulawak or Javanese turmeric in Indonesia), is native to tropical South-Asia and is found both in the wild and cultivated in Indonesia. The essentials oils of OC and CX contain high amounts of simple phenols, and their antioxidant activity is well documented (Sinurat et al., 2009; Luna et al., 2010). The antioxidant activity of OC is mainly attributed to its main components carvacrol and thymol, and for CX mainly refers to ar-curcumene, β-curcumene, and xanthorrizhol. Carvacrol, thymol, and xanthorrizol are simple phenolic compounds bearing different aliphatic side chains on the aromatic ring. In previous works, we showed that using plant extracts rich in phenolic compounds could positively affect broiler gut health and metabolic parameters when reared under high ambient temperatures (Akbarian et al., 2013a,b). The positive effects of oregano, curcuma, or their essential oils on the antioxidant status of poultry have already been reported (Sinurat et al., 2009; Avila-Ramos et al., 2012), but to the best of our knowledge, no information is available concerning the potential effects of these products in heat-challenged broilers. Thus, it was hypothesized that these 2 essential oils may maintain the reducing state of the cells through improving the antioxidant capacity of broilers when subjected to daily increased

temperatures. Therefore, this study was set up to assess the effects of OC and CX on performance, oxidative status, and meat quality of broilers reared under hot conditions.

MATERIALS AND METHODS

The experiment was approved by the Ethics Committee of Ghent University (Belgium) for the humane care and use of animals in research (number EC2011-194).

Essential oils

The CX essential oil was obtained from PT. PHYTOCHEMINDO REKSA (Bogor, Indonesia). According to the compositional data provided by the supplier the main bioactive compounds were: ar-curcumene (11.4%), β-curcumene (8.5%), and xanthorrhizol (hydroxy-ar-curcumene) (28.0%). The OC essential oil was provided by Pranarôm International SA (Ghislenghien, Belgium). According to the compositional data provided by the supplier the main bioactive compounds were carvacrol (44.9%) and thymol (16.4%).

Chicks and housing

Three hundred one-d old broiler chicks (Ross 308; mixed sexes) were purchased from a commercial hatchery (Vervaeke-Belavi, Tielt, Belgium). At setting, (d 0) chicks were allocated randomly to 15 pens with 20 chicks each representing an initial density of 14.8 birds per m^2. The floor was covered with fresh wood shavings and no additional filling or cleaning of the floor was executed during the trial. Heat was provided with heating lamps and automatically stirred heating elements maintaining a stable temperature of 32°C to 28°C, 28°C to 25°C, and 25°C to 22°C during the first 10 d, from d 11 until d 17, and from d 18 until d 28, respectively. The lighting program was 23L:1D during the entire period. The broilers were vaccinated against Newcastle Disease (spray) and Infectious Bronchitis (spray) at the hatchery. At d 16, the vaccination against Newcastle Disease was repeated (Nobilis ND

Clone 30) and the chicks were vaccinated against Gumboro (Nobilis Gumboro D78). Twice daily, animals and housing facilities were inspected for the general health status, constant feed and water supply as well as temperature and ventilation, dead birds, and unexpected events.

Diets and heat challenge

The chicks were raised up to 25 d of age on a basal diet. The basal diet was formulated to correspond to nutrient requirements that were equal to or slightly lower than those outlined by the Ross 308 broiler management guide (Aviagen, 2011), but adapted to the Iranian situation. Based on the Nutrient Specifications for Ross 308 Broilers, the requirement for metabolizable energy, total methionine + cystine and lysine during the finisher phase are 3200 Kcal, 0.86%, and 1.09%, respectively. According to the Ross-Nutrient-Supplement guidelines, an adjustment for local conditions is required and a slightly lower specification can be formulated. The basal diet in the current trial was formulated based on local conditions and markets with slightly lower levels of metabolizable energy, methionine + cystine, and lysine, i.e., 3000 Kcal, 0.80%, and 1.05%, respectively.

Ingredients, chemical, and analyzed composition of the basal diet are shown in Table 5.1. From d 25 until the end of the trial (d 42), 5 experimental diets were fed to 3 replicate pens in a completely randomized design. These experimental diets were prepared through including different levels of the essential oils into the basal diet. The 5 dietary treatments were as follows: control diet (CON) (= basal diet, without any of essential oils), CON + 200 mg/kg CX (CX200), CON + 400 mg/kg CX (CX400), CON + 200 mg/kg OC (OC200), and CON + 400 mg/kg OC (OC400). The essential oils were first mixed intensively with the associated corn oil and then gradually added to the basal diet. Feed and water were offered *ad libitum.* In order to accustom the chickens to the experimental diets; a three d adaptation period was included before imposing the heat challenge. Starting on d 28, a cyclic chronic heat challenge model (34°C - 22°C - 34°C) was applied. The basal temperature was 22°C. Between 8:00 and 10:00 AM, the temperature was gradually increased to 34°C and this high

temperature was then maintained for 5 hours. Afterwards, the temperature was decreased to the basal level of 22°C within 2 h (by 5:00 PM) and then maintained at this level for the rest of the day. Air humidity was kept at 50-60% during the experimental period.

Table 5.1. Ingredients, calculated, and analysed composition of the basal diet

Item	Amount in basal diet
Ingredients (g/kg)	
Corn	561.9
Soybean meal 48HP	360.7
Corn oil	40.0
Limestone	11.2
Dicalcium phosphate	14.7
Common salt	4.3
L-Lysine HCL	1.0
DL-Methionine	1.2
Vitamin and mineral premix[1]	5.0
Calculated composition (g/kg, unless othervise stated)	
ME (Kcal/kg)	3000
Crude protein	201
Calcium	8.6
Ava. Phosphorus	4.3
Ileal digestible lysine	10.5
Ileal digestible methionine	4.0
Ileal digestible Met + Cys	8.0
Analyzed composition	
Dry matter (g/kg)	888
Crude ash (g/kg)	56.1
Crude fat (g/kg)	68.4
Crude protein (g/kg)	204
Crude fiber (g/kg)	51.8
Calcium (g/kg)	9.5
Phosphorus (g/kg)	6.9
Saturated fatty acids (g/100 g FAME[2])	11.8
Monounsaturated fatty acids (g/100 g FAME)	28.0
Linoleic acid (g/100 g FAME)	53.2
Alpha-linolenic acid (C18:3n-3)(g/100 g FAME)	3.36
Total fatty acids (g/100 g FAME)	96.9

[1]vitamin and mineral premix supplied per kilogram of diet: vitamin A (from vitamin A acetate), 10000 IU; vitamin D_3, 9790 IU; vitamin E (DL-α-tocopheryl acetate), 30 IU; vitamin B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamin, 4 mg; zinc sulfate, 60 mg; copper sulfate, 100 µg; selenium (sodium selenate), 0.2 mg; iodine, 1 mg; manganese oxide.

[2] Fatty acid methyl esters

Animal performance

Average pen weight was recorded at d 25, 31, and 42. Feed intake was recorded for each period (d 25 - 31 and 31 - 42 d) and for the entire experimental period (d 25 - 42). Broiler performance at pen level (daily weight gain, g/d; daily feed intake, g/d, and feed:gain, g/g) was calculated per period. Mortality was recorded for each pen and per period. Corrections for average daily feed intake, when calculating animal performances, were done using the number of 'broilerdays' (number of broilers x d alive). Broilerdays for the animals that died were calculated counting the number of d alive minus one, due to the unknown moment of the death and the expected reduction in feed intake of a sick animal.

Sampling

On d 31 (3 d after starting the heat challenge), 4male birds per pen (12 per treatment) were sampled at random between 11:00 AM and 2:00 PM. Blood samples were taken with a 23G needle from the vena brachialis and collected in 2 tubes. The first tube containing EDTA, was centrifuged(3000 *g*, 15 min) to obtain plasma for determination of Ferric Reducing Ability of Plasma (FRAP), Malondialdehyde (MDA), and GSH-Px and was stored at -20°C pending analysis. The second tube containing heparin together with BPDS was used to obtain red blood cells (RBC) for glutathione redox quantification, i.e., for the determination of reduced (GSH) and oxidized (GSSG) glutathione. Terminal anaesthesia was induced by sodium-pentobarbital (250 mg sodium-pentobarbital/kg BW) in the vena brachialis prior to exsanguination. Then heart, liver, and kidneys were carefully excised. Two subsamples of each tissue were snap frozen in liquid N_2 and stored at -80°C for determination of HSP70 and antioxidant enzyme gene expression and for measuring the activity of these enzymes (SOD, GSH-Px, and catalase, CAT). Six samples (out of twelve birds that had already been sampled) of each tissue were taken at random for pursuing the analyses of gene expression and activity of antioxidant enzymes in heart, liver, and kidney.

On d 42 (2 wk after starting the heat challenge, end of trial), another 4male birds per pen were sampled at random (12 per treatment). The procedure and sampling was identical as described above. In addition, meat quality and deterioration of meat quality upon simulated retail display (either or not after long term storage) were monitored. Therefore, pH of the right breast muscle was immediately determined after slaughter. The eviscerated carcasses were chilled at 4°C. The pH and color of the right breast muscle was determined 24 hours post-mortem and the right breast was employed to determine the water holding capacity (WHC). Then, subsamples of the right breast muscle and right thigh were taken and snap frozen in liquid N_2 and stored at -80°C for determination the antioxidant enzymes activities (GSH-Px and SOD).These samples were not subjected to simulated retail display. Finally, another set of subsamples of the right breast muscle and right thigh were subjected to simulated retail display while subsamples of the left breast muscle and left thigh were vacuum packed and stored at -20°C for 7 months before being subjected, after thawing, to a simulated retail display. For the simulation of retail display, samples were wrapped in oxygen permeable foil and continuously displayed at 4°C under fluorescent light (approximately 1200 lux) for 10 d.

Meat quality variables

Meat quality was assessed as described by Michiels et al. (2012a). At 0 and 24 h post-mortem, pH was measured in the right breast muscle, using a portable pH meter (Knick Portamess 654 with Schott N5800A electrode). The pH meter was calibrated by measuring buffer solutions (pH = 4 and pH = 7). Water-holding capacity (WHC) was determined based on the volume of free water squeezed from the ground right breast samples using a filter paper press method described by Grauand Hamm (1953). Color of thigh and breast muscles were determined with a Hunterlab Miniscan color meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard). Lipid oxidation was assessed spectrophotometrically at the wavelength of 532 nm by the thiobarbituric acid reactive substances (TBARS) method as described by Tarladgis et al. (1960) and expressed as ng malondialdehyde (MDA) per g meat.

Plasma oxidative status and glutathione in erythrocytes

FRAP, GSH-Px activity, and MDA concentration in plasma were determined according to Michiels et al. (2012b). The FRAP assay is considered as a measure of the "total antioxidant capacity". The MDA concentration measured by the TBARS method is commonly used to assess lipid peroxidation. Glutathione redox status in erythrocytes (red blood cells, RBC)(ratio between GSH and GSSG) was quantified as described by Degroote et al. (2012). Briefly, hemolyzed RBCs were homogenized and an acid extract was made. This was followed by a derivatization procedure including the reaction of iodoacetic acid with thiols to form S-carboxymethyl derivatives and the formation of chromophores of the primary amines with Sanger's reagent, 2,4-dinitrofluorobenzene. Finally, derivatized thiols were separated on an EC250/4.6 Nucleosil 120-7 NH_2 column (aminopropyl column; Machery-Nagel, Düren, Germany) protected by the same NH_2 guard column (CC8/4). The concentration of GSH and GSSG were determined by high performance liquid chromatography (Agilent Technologies, 1200 series, Degasser, Germany).

Tissues antioxidant enzymes activities

SOD, GSH-Px, and CAT activities were determined in liver, heart, and kidney samples taken at d 31 and 42 and on stored breast and thigh (not CAT) which were taken only at d 42. After thawing, all samples were kept on ice during the procedure. A 2 g sample of liver, heart, and kidney and 5 g sample of fresh breast and thigh muscles were homogenized in 10 mL of 0.05 M phosphate buffer (pH = 7.0) and centrifuged at 4°C for 20 min at 7000 g. The supernatant fraction was filtered through glass wool before determining enzyme activities. The CAT activity was determined according to the method of Aebi (1983). One unit of CAT activity was defined as the amount of extract needed to decompose 1 µmol of H_2O_2 per min at room temperature. The activity of GSH-Px was determined by measuring the oxidation of NADPH according to Hernandez et al. (2004). One unit of GSH-Px activity was defined as the amount of extract needed to oxidize 1 µmol of NADPH per min at 25°C.

The SOD activity assay was performed as described by Marklund and Marklund (1974) by measuring the inhibition of pyrogallol autoxidation. One unit of enzyme activity was defined as the amount of extract needed to inhibit the rate of oxidation by the control (no SOD) by 50%. In the case of kidney, no activity of CAT was detected.

HSP70 and antioxidant enzymes mRNA levels in heart, liver, and kidney

Quantitative real-time PCR was performed to determine the levels of inducible HSP70 and antioxidant enzymes mRNA in different tissues. Tissues of heart, liver and kidney were disrupted and homogenized. Tissues were dissolved in TRIZOL reagent for total RNA extraction according to standard instructions. The total RNA concentration was quantified by Nanodrop photometer (ND-1000 Spectrophotometer, NanoDrop Technologies, USA). Ratios of absorption (260/280 nm) of all preparations were in the range of 1.9 and 2.1. In order to verify the integrity of ribosomal RNA bands, 5 µL of each obtained RNA was separated by electrophoresis on agarose gels under denaturing conditions.

After extraction of total RNA, AMV reverse transcriptase (Promega, USA) was used for reverse transcription (RT). Reverse transcription was performed according to the manufacturer's instruction.

The pooled sample, made by mixing equal quantities of total RNA from all samples, was used for optimizing PCR conditions and tailoring standard curves for the target gene. Two µL of 10-fold dilution RT products was used for PCR in a final volume of 25µL containing 0.4 - 0.8 µM primers and 12.5µLQuantiTect SYBR® Green master mix (Cat. no. 204143+204163, Qiagen). Real-time PCR was performed in StepOnePlus® Real-Time PCR System (AB applied biosystems, USA). The PCR cycling program was as follows: 1 min at 95°C then followed by 42 cycles of 1 min at 95°C, 20s at 60°C. Melting curves were executed to ensure a single specific PCR product for each gene. Numbers of controls were set in order to monitor the possible genomic and environmental contamination of DNA both at the stages of RT and PCR. The special forward and reverse primers were designed using Primer Express Software for HSP70 and antioxidant enzymes of CAT, SOD, and GSH-Px

amplification. TATA box binding protein (TBP) and beta-2-microglobulin (B2M) were used as reference genes for normalization purposes. Primer sequences and information are listed in Table 5.2. The $2^{-\Delta\Delta Ct}$ method was used to analyze the real-time PCR data relative to the average value of control.

Chemical analysis

Feed samples were analyzed in triplicate for dry matter (71/393/EEC), crude ash (71/250/EEC), ether extract (71/393/EEC), and crude fiber (92/89/EEC) according to Directives of the European Community. Crude protein (N x 6.25) was measured according to ISO 15670. Ca and total P were determined using ICP-AES after ashing (450°C, 3 h) and solubilization in 6 M HCl. The content of fatty acids in diets was analyzed by gas chromatography (HP6890, Brussels, Belgium) on a CP-Sil88 column for FAME (100 m · 0.25 mm · 0.2 lm; Chrompack, The Netherlands) according to the method described by Raes et al. (2001). Peaks were identified based on their retention times, corresponding with standards (NuChek Prep., IL, USA; Sigma, Bornem, Belgium). Results are shown in Table 5.1.

Table 5.2. Nucleotide sequences of specific primers

Target gene	Product length (bp)	Primer sequence (F: forward, R: reverse)	Gene bank accession
HSP70[1]	145	F: ATGCTAATGGTATCCTGAACG R: TCCTCTGCTTTGTATTTCTCTG	NM_001006685.1
B2M[2]	1038	F: ACCAAGAACGTCCTCAACTGC R: CGGGATCCCACTTGTAGACC	Z48921
CAT[3]	245	F: ACCAAGTACTGCAAGGCGAA R: TGAGGGTTCCTCTTCTGGCT	NM_001031215.1
GSH-Px[4]	141	F: TTGTAAACATCAGGGGCAAA R: ATGGGCCAAGATCTTTCTGTAA	NM_001163245.1
SOD[5]	122	F: AGGGGGTCATCCACTTCC R: CCCATTTGTGTTGTCTCCAA	NM_205064.1
TBP[6]	196	F: TTTAGCCCGATGATGCCGTATG R: CTGTGGTAAGAGTCTGTGAGTGG	NM_205103

[1] Heat shock protein 70; [2] beta-2-microglobulin; [3] catalase; [4] glutathione peroxidase; [5] superoxide dismutase; [6] TATA box binding protein

Statistical analysis

Statistical comparisons were performed using ANOVA followed by Tukey's multiple comparison test (SAS Institute Inc., vers. 9.1, Raleigh, NC, USA). All statements of significance are based on probability $P < 0.05$. Orthogonal contrasts were applied to explore the effect of each individual EO in the case of performance and meat quality traits. The activity of antioxidant enzymes in tissues and oxidative status of blood were analyzed using a model with the fixed effects of diet (treatment), day of sampling, and diet × day of sampling.

RESULTS

Animal performance

Mortality data were not subjected to statistical analysis because just one case of mortality in the control treatment was observed throughout the trial. Dietary treatments did not affect significantly daily weight gain, daily feed intake, and feed:gain of chickens during d 25 to 31, d 31 to 42, and the total period of feeding the experimental diets (Table 5.3) ($P > 0.05$). However, there was a tendency for improved feed:gain in those chicks fed with OC, irrespective of doseas compared to the control group during 31-42 d of age and the whole experimental period (d 25 - 42) (1.68 vs. 2.10 for OC and CON, respectively in period d 31 - 42, $P = 0.081$; 1.56 vs. 1.80 for OC and CON, respectively in period d25-42, $P = 0.065$).

Table 5.3. Effect of *C. xanthorrhiza* and *O. Compactum* essential oils on performance of broiler chickens during 25-42 d of age subjected to heat challenge.

Parameter	Control	*C. xanthorrhiza*		*O. compactum*		*P* value		
		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	*diet*	*CX*[1]	*OC*[2]
Daily weight gain (g/day)								
25-31	74	74	74	78	76	0.193	0.780	0.085
31-42	69	75	66	78	76	0.471	0.857	0.224
25-42	71	75	70	78	76	0.344	0.772	0.113
Feed consumption (g/day)								
25-31	102	101	89	108	99	0.501	0.486	0.838
31-42	141	148	132	125	131	0.316	0.959	0.213
25-42	121	125	111	117	115	0.342	0.582	0.393
Feed conversion ratio								
25-31	1.37	1.37	1.21	1.38	1.31	0.733	0.490	0.836
31-42	2.10	1.99	2.02	1.62	1.74	0.275	0.835	0.081
25-42	1.80	1.77	1.71	1.53	1.59	0.269	0.629	0.065

[1] Orthogonal contrast of *C. xanthorrhiza* vs. control; [2] Orthogonal contrast of *O. compactum* vs. control

Meat quality variables and antioxidant enzymes

With regard to breast muscle characteristics, there were no differences for WHC, pH, and color variables ($P > 0.05$), except for the a^* value for which the birds fed the control diet had lower values than those fed OC400 after storing the breast meat for 7 months and then exposing for 10 d to simulated retail display ($P < 0.05$; data for other color measurements, b* and L* are not shown) (Table 5.4). The a* values for fresh breast and thigh of the other diets were numerically higher than for the control diet, as was also the case for stored thigh ($P > 0.05$). Regarding TBARS values in fresh breast, the orthogonal contrast of OC vs. CON was significant ($P = 0.005$) indicating a reduction in lipid peroxidation. TBARS values increased substantially upon storage at -20°C for 7 months, in particular for thigh.

SOD activity in breast and thigh muscle was not affected by treatment ($P > 0.05$; Table 5.3). In contrast, several treatments (mainly CX400 and OC400) increased GSP-Px activity compared to the CON, both in breast and thigh, with the latter muscle showing an approximately 2-fold higher enzyme activity.

Plasma oxidative status in erythrocytes

In general, dietary effects were most pronounced on d 42 (Table 5.5). Only feeding CX400 decreased MDA at d 31 of age compared to the CON whereas supplementing CX at both levels and OC200 decreased MDA at d 42 of age ($P < 0.05$). None of the treatments exerted an effect on plasma GSH-Px activity at both ages. A similar outcome was found for FRAP at d 31, whereas feeding CX at both levels caused a decrease at d 42 ($P < 0.05$). Interestingly, whereas GSH-Px activity in plasma increased with age (and heat challenge), MDA concentrations declined (both $P = 0.001$). Feeding both levels of CX and OX noticeably elevated the concentration of GSH in RBC compared to the CON at d 42 ($P = 0.001$). There was no difference for the GSH/GSSG ratio among treatments ($P > 0.05$).

Table 5.4. Effect of *C. xanthorrhiza* and *O. compactum* essential oils on fresh and stored meat characteristics and activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in broilers subjected to heat challenge (n = 12)

Parameter	Control	*C. xanthorrhiza*		*O. compactum*		*P value*			Pooled SEM
		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	Model	CX[1]	OC[2]	
				Fresh breast					
Water Holding Capacity (%)	24.5	24.5	23.8	23.0	21.8	0.378	0.804	0.127	1.11
pH (0 h post-mortem)	6.71	6.63	6.71	6.64	6.56	0.124	0.461	0.053	0.052
pH (24 h post-mortem)	5.68	5.69	5.78	5.70	5.70	0.095	0.101	0.464	0.031
a*[3]	5.42	5.92	5.33	4.75	5.17	0.093	0.499	0.229	0.328
TBARS[4] (ng MDA/g)	258	224	231	180	186	0.061	0.234	0.005	29.9
				Fresh thigh					
a*	7.00	7.33	7.83	7.83	7.67	0.190	0.107	0.035	0.239
TBARS (ng MDA/g)	221	223	238	186	206	0.712	0.779	0.451	26.7
				Breast after 7 months of storage at -20°C					
a*	5.41^{c}	5.60^{bc}	6.56^{ab}	6.45^{ab}	6.85^{a}	0.017	0.120	0.005	0.351
TBARS (ng MDA/g)	356	361	371	330	311	0.885	0.855	0.535	46.1
				Thigh after 7 months of storage at -20°C					
a*	8.10	8.33	8.42	8.66	8.27	0.678	0.394	0.263	0.272
TBARS (ng MDA/g)	1432	1176	1292	1473	1272	0.722	0.345	0.776	169.2
GSH-Px (U/g)	178^{b}	222^{ab}	294^{a}	264^{ab}	283^{a}	0.005	0.001	0.006	53.3
SOD (U/g)	33.9	38.2	38.5	35.9	37.1	0.873	0.526	0.295	8.32
GSH-Px (U/g)	402^{b}	457^{ab}	497^{a}	489^{ab}	446^{ab}	0.029	0.018	0.008	29.9
SOD (U/g)	39.2	39.3	39.1	39.4	39.6	0.289	0.157	0.868	0.22

[a-c] Means within a row lacking a common superscript differ ($P < 0.05$).
[1] Orthogonal contrast of *C. xanthorrhiza* vs. control
[2] Orthogonal contrast of *O. compactum* vs. control
[3] Redness, determined after 10 d of simulated retail display
[4] Thiobarbituric acid reactive substances: determined after 10 d of simulated retail display

Antioxidant enzyme activities in heart, liver, and kidney

Effects of dietary essential oils on antioxidant enzyme activities in different tissues are given in Table 5.6. In general, supplemented diets appeared to enhance antioxidant enzyme activities; however this effect was largely dependent on the tissue and sampling day, i.e., time after starting the heat challenge. Here, more effects were found on d31 than on d42. Day had in all cases an effect on the antioxidant enzyme activities (all $P < 0.05$) and significant interactions between diet and day were found on several occasions.

In heart, at d 31 of age, both levels of CX and OC400 resulted in higher GSH-Px activity compared to CON ($P < 0.05$). The CAT activity in heart at 31 d of age was increased by feeding CX400 and OC400 ($P < 0.05$). Feeding CX400 increased SOD activity of liver, kidney and heart at d 31 ($P < 0.05$). Also, dietary supplementation with OC400 resulted in higher SOD activity in kidney at d 31 ($P < 0.05$). None of the treatments affected the liver CAT and both the liver and kidney GSH-Px activities at d 31 ($P >0.05$).

Fewer effects were observed at d 42. There was no effect of treatment on the kidney and heart enzyme activities at d 42 of age. Feeding CX200 increased the activity of GSH-Px in liver at d 42 ($P < 0.01$). A higher activity of CAT was observed in groups CX200 and OC400 ($P < 0.01$) at d 42. None of the treatments affect the activity of SOD in liver at d 42 ($P >0.05$).

Table 5.5. Effect of *C. xanthorrhiza* and *O. compactum* essential oils on plasma oxidative status and glutathione in erythrocytes of broilers at 31 and 42 d of age subjected to heat challenge (n = 12)

Parameter	Control	*C. xanthorrhiza*		*O. compactum*		Control	*C. xanthorrhiza*		*O. compactum*		*P*value			Pooled SEM
		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	Diet	Day	Day*Diet	
	31 d of age (3 d after heat challenge)					42 d of age (2 wk after heat challenge)								
Plasma														
MDA[1] (nmol/ml)	14.7[a]	14.1[a]	12.5[b]	13.4[ab]	13.5[ab]	12.9[a]	11.9[b]	11.7[b]	11.7[b]	12.3[ab]	0.019	0.001	0.639	0.28
GSH-Px[2] (U/l)	995	921	934	946	842	1318	1123	1240	1311	1196	0.303	0.001	0.859	50.3
FRAP[3] (µmol Fe^{2+}/l)	869	832	896	760	763	931[a]	661[b]	688[b]	851[ab]	734[ab]	0.043	0.818	0.346	43.2
Red Blood Cells														
GSH[4] (nmol/ml)	0.45	0.34	0.43	0.54	0.53	0.57[b]	0.78[a]	0.84[a]	0.78[a]	0.75[a]	0.001	0.003	0.002	0.052
GSH/GSSG[5]	10.4	9.7	12.4	11.6	11.3	7.54	9.59	7.05	6.54	9.63	0.647	0.906	0.576	0.066

[a-b] Means within a row for each day lacking a common superscript differ ($P < 0.05$).
[1] Malondialdehyde
[2] Glutathione peroxidase
[3] Ferric reducing ability of plasma
[4] Reduced glutathione
[5] The ratio of reduced glutathione to oxidized glutathione

HSP70 and antioxidant enzymes mRNA levels in heart, liver, and kidney

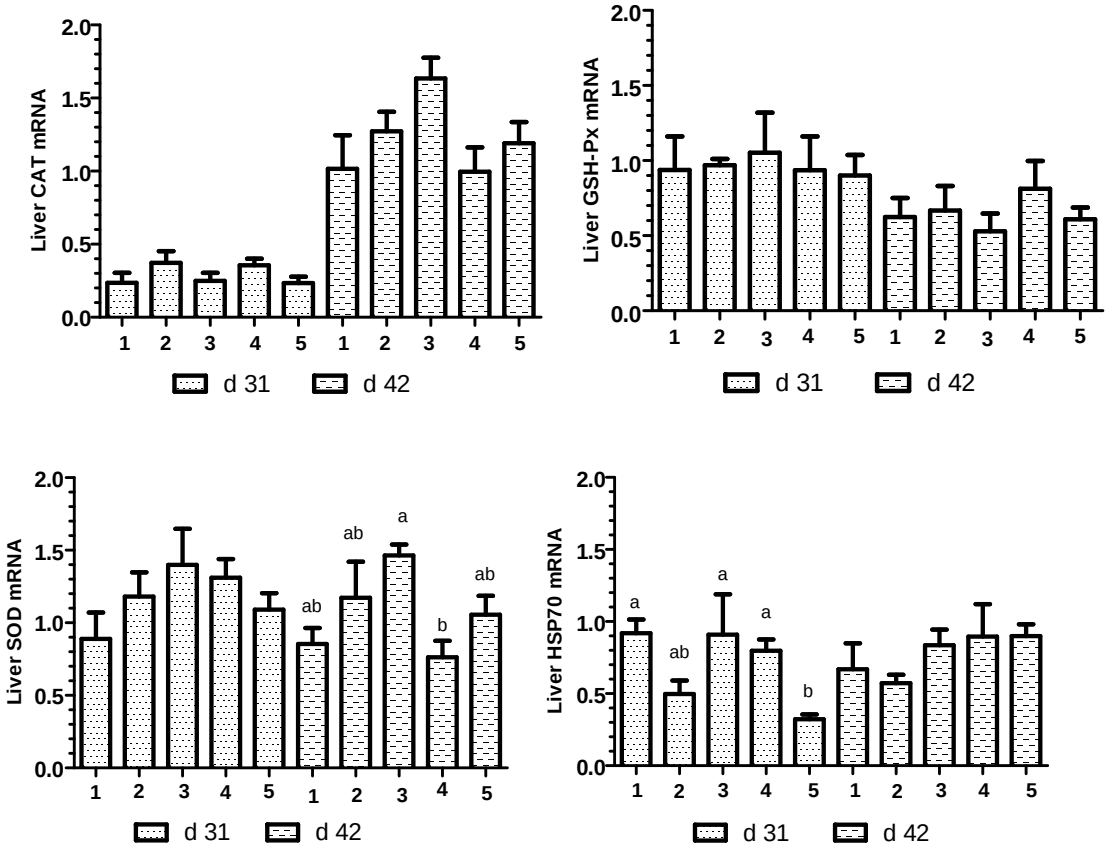
Effects of dietary supplemental essential oils on mRNA levels of inducible HSP70 and antioxidant enzymes of heart, liver, and kidney are presented in Figure 6.1. Compared to CON, mRNA levels of HSP70 were reduced at d 31 of age by feeding CX400 and OC400 in kidney and liver tissues, respectively ($P < 0.05$). The mRNA levels of SOD were increased at d 31 in the OC400 group in kidney and CX400 diet in heart ($P < 0.05$). Feeding CX400 and OC400 increased the mRNA levels of CAT at d 31 of age ($P < 0.05$). At d 42, only CX400 decreased HSP70 mRNA levels in the heart ($P < 0.05$). None of the treatments affects the GSH-Px mRNA levels in all tissues.

Table 5.6. Effect of *C. xanthorrhiza* and *O. compactum* essential oils on activities (U/g) of catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in heart, liver, and kidney of broilers at 31 and 42 d of age subjected to heat challenge (n = 6)

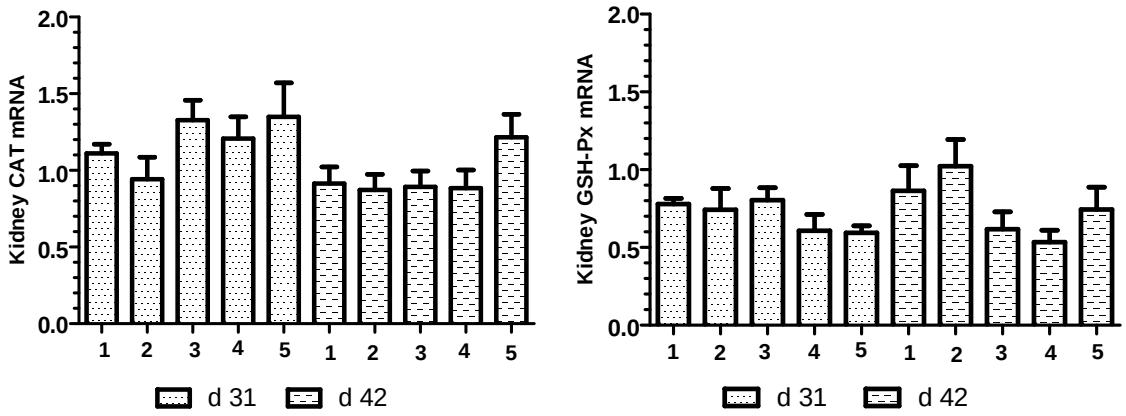
Parameter	Control	*C. xanthorrhiza*		*O. compactum*		Control	*C. xanthorrhiza*		*O. compactum*		*P*value			Pooled SEM
		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg		200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	Diet	Day	Day*Diet	
	31 d of age (3 d after heat challenge)					42 d of age (2 wk after heat challenge)								
Liver														
CAT	198	218	189	180	193	50.1^{b}	86.1^{a}	72.9^{ab}	63.1^{ab}	85.2^{a}	0.008	0.001	0.608	8.61
GSH-Px	142	146	139	130	138	77^{b}	141^{a}	120^{ab}	95^{ab}	111^{ab}	0.004	0.001	0.030	5.8
SOD	70.4^{b}	70.3^{b}	74.1^{a}	71.5^{b}	71.1^{b}	78.7	79.0	78.9	80.3	81.0	0.002	0.001	0.001	0.34
Kidney														
GSH-Px	554	552	527	546	533	598	633	617	663	584	0.317	0.001	0.520	14.7
SOD	81.1^{b}	106^{ab}	114^{a}	112^{ab}	122^{a}	76.5	83.8	78.3	79.6	78.7	0.004	0.001	0.012	3.6
Heart														
CAT	269^{b}	290^{ab}	313^{a}	292^{ab}	320^{a}	282	281	269	264	265	0.003	0.004	0.089	8.3
GSH-Px	637^{b}	702^{a}	714^{a}	672^{ab}	698^{a}	593	626	638	568	609	0.003	0.001	0.907	19.6
SOD	64.6^{b}	66.4^{a}	66.6^{a}	64.9^{b}	65.7^{ab}	78.1	78.3	77.7	78.6	79.7	0.001	0.001	0.127	0.41

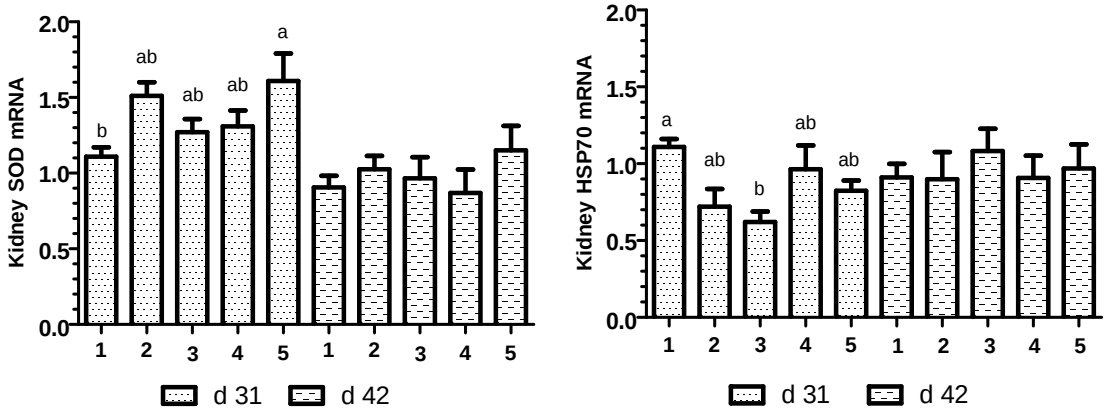
$^{a-b}$ Means within a row for each day lacking a common superscript differ ($P < 0.05$).

A) Liver



B) Kidney





C) Heart

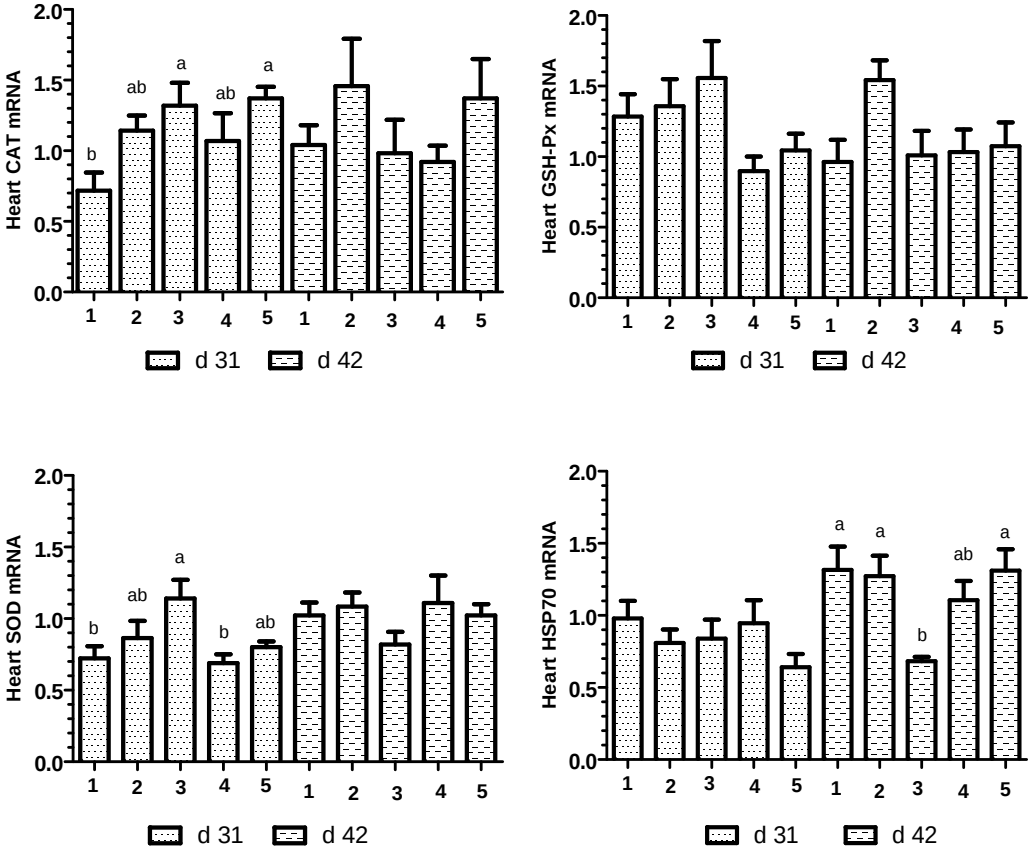


Figure 6.1. Effect of *C. xanthorrhiza* and *O. compactum* essential oils on gene expression of heat shock protein 70 and antioxidant enzymes in heart, liver, and kidney (n = 6) of broilers at 31 and 42 d of age subjected to heat challenge. A, liver; B, kidney; C, heart. Each bar on the graph represents the treatment mean ± SEM

[a-b] Means within d of sampling lacking a common superscript differ ($P < 0.05$).

1) Cont., 2) *C. xanthorrhiza* essential oil at 200 mg/kg,3) *C. xanthorrhiza* essential oil at 400 mg/kg, 4)*O. compactum* essential oil at 200 mg/kg, 5) *O. compactum* essential oil at 400 mg/kg.

DISCUSSION

In the current study, the effects of two essential oils rich in simple phenolic compounds (xanthorrhizol in CX oil, and the monoterpenes thymol and carvacrol in OC oil) on the gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, meat quality, and animal performance were tested in heat challenged finishing broilers. Two main outcomes can be deduced from the results. First, these essential oils could alleviate some of the negative effects on the bird oxidative status associated with the patho-physiology of heat stress. Secondly, these essential oils may improve the oxidative stability of meat and, there were indications that feed efficiency is improved in OC fed broilers. These effects might be a consequence of the beneficial effects on the antioxidant system.

The antioxidant properties of phenolic compounds are well established, and involve radical scavenging, hydrogen or electron donating and metal chelating activity (Bravo, 1998; Balasundram et al., 2006). Their efficiency of antioxidant activity *in vivo* or in food systems depends among other factors mainly on the molecular structure, the bio-availability and the tissue or matrix considered. In addition, polyphenols have a large array of biological activities including modulation of cell signaling pathways and gene expression effects (Rodrigo et al., 2011). Hence, their overall effects are very complex and non consistent among studies. Their use in chicken diets should be critically evaluated (Surai, 2014).

Plasma and RBC oxidative status

In the present study, feeding CX400 and both levels of OC caused a decrease in plasma MDA. Consistent with our results, Seven et al. (2009) reported that using plant extracts

rich in phenolics leads to a decrease of the MDA concentration in heat stressed broilers, confirming the direct antioxidant activity of phenolic compounds.

Glutathione is a tripeptide present at high concentrations intracellularly and known as a protector against toxic effects of lipid peroxidation (Nordberg and Arner, 2001). Interestingly, an enhancement in GSH concentrations had arisen after 14 d of dietary supplementation with essential oil. In other words, the antioxidant system had been successful in adapting itself via up-regulation of glutathione synthesis through the phenolic compounds.

In the present study, FRAP levels were significantly reduced by feeding CX. The FRAP assay is considered as a measure of the 'total antioxidant power', referred analogously to as the 'ferric reducing ability of plasma'. This parameter measures the reduction of the ferric ion to ferrous ion. Lin et al. (2006) reported that FRAP levels increased by exposing broilers to heat stress. In another work, Song et al. (2009) tested different levels of copper (as an antioxidant) on FRAP in chickens challenged with lipopolysaccharide. In line with our results, they found that chickens on a basal diet had higher plasma FRAP values. These authors could not provide a reasonable explanation for this finding. In this respect, the lower levels of FRAP in the CX group after 2 wk of high temperature could be interpreted as a reflection of reduced harmful consequences of increased temperature in chickens fed with CX compared to control chickens. However, it should be said that higher FRAP values are generally associated with an improved antioxidant status (Benzie and Strain, 1996). Therefore, the biological significance of the FRAP values in the present study can be questioned.

Effects of the essential oils on the antioxidant system in tissues of heat challenged broilers

The applied heat stress model can be ascribed as a cyclic chronic heat challenge protocol. Animals were sampled between 1 and 4h after the daily increased temperature (34°C) was achieved. As such, physiological responses of sampled birds can be considered acute heat stress responses and effects described by Lara and Rostagno (2013) can be expected.

In brief, it has been reported that elevated temperatures lead to mitochondrial damage and consequently excessive production of free radicals, hence oxidative stress (Lin et al., 2006). Exposing broilers to acute heat shock leads to an increase in the activity of antioxidant enzymes as a protective mechanism (Lin et al., 2006). In contrast, after a longer term of heat challenge (i.e., cyclic or constant chronic heat exposure)there might be a decrease in their activity rendering it insufficient to counteract the induced oxidative damage (Sahin and Kucuk, 2003; Sahin et al., 2013).This was confirmed by the findings of the present study, i.e., a drastic reduction in the activities of CAT (~ 4-fold) and GSH-Px (~ 2-fold) in the liver at d 42 compared to d 31 in control groups was observed.

In the course of the trial, the effect of age of the birds and the adaptation to the daily heat stress might superimpose to the acute heat stress effects. This may explain the clear differences in oxidative status between d 31 and 42 in the current study, shown by alteration of SOD activity in liver, kidney, and heart after 3 d of heat shock (as an acute heat stress model) compared with 2 wk after heat challenge (as a cyclic chronic heat stress model).These findings suggest that effects of the essential oils on the antioxidant enzymes were modified by the duration of the heat challenge, or from another view, it sounds that those birds in the control group that did not receive plant oils had become acclimated to high ambient temperature. A stimulating effect of dietary supplementation with phenolic compounds (gallic acid, ferulic acid and p-coumaric acid) on the activity of GSH-Px,

SOD and CAT in heart of male Sprague-Dawley rats was reported by Yeh et al. (2009), and was accompanied by similar changes in mRNA levels.

Effects of the essential oils on meat quality parameters

Some positive effects of essential oils on meat characteristics such as reducing the MDA concentration and stabilizing the redness of the meat (a* value) during storage were found in the present study. Avila-Ramos et al. (2012) reported a reduction in lipid oxidation when diets were supplemented with oregano essential oil. As described by Yanishlieva et al. (1999), the preventive effects of oregano on lipid peroxidation could be due to blocking the radical chain process by intervening with peroxide radicals and by enhancing the activity of antioxidant enzymes. Also, these authors suggested that during the peroxidation of lipids at high ambient temperature, thymol is a more effective antioxidant than carvacrol, due to the fact that the former has greater steric hindrance of the phenolic group than the latter.

According to Gregory (2010), elevated temperatures may affect meat quality in 2 ways: 1) a direct effect of high temperature on muscle metabolism which might last after slaughter, and 2) indirect effects of high temperature related to management practices of livestock and poultry that could lead to changes in meat quality. Dai et al. (2009) showed that high ambient temperature caused a decrease in the a* value of meats harvested from broilers. The higher a* value in CX400 and both OC groups compared to the CON in our study, are in line with this when one assumes a role of the essential oils antioxidants in retarding oxidation of myoglobin to metmyoglobin and hence maintaining meat redness longer during fresh or frozen storage. In line with our results, Sayago-Ayerdi et al. (2009) reported that chickens fed grape antioxidant fiber had redder meat with lower lipid oxidation.

Pastsart et al. (2013) described a negative relationship between GSH-Px activity and metmyoglobin formation, which is inversely related to color stability as mentioned before. In line with this, our results showed that those meats with higher color stability (breast after 7 months of storage; OC400 and CX400) had a higher GSH-Px activity.

HSP70 and antioxidant enzymes mRNA levels

The synthesis of HSP70 is temperature dependent and is induced to conserve cells which are exposed to stress, so that the role of HSP70 is considered as cellular thermometer. It is well documented that high temperatures can induce HSP70 mRNA synthesis via increasing either the amount or the activity of the heat shock transcription factor and consequently the HSP70 concentration (Craig and Gross, 1991). On the other hand, most of the factors that induce the HSP response are also involved in the production of reactive oxygen species (Mahmoud et al., 2004). Thus, oxidative stress has been proposed as an indirect mechanism to induce HSP synthesis. Thereupon, the heat shock response may be used as an analytical tool to better understand the animals' response to elevated temperatures on a molecular basis.

Higher levels of HSP70 and oxidative stress in different tissues of poultry after environmental stressors such as increased temperature have been reported (Craig and Gross, 1991). A strong relationship between lipid oxidation and HSP70 synthesis in stressed cells has also been noticed (Mahmoud et al., 2004). Based on these findings it can be concluded that the supplementation with essential oils in the current study was protective against the onset of oxidative damage. Supplementation of plant extracts caused a decrease in both plasma MDA concentration and kidney and heart HSP70 mRNA levels in the CX400 group. The abundance and inducibility of heat shock proteins has been shown to vary according to organ and developmental stage in different

organisms (Givisiez et al., 2003). The abundance of HSP70 mRNA at d 42 in different tissues was as follows in the present study: heart > kidney > liver. Chickens given the diet without essential oils had higher inducible HSP70 mRNA levels in liver and kidney than the other groups at d 31 of age. In other words, by the end of 3 d of heat challenge, the resistance to high temperature was improved in the birds fed plant extracts as evidenced by lower HSP70 gene expression in OC400 and CX400. At d 42, although adding CX400 decreased the inducible HSP70 gene expression in heart, there were no significant differences for HSP70 mRNA concentration among the treatment groups in the case of liver and kidney. The increase in HSP70 levels in heat challenged animals at higher age demonstrates that even not a very severe but permanently increased environmental temperature leads to a cellular stress response. From Figure 6.1 it can be seen that an adaptive response to HSP70 mRNA had occurred in liver and kidney after prolonged high temperature exposure, but heart was still suffering from long term exposure to high temperature. The varying changes of HSP70 mRNA levels in heart tissue compared to liver and kidney may be attributed to differences in heart tissue antioxidant capacity (Cao et al., 1996). In this regard, Lin et al. (2006) noted that liver has more antioxidant power than heart (as reflected by higher FRAP levels in liver than heart), possibly due to the role of liver in nutrient storage and metabolism.

There was no consistent link between the antioxidant enzyme activities and their corresponding gene expression data. For instance, there was no difference among treatments for GSH-Px activity in kidney for both 31 and 42 d of age, which is supported by the lack of an effect on gene expression of this enzyme. The reason could be due to a lower susceptibility of this organ to increased ambient temperature. However, a different observation was made for HSP70 and SOD gene expression in kidney; i.e., compared to CON and after 3 d of high temperature, OC400 increased the mRNA levels of SOD and

CX400 decreased the levels of HSP70; whereas these parameters did not differ among the treatments after 2 wk of heat conditioning. In heart, the CAT and SOD activities followed the same trend as their gene expression data. On the other hand, regarding CAT activity in liver, a large difference between the times of sampling was observed but this was not paralleled by a difference in the mRNA levels. The activity of CAT in liver at d 42 was much lower than at d 31, whereas the mRNA levels displayed the opposite trend. It is well-known that up-regulation or down-regulation of a gene will not necessarily result in increased or decreased enzyme quantity or activity, because mRNA and resultant protein levels are affected by many factors (Steel et al., 2008). The response of antioxidant enzyme activities in tissues under oxidative stress could be less pronounced due to a reduction in intracellular translational efficiency (Lambertucci et al., 2007).

CONCLUSIONS

To summarize, even though dietary essential oils did not exert a pronounced effect on chicken performance, evidence was present that feeding CX and OC at 200 and 400 mg/kg in the diet is beneficial for the broiler antioxidant system when subjected to long term cyclic heat challenge. In particular, CX and OC at 400 mg/kg showed desirable effects on meat quality and activity of GSH-Px in both breast and thigh. Effects of the dietary supplementation on the activity and gene expression of antioxidant enzymes and HSP70 in different tissues displayed a complex pattern, illustrating that the effects depend on age of the birds, tissue, and duration of high ambient temperature.

CHAPTER 6

GENERAL DISCUSSION AND FUTURE PROSPECTS

CHAPTER 6

GENERAL DISCUSSION AND FUTURE PROSPECTS

INTRODUCTION

The environmental temperature in some regions of the world like south of Iran remains well beyond the upper limit of the bird's comfort zone during the greater part of the year. The elevated ambient temperature increases the chance of heat stress (HS) and causes enormous economic losses, which has been a great concern for poultry producers over the years. Thus, taking appropriate measures to mitigate this phenomenon seems necessary. The current PhD research was set up to contribute to this objective.

The HS model to which broilers were exposed in the present study was based on the literature and can be considered an appropriate model for evaluating the effects of plant extracts under HS conditions. For practical reasons, we did not include a negative control treatment without exposure to elevated ambient temperature in order to check whether the birds really experienced heat stress under the present experimental conditions. However, the severity of the HS model can be estimated from the THI index proposed in chapter 1. When including the ambient temperature and relative humidity imposed in the present experiments in the equation, a THI index of 100 is obtained, which is higher than the threshold level of 90 for HS in broiler. Therefore, we can assume that the model applied in this PhD research did induce some level of HS in the birds.

Generally, to overcome HS and its deleterious effects on health and overall physiology of broilers, several methods have been proposed. Some effective and well established strategies such as air conditioning to cool the barn might be costly and water or electricity is not permanently available in all regions around the globe. In some countries like Iran, there has been power supply problems (severe shortage of water and power cut) for years, i.e., during the hottest seasons due to high pressure on electricity systems. Power cuts happen often, spare generators are also available but sometimes the power cut persists for a long time and the generators are not working properly. Therefore, dietary manipulation has been proposed as a practical and cheaper strategy to combat the effect of high ambient temperature. Recently, there has been growing interest to incorporate natural products into poultry diets owing to their beneficial effects on overall health parameters of birds. A sub set of these compounds are phenolics with several biological activities such as anti-bacterial, anti-inflammatory and antioxidant potential. This PhD research was designed to test whether plant extracts rich in phenolic compounds are able to offer a potential beneficial effect to broilers when submitted to a cyclic chronic HS or not. The parameters that have been evaluated in this PhD research are discussed in the next sections.

MEASURED VARIABLES

Composition of Citrus peel extracts

Before discussing the animal variables measured in this PhD research, some notes regarding the methods by which plant extracts of OPE and LPE were obtained should be addressed. In this PhD research, tannic acid was employed as a standard for determination of total phenolic compounds. The chemical nature (i.e., the presence of OH groups) of this compound has been shown to vary across batches. Thus using tannic acid as a standard

holds the risk that measurements with different batches of tannic acid will not yield the same results. Moreover, it implies that it is difficult to compare results from different labs. In the current PhD, only one batch from either LPE and OPE was prepared and the total content of phenolic compounds was determined only once. These batches of LPE and OPE were used in the experiments described in chapter 2, 3 and 4.

Performance and carcass composition

In this PhD research, none of the dietary treatments did exert a pronounced effect on broilers performance; although a tendency for improved feed conversion by feeding OCEO was found. Our hypothesis was to observe an indirect effect of phenolic compounds on the production performance of broilers through improving the oxidative status and gut health parameters. Many studies have been conducted with plant extracts rich in phenolics or combinations on the broiler performance, and the data obtained from these studies do not draw a consistent picture. In agreement with our results, Reisinger et al. (2011) found that supplementation of broiler chicken diets with a mixture of phytogenics containing oregano, anise, and citrus peel oils did not affect FI or FCR significantly. The same result was found in a study of Lee et al. (2013) who reported no differences in FI, BWG and FCR of broiler chickens fed thymol, cinnamaldehyde and a commercial mixture of essential oil components.

According to Deyoe et al. (1962), the flavor of diets can positively or negatively affect feed intake, although poultry have lower sensitivity of smell and taste than mammals. The phenolic compounds may give the diets a taste that may be unpleasant to birds, as reflected by a linear reduction in feed intake with increasing levels (0, 1, 3, 5 g/kg) of thyme oil (Cross et al., 2003). Anyway, the efficacy of plant extracts to impact on animal

performance depends on several factors, e.g., dose of the plant extract used and concentration and profile of active components present in the extracts, physiological state of the animal, background diet and interaction with other compounds in the diet, housing conditions, etc. (Basmacioğlu et al., 2010; Brenes and Roura, 2010).

In the current PhD research there was no effect on carcass composition of broilers. The literature does show different effects of HS on carcass composition depending on the breed of broilers. It has been shown that heat stress negatively affects carcass composition. Lower carcass weight and breast meat proportion and increased deposition of abdominal fat (visceral fat; in fact it refers to “deep” fat that wraps around the inner organs which is linked to get health problems) in the broiler body has been shown in several studies. However, it has been reported that the effect of HS on carcass composition depends on the breed, i.e., Lu et al. (2007) reported that HS increased the fat deposition in Beijing You broilers, whereas fat deposition in Arbor Acres was decreased after HS. The authors concluded that some breeds like theBeijing You broilers are more resistant to high temperature, possibly due to their increased feed efficiency and deposition of abdominal fat under heat exposure.

Blood constituents

Heat stress increases the excretion of minerals and alters the electrolyte balance in broilers. Exposing broilers to HS increases water loss through urine and panting which decreases the heat dissipation capacity by evaporation and increases the osmotic stress inbody cells. Romijn and Lokhorst (1961) noted that heat loss through evaporation accounts for more than 80% of total heat loss at high (32ºC) temperatures. On the other hand, under heat stress conditions, water retention is reduced due to the increased electrolyte excretion in urine and faeces. The excretion varies according to the mineral

and HS severity (Belay and Teeter, 1996). High ambient temperatures lead to higher respiration through panting which in turn leads to respiratory alkalosis. Belay et al. (1990) reported that respiratory alkalosis is related to a relative increase in K+ and Na+ as compared to Cl^-, which are key minerals in governing plasma pH and optimum osmotic relationships as well as maintaining the body fluid volume. However, it should be stated that literature regarding the effects of high ambient temperatures on various blood minerals is contradictory. Bogin et al. (1996) demonstrated that even in chickens having same age, breed, and size, large variation in their responses to high ambient temperature could be observed. In this respect, there was an increase in serum levels of Cl^- in all dietary treatments compared to the control group (chapter 4). This can be considered as a positive effect, because it has been reported that because of respiratory alkalosis (higher blood pH) arising after HS, more $Cl^-$is needed in order to exert an acidic effect to normalize perturbed blood pH (Borges et al., 2004). In agreement with our results, Seven et al. (2009) found that feeding plant extracts rich in phenolic compounds (e.g., caffeic acid, cinammic acid, chrysin, etc.), under chronic HS, did not affect serum sodium and potassium levels.

This PhD research points the chicken as a potentially good model for human for evaluating the interest of plant extracts for reducing coronary diseases. Therapeutic strategies aimed at enhancing cholesterol efflux from the arterial wall may be of additional benefit for patients with atherosclerosis. Numerous epidemiological studies have associated decreased cholesterol with an inverse risk for coronary artery disease. An increase of HDL-cholesterol and a reduction of triacylglycerols, total cholesterol and LDL-cholesterol are considered to reduce coronary artery disease (Castelli et al., 2005). In the present study, feeding LPE and CXEO (200 mg/kg and 400 mg/kg diets, respectively)

and 400 mg/kg CXEO diet for 13 days resulted in a significant decrease in LDL-cholesterol and total cholesterol, respectively.

Endocrine function in broilers is depressed following HS. In this concern, chronic heat stress has been shown to reduce the activity of the thyrotrophic axis in poultry as reflected by reduced plasma T_3 concentrations resulting in functional hypothyroidism (Mitchell and Carlisle, 1992). In fact, thyroid gland hormones play an important role in adaptation to high ambient temperature through their central role in the regulation of metabolic rate of birds during growth period (May et al., 1986). The inverse relationship between plasma concentration of T_3 and increased temperature is well known (Sahin and Kucuk, 2003). Sahin et al. (2003) conducted an experiment with broiler chickens reared under high ambient temperature and antioxidant supplementation (vitamin C and chromium) and found a significant increase in T_3 levels in chickens fed vitamin C and chromium compared to their control. This indicates favorable effects of antioxidant supplementation to counteract the negative effects of heat stress on the thyrotrophic axis. This is in agreement with the results of the current PhD, in which the treatments that showed a clear up-regulation of GSH-Px and SOD (400 mg/kg CXEO and non-significant 400 mg/kg LPE) also resulted in increased T_3 concentrations. Therefore, it can be inferred that supplementation with these extracts may provide tolerance to stress and/or attenuate the negative responses of high temperature in broiler chickens.

A number of enzymes are used in clinical biochemistry as tools for differential diagnosis, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Since the bulk of each is located in different tissues, their abnormal appearance in the blood plasma can give a hint to specific muscle or organ damages (Pech-Waffenschmidt et al., 1995). The increase in the activities of AST and ALT enzymes in plasma is an indication of liver damage and thus causes alterations in liver function (Kim et al., 2008).

The effect of HS on the activity of these enzymes was found to be inconsistent in relation to age and genotypes. According to the results obtained by Pech-Waffenschmidt, (1995), heat exposure did not change the enzyme activities in the chicken's serum, whereas Zaidi et al. (2005) showed that the AST activity in blood significantly increased under stressful conditions. In this PhD, no significant effect of plant extracts on the activity of these enzymes was found. However, it was found that AST activity tended to decrease in birds fed with 400 mg/kg CXEO compared to other treatments. In agreement with this partial effect, Seven et al. (2008) reported that AST activity in broilers fed vitamin C and phytogenics rich in phenolic compounds (e.g., caffeic acid, cinammic acid, chrysin, etc.), was significantly decreased when compared to chronically heat exposed control birds. The decreased AST activity found in our study supports the assumption that CXEO, particularly when supplemented at 400 mg/kg, may decrease the high temperature responses in broiler chickens.

Oxidative status

The model used in the current PhD, has been shown to cause chronic HS. Considering that there is an association between HS and OS, this PhD research was set up to evaluate the potential of plant extracts rich in phenolics in alleviating HS through improving oxidative status.

Heat stress has been shown to create a redox imbalance in favor of pro-oxidants, hence inducing OS.In fact, in order the improve the oxidative status of heat challenged broilers, with feeding these extracts consisting of xanthorrhizol, thymol, carvacrol, protocatchic, catechol, and quercetin, this PhD was put in the right direction. In other words, achieving this goal was expected because the plant extracts were chosen for their antioxidant properties, therefore their ability to improve the antioxidant system and hence reducing

OS via improving/enhancing the activity of intrinsic antioxidants and consequently preventing induction of HSPs.

In this PhD, several beneficial effects of dietary plant extracts on the oxidative status were found. In Chapter 4, we did examine the effectiveness of plant extracts, i.e., LPE, OPE, and CXEO towards improving the antioxidant system after 10 d of chronic HS. The results showed that dietary plant extracts could increase the activity of antioxidant enzymes (GSH-Px and SOD) as compared to control birds. These findings encouraged us to go further and examine more parameters of oxidative status, e.g., the concentration of MDA, FRAP, GSH, GSSH/GSH, and the activities of CAT, GSH-Px, SOD and the mRNA levels of heat shock protein 70 (HSP70) in Chapter 5. In this Chapter, we did see that OCEO and CXEO, in most cases at 400 mg/kg level, were able to improve the oxidative status of broilers after 3 and 14 d of chronic HS via reducing the concentrations of MDA, increasing the activity of CAT, GSH-Px, and SOD and reducing the mRNA levels of HSP70 in different tissues consisting of blood, meat and metabolically highly active tissues (heart, liver, and kidney). Moreover, an up-regulation of these antioxidant enzymes at mRNA level was also found. However, these effects were largely tissue-/time-dependent. In other words, there was not a similar trend in increasing the activities and/or up-regulation of enzymes in all different tissues.

To support the effect of the phenolic compounds used in this PhD on transcription factors, some examples are given here. Kim et al. (2004) showed that xanthorrhizol could attenuate oxidative stress induced by high dose of cisplatin via regulation of gene expression, i.e., through modulation of the DNA-binding activities of transcription factors in liver of mice. In another study, catechol derivatives were able to inhibit NF-κB transcription factor activity, hence preventing oxidative stress in acute human leukemia

cells (Suzuki and Packer, 1994). Inhibition of NF-κB transcription factor in intestinal epithelial cells of mice by quercetin has also been reported (Ruiz et al., 2007).

Heat shock proteins

All organisms, i.e., animals, plants, or microbes respond to elevated temperature through increasing the synthesis of a group of proteins known as the heat shock proteins (HSP). The HSPs are groups of proteins having a wide range of functions such as folding and unfolding other proteins by affecting their conformation and location (Nover, 1991).

In fact, we should differentiate between 'constitutive' and 'inducible' HSPs. The formers are expressed under normal conditions to fulfill the vital functions in normal cells, whereas the latter is merely required to withstand harmful effects of increased temperatures (Morimoto et al., 1990). Several studies have shown that a group of this family, 70 kDa heat shock proteins (HSP70), are directly involved in HS response. The synthesis of HSP70 is temperature dependent and is induced to conserve cells which are exposed to stress, so that the role of HSP70 is considered as a cellular thermometer (Yahav et al., 1997).

In a heat stressed cell, HSP70 binds to cellular proteins (e.g., newly synthesized proteins that are just being released from ribosome) that have been denatured by heat and protect them from degradation and precipitations and therefore affect cell viability. It is known that HSP70 are able to distinguish folded from unfolded proteins (Gething and Sambrook, 1992). Transcription of HSP-encoding-genes is regulated by a heat shock factor (HSF), which interacts with the heat-shock element (HSE, a conserved DNA sequence located in the 5' flanking regions of the genes). Binding higher levels of HSF to HSE sequences

(arisen after elevated temperatures) leads to conversion of small and inactive forms to an active multimer protein (Westwood et al., 1991).

In this PhD research, positive effects of dietary essential oils on HSP70 mRNA levels, however inconsistent and tissue- and time-dependent, were found. In this regard, chickens given the diet without essential oils had higher inducible HSP70 mRNA levels in liver and kidney than the other groups at d 31 of age. In other words, by the end of 3 d of heat challenge, the resistance to high temperature was improved in the birds fed essential oils as evidenced by lower HSP70 gene expression in OC400 and CX400. At d 42, although adding CX400 decreased the inducible HSP70 gene expression in heart, there were no significant differences for HSP70 mRNA concentration among the treatment groups in the case of liver and kidney. The increase in HSP70 levels in heat challenged animals at higher age demonstrates that prolonged increased environmental temperature leads to a cellular stress response. It has been proposed that activating HSP genes arises upon excessive production of ROS (Ananthan et al., 1986), which in turn is a consequence of antioxidant enzymes deficiency. Thus, any strategy towards improving the antioxidant system under heat stress conditions leads to reduced ROS production, hence lower induction of HSP70. In this regard and in line with the results of the present PhD, Mahmoud et al. (2004) showed that dietary supplementation with ascorbic acid significantly reduced the expression of HSP70. What can be concluded here is that lower expression of HSP70 is a reflection of lower ROS production in cells, therefore lower damage to the cellular structure and functions, hence possibly improving animal performance.

The different changes of HSP70 mRNA levels in heart tissue compared to liver and kidney may be attributed to differences in heart tissue antioxidant capacity (Cao et al., 1996). In this regard, Lin et al. (2006a) noted that liver has more antioxidant power than

heart (as reflected by higher FRAP levels in liver than heart), possibly due to the role of liver in nutrient storage and metabolism.

Gut health parameters

Good intestinal health in broilers is of utmost importance to achieve target growth rates and feed efficiency (Montagne et al., 2003). The gastrointestinal tract is particularly responsive to stressors like HS, which causes a disruption in the structure and function of intestinal epithelium, including reduced regeneration and integrity of the intestinal epithelium (Burkholder et al., 2008). The latter in turn can affect the absorption of nutrients, impairing the productive performance of animals (Liu et al., 2009). Moreover, a thicker mucus layer in turn reduces the chances of epithelial microbial adhesion, hence indirectly changing the gut microbial population. In this PhD research, some effects of plant extracts on intestinal morphogy were found. In this respect, reduced crypt depth and increased villus height might be considered as a positive effect. The effect of plant extracts on muscular layer has been found to be very difficult to interprete.In fact the meaning of this finding still remains unclear and further investigation is needed to cast more light on this criterion.

However, phenolic compounds have been shown to exert considerable antimicrobial activity, which in turn enables them to modulate the gut ecosystem to affect feed efficacy (Jamroz et al., 2005; Si et al., 2006; Jang et al., 2007). It has been suggested that their antibacterial mechanism consists of creating disturbance of the cytoplasmic membrane, disruption of the proton motive force, electron flow and active transport and coagulation of cell contents (Lambert et al., 2001; Burt, 2004). Compounds with phenolic structures (compounds with a hydroxyl group (-OH) attached to a phenyl ring) have been shown to

disintegrate the outer membrane of Gram-negative bacteria and to release lipopolysaccharides (reviewed by Lee et al., 2004).

Michiels et al. (2007) concluded that carvacrol, thymol and cinnamaldehyde are fastly absorbed in the proximal parts of the GIT and can specially be used to reduce the bacterial population in the proximal and more acidic parts of the GIT. In the current study, positive effects of plant extracts (LPE and CXEO) were seen in the ileum and cecum as reduced coliforms counts in these distal parts. Therefore, it can be speculated that these plant extracts did already reduce the coliforms counts in proximal parts of the GIT and this effect has been extended to the distal parts. However, it is still remains without answer whether the dosis of plant extracts were high enough to provoke such effect or other factors are involved.

Meat quality

Dietary application of antioxidant materials has been shown to improve the oxidative stability of meat which is very important to avoid or delay development of rancidity in meat products. In fact, rancidity is mimicked by lipid peroxidation and can be influenced by several factors such as: concentration and composition of lipids, composition and amount of natural antioxidants, activities of enzymatic and non-enzymatic antioxidants, concentration of transition metals like iron and copper having pro-oxidant effects, etc. (Surai, 2006).

Some positive effects of essential oils on meat characteristics such as reducing the MDA concentration and stabilizing the redness of the meat (a* value) during storage were found in the present PhD research. The higher a* value in CX400 and both OC groups compared to the CON in our study, are in line with this when one assumes a role of the essential oils

antioxidants in retarding oxidation of myoglobin to metmyoglobin and hence maintaining meat redness longer during fresh or frozen storage.

As described by Yanishlieva et al. (1999), the preventive effects of oregano on lipid peroxidation could be due to blocking the radical chain process by intervening with peroxide radicals and by enhancing the activity of antioxidant enzymes. Also, these authors suggested that during the peroxidation of lipids at high ambient temperature, thymol is a more effective antioxidant than carvacrol, due to the fact that the former has greater steric hindrance of the phenolic group than the latter. However and importantly the deposition of these essential oils may play an important role in their effect on meat quality. In the present PhD research, we did not measure their residues in the meat to really show whether they are still available in thigh and breast meats post-mortem to exert their direct protective effects or not. However, the form of which they are absorbed and deposited in the tissues is also another important factor. In other words, because of their possible biotransformation in the small intestine and hepatic metabolism upon absorption, therefore, these metabolic modifications happening *in vivo* may affect their antioxidant activity in tissues (Prochazkova et al., 2011). Therefore, many questions remain whether protective effects of experimental diets against lipid peroxidation are phenolic-dependant or an indirect one.

CONCLUSIONS

The objective of this PhD was to investigate the effect of high ambient temperatures on performance, gut health parameters and oxidative stress and to evaluate the potential of dietary plant extracts rich in antioxidant phenolic compounds in this respect. Several

experiments were conducted, using LPE, OPE, and their combination, CXEO, and OCEO at inclusion levels of 200 and 400 mg/kg of diet. A cyclic chronic heat stress model was installed by increasing the room temperature from 22°C to 34°C for 5 h/d during finishing phase from d 28 to d 38 of age. The results ofthis PhD demonstrate that even though dietary plant extracts did not exert a pronounced effect on chicken performance, it is clear that dietary LPE, OPE, CXEO and OCEO are potential additives to improve some blood components, the proximal intestinal morphology and oxidative status of broilers chickens when exposed to a high ambient temperature. However, it should be stated that several factors such as the age of the birds, tissue, and duration of high ambient temperature interfered with the effect of dietary supplementation.

Monitoring the real situation in Iran and according to the results of this PhD research, some indications with regard to application of these plant extracts (Citrus peel extracts and *Curcuma xanthorrhiza* and *Origanum compactum* essential oils) in broiler diets under hot conditions are delivered. Positive effects of Citrus peels extracts on microbiota and the antioxidant system under the condition of this PhD research are clear. In order to offset heat stress consequences in broilers, it is always recommended to add antioxidant additives to the broiler diets under hot conditions. Therefore, considering the high production of citrus products in Iran, the application of their extract in the broiler diets can be advised to the poultry industry in Iran. It is recommended to use these extracts in the finishing phase, which is closer to market age and in which there is a higher susceptibility of broilers to external/environmental stressors. Furthermore, and depending on our target, i.e., improving oxidative status, meat quality, gut health parameters, etc. application of these products should be re-considered. In other words, during the hot months of the year (Spring and Summer), inclusion of these products has a beneficial effect, and for the rest of the year further research and investigations are absolutely needed. In this PhD research,

mostly the highest dose of plant extracts (i.e., the inclusion of 400 mg/kg of plant extracts) showed beneficial effects on guthealth and oxidative status parameters of broilers, which suggests that higher inclusion rate of these products (e.g.,800, 2000 mg/kg) may offer a potential to improve and promote performance criteria of broilers under hot conditions.

FUTURE PROSPECTS

Besides factors evaluated in this PhD research, there are still several issues that are worthwhile considering in future research.Relying on the data observed in this PhD research and according to my opnion, the following topics are the most important issues that should be addressed through future investigations to better understand the mechanism of action of plant extracts and their interaction with high temperatures in broilers:

- Currently, there is no information available about the effect of citrus peel extracts on different parameters of broilers under normal conditions; therefore it is suggested to further explore the effects of these extracts under normal temperature conditions to take their possible advantages in the poultry industry. Measuring the expression of HSP70 in broilers in response to feeding citrus peel extracts in HS conditions is also suggested.
- What is the best model to study the effect of antioxidants and other components to alleviate HS consequences and what are the best indicators for HS? In this respect, different models of increasing the rate of increasing the temperature (i.e., rise form 22°C to 34°C in 1 h or in 4 h) could be tested.
- Removing the feed during HS and supplying plant extracts through drinking water to chickens is another strategy that deserves research. This is to limit heat production

upon feed consumption, meanwhile making sure of that the plant extracts are consumed by the chickens during HS.

- Many studies have shown that phenolic compounds are rapidly excreted from the body and even if they are deposited in the tissues, they are not present in the same form as what they were before administration. Therefore, it remains unclear how these plant extracts exactly do relieve HS in broilers. Is there a direct effect or is an indirect effect involved by stimulating other factors? Are the secondary products formed after metabolism in the body, still active to exert a pronounced effect on the oxidative status of broilers?
- Attention should be given to the amount of plant extracts that are deposited in broiler meat and tissues under HS conditions and to their availability in tissues to examine their biological function at cellular level. Having this information would also allow to test the impact of dietary plant extracts on the quality and safety of broiler products in the human food chain.
- Both from a biological and economic perspective, more research is needed to determine optimal doses for the inclusion of plant extracts in the diet of farm animals. Synergistic effects of plant extract and essential oils, and the interaction with other dietary components, should be investigated. Synergistic effects would allow minimizing the concentrations required to achieve a particular effect and maximize their potential.

SUMMARY – SAMENVATTING - چکیده

SUMMARY

The effect of high ambient temperatures during some months of the year on poultry production has been of great concern in many countries. It is well known that exposing broilers continuously to high ambient temperatures, especially during the finisher phase exerts profound effects on physiology, health and performance. Several management approaches have been used to minimize the deleterious impact of heat stress. Plant extracts rich in antioxidants like phenolic compounds are considered as a possible therapy. Studies have shown that fruits of the Citrus family (particularly orange and lemon), and herbs of the *Zingiberaceae* family (particularly turmeric) and mint family (*Lamiaceae*; particularly Origanum), amongst many others, are rich in phenolic compounds. Therefore, the objectives of the present PhD research were to investigate the effects of plant extracts in broilers on: [1] performance parameters, [2] gut health parameters, [3] oxidative stress parameters and meat quality.

To test the potential of phenolic compounds to mitigate heat stress, an extract obtained from the named plants was included in the finishing diet of Ross 308 broilers. Due to the higher susceptibility of broilers to high ambient temperatures at market age, the emphasis was placed on the finishing phase. To simulate the real conditions in Iran, a cyclic chronic heat stress model was applied, whereby the birds are exposed to a high ambient temperature during several hours per day over a long period. The occurrence of heat stress was not verified in the present study, but it is known from the literature that the applied conditions normally lead to heat stress. Three extensive experiments were conducted, using lemon peel extract (LPE), orange peel extract (OPE), and their combination,

Curcuma xanthorrhiza essential oil (CXEO), and Origanum compactum essential oil (OCEO) at inclusion levels of 200 and 400 mg/kg of diet.

A literature review on heat stress, oxidative stress and the potential of antioxidants is presented in Chapter 1. It covers the information on different models of heat stress, its effect on poultry parameters, and the potential of antioxidant additives to alleviate heat stress. To summarize the information from the literature, several tables were compiled.

In the first experiment (Chapter 2), a blend of LPE and OPE at different inclusion levels was tested on broiler performance, serum components and intestinal morphology under cyclic chronic heat stress (increasing the room temperature form 22°C to 34°C for 5 h/d during finishing phase from d 28 to d 38 of age). An extract of lemon peel and orange peel was prepared and 2 levels of OPE (0 and 200 mg/kg) × 3 levels of LPE (0, 200, and 400 mg/kg) were added to 6 experimental diets. This was done to test their possible synergistic effect on different parameters. None of the dietary treatments did affect weight gain, feed intake, and feed conversion ratio of broilers. The inclusion of 200 mg/kg OPE increased serum total protein, but reduced serum lactate dehydrogenase and creatine phosphokinase activity in broilers. Lemon peel extract supplementation decreased the activity of lactate dehydrogenase quadratically and creatine phosphokinase linearly. No differences in the other blood characteristics and intestinal traits were observed with the exception of muscularis thickness of duodenum, which was reduced when LPE was added to the diet.

A second experiment with lemon and orange peel extracts was conducted in which LPE, OPE and also CXEO were included separately at 2 levels (200 and 400 mg/kg) in 6 dietary treatments. The heat stress model and feeding period was the same as in the first experiment. Chapter 3 deals with the results of this experiment on performance parameters, intestinal microbiology and morphology. Plant extracts did not affect the birds performance. The bursa weight of the control birds was lower compared to those fed 400

mg/kg OPE and 200 and 400 mg/kg CXEO diets. Feeding 400 mg/kg of LPE decreased the duodenal villus:crypt ratio compared to control and 200 mg/kg OPE fed birds. Plant extracts did not affect the ileal histo-morphology. Feeding with 400 mg/kg of LPE and CXEO caused a decrease in coliform counts in ileum and feeding of 400 mg/kg CXEO diet decreased coliform counts in caecum compared to control birds.

Chapter 4 describes the effects of the aforementioned extracts in the second experiment on antioxidant enzymes, immune system, plasma hormones and serum metabolites of finishing broiler chickens. Compared to the control diet, supplementation with OPE at 400 mg/kg and CXEO significantly increased erythrocyte GSH-Px and SOD activity, plasma growth hormone concentrations and serum phosphorus, total protein and chloride concentrations and decreased serum LDL-cholesterol and total cholesterol concentrations in chickens at 38 d of age. CXEO supplementation at 400 mg/kg caused a significant increase in Bronchitis antibody titres. Supplementation with LPE and OPE yielded inconsistent results. Most interesting, 400 mg/kg LPE significantly increased serum 3,5,3′-triiodothyronine and growth hormone levels compared to the control.

A third experiment was designed to explore in more detail the molecular mechanisms through which essential oils may mediate oxidative stress in broilers under high ambient temperatures (Chapter 5). CXEO and OCEO were added at 2 levels (200 and 400 mg/kg) to 4 dietary groups plus a control diet. Oxidative status parameters including the levels of malondialdehyde, FRAP, GSH, GSSH/GSH, and the activities and the mRNA levels of CAT, GSH-Px, SOD and the mRNA levels of heat shock protein 70 (HSP70) were assessed. Meat quality variables were also measured in this experiment. In this experiment, birds were also sampled after 3 d exposure to increased temperature, which is considered as a model for acute heat stress. The remaining birds were further exposed to daily increased temperatures for another 2 weeks, after which they were sampled for

investigating the effects of cyclic chronic heat stress. This approach allowed us to compare different heat stress models in broilers. Dietary CXEO or OCEO did not affect zootechnical performance of the chickens. Compared to control, mRNA levels of HSP70 were reduced at d 31 by feeding CXEO400 and OCEO400 in kidney and liver, respectively. The mRNA levels of SOD were increased at d 31 on the 400 mg/kg OCEO diet in kidney and on the 400 mg/kg CXEO diet in heart. Feeding both 400 mg/kg of CXEO and OCEO increased the mRNA levels of CAT at d 31. In heart, at d 31, both dietary levels of CXEO and 200 mg/kg OCEO resulted in higher GSH-Px activity. Feeding CXEO at 400 mg/kg increased SOD activity in liver, kidney, and heart at d 31. A higher activity of CAT was observed in the 200 mg/kg of CXEO and 400 mg/kg of OCEO groups at d 42. Feeding CXEO at both levels decreased plasma FRAP values at d 42. Feeding CXEO at both levels and OCEO at 200 mg/kg decreased plasma malondialdehyde concentrations at d 42. There were no differences in breast water holding capacity, pH, and the a* value in fresh breast meat, whereas feeding 400 mg/kg CXEO and both levels of OCEO increased the a* value in previously frozen and thawed breast meat. Feeding OCEO diets tended to decrease the TBARS values in fresh breast muscle.

Finally, a general discussion regarding the effect of heat stress on different parameters of broilers, with emphasis on plausible mechanisms through which plant derived compounds exert their effects and also some future prospects are delivered in Chapter 6.

In conclusion, the results of this PhD research demonstrate that even though dietary plant extracts and essential oils did not exert a pronounced effect on chicken performance, it is clear that feeding OPE, LPE, and their combination might positively modify some blood components and the proximal intestinal morphology. Dietary LPE, OPE, CXEO and OCEO are potential additives to improve the broiler antioxidant system when subjected to

long term cyclic heat challenge. The expression of HSP70 was reduced in response to dietary CXEO and OCEO supplementations. In the three experiments, the higher dose of 400 mg/kg diet seemed to exert a greater response than the lower dose. However, it should be stated that several factors such as the age of the birds, tissue, and duration of high ambient temperature interfered with the effect of dietary supplementation on several parameters.

SAMENVATTING

Het effect van hoge omgevingstemperaturen gedurende enkele maanden van het jaar op de pluimveeproductie is in vele landen van groot belang. Het is algemeen bekend dat het blootstellen van vleeskippen aan hoge omgevingstemperaturen, vooral in de laatste afmestfase, tot hittestress kan leiden en een groot effect uitoefent op de fysiologie, gezondheid en prestaties van de dieren. Verschillende management maatregelen werden reeds toegepast om de nadelige effecten van hittestress te minimaliseren. Plantenextracten die rijk zijn aan antioxidanten, zoals fenolische componenten, worden beschouwd als een mogelijke oplossing. Verschillende studies toonden reeds aan dat onder andere vruchten van de citrus familie (in bijzonder sinaasappel en citroen), kruiden van de *Zingiberaceae* familie (in bijzonder kurkuma) en de munt familie (*Lamiaceae*; in het bijzonder *Origanum*) rijk zijn aan fenolische componenten. Daarom was het doel van dit doctoraatsonderzoek om het effect van planten extracten te onderzoeken bij vleeskippen op: [1] prestatieparameters, [2] parameters voor de darmgezondheid, [3] oxidatieve stress parameters en vlees kwaliteit.

Om het potentieel van fenolische componenten te testen tegen hittestress bij Ross 308 vleeskippen, werd een extract van de genoemde planten in hun laatste fase voeder ingemengd. Wegens de grotere vatbaarheid van vlees kippen voor hoge omgevings temperaturen rond hun slacht gewicht, werd de nadruk gelegd op de afmestfase. Om de condities in Iran te simuleren, werd er gebruik gemaakt van een cyclisch chronisch hittestress model, waarbij dieren over een langere periode dagelijks blootgesteld worden aan verhoogde temperaturen gedurende enkele uren. Het optreden van hittestress werd als dusdanig niet geverifieerd, maar uit de literatuur is bekend dat deze omstandigheden

normaal tot hittestress leiden. Drie uitgebreide proeven werden uitgevoerd met antioxidant supplementatie, gebruik makend van een ‘lemon peel’ extract (LPE; extract van limoenschillen), een ‘orange peel’ extract (OPE; extract van sinaasappelschillen), combinaties van LPE en OPE, een etherische olie van *Curcuma xanthorrhiza* (CXEO) en een etherische olie van *Origanum compactum* (OCEO) aan gehalten van 200 en 400 mg/kg voeder.

Hoofdstuk 1 bevat een literatuurstudie aangaande hittestress, oxidatieve stress en het potentieel van antioxidanten in dit verband. Deze literatuurstudie geeft informatie over verschillende hittestress modellen, hun effect op dierparameters en het potentieel van antioxidant bevattende additieven om hittestress te milderen. Uit de beschikbare literatuur werden enkele tabellen samengesteld om de informatie overzichtelijk samen te vatten.

In het eerste experiment (Hoofdstuk 2), werden LPE en OPE aan verschillende inmengdosissen getest op hun effect op dierprestaties, bloedwaarden en intestinale morfologie onder cyclische chronische hittestress (door verhogen van de kamertemperatuur van 22°C naar 34°C gedurende 5h/dag in de afmestfase van dag 28 tot dag 38). Een extract van limoenschillen en sinaasappelschillen werd bereid en 2 gehalten van OPE (0 en 200 mg/kg) x 3 gehalten van LPE (0, 200 en 400 mg/kg) werden toegevoegd aan 6 experimentele voeders. Hierdoor was het mogelijk om hun eventueel synergetisch effect te testen op de verschillende parameters. Geen enkele behandeling had een effect op de gewichtstoename, voederopname en voederconversie van de kippen. De toevoeging van 200 mg/kg OPE verhoogde wel het totaal serum eiwitgehalte, maar reduceerde lactaat dehydrogenase en creatine fosfokinase activiteit. Supplementatie met LPE verminderde de activiteit van lactaat dehydrogenese en creatine fosfokinase volgens een kwadratisch en lineair verband respectievelijk. Voor de andere bloedwaarden en intestinale parameters werden geen significante verschillen gevonden, behalve voor de

spierlaagdikte van het duodenum die dunner was wanneer LPE werd toegevoegd aan het rantsoen.

Een tweede proef werd uitgevoerd metLPE en OPE, waarin LPE, OPE maar ook CXEO in 2 gehalten (200 en 400 mg/kg) werden toegevoegd aan 6 voederbehandelingen. Het gebruikte hittestress model was hierbij identiek aan dat van het eerste experiment. Hoofdstuk 3A geeft de resultaten van dit experiment weer op de dierprestaties, en de darmmicroflora en –morfologie. De plantenextracten hadden geen effect op de prestaties van de kippen. Het gewicht van de bursa van de controle dieren was lager in vergelijking met de bursa van dieren aan wiens rantsoen 400 mg/kg OPE en 200 of 400 mg/kg CXEO werd toegevoegd. Het rantsoen met 400 mg/kg LPE verlaagde de duodenale villus:crypte verhouding in vergelijking met de controle groep en de groep gevoederd met 200 mg/kg OPE. De plantenextracten hadden geen effect op de histo-morfologie van het ileum. Het voederen van de dieren met 400 mg/kg LPE of 400 mg/kg CXEO resulteerde in een verlaging van het aantal kolonie vormende eenheden van coliformen in de inhoud van het ileum in vergelijking met de controle dieren, voor de groep 400 mg/kg CXEO zette dit effect zich door in de caeca.

Hoofdstuk 3B behandelt de resultaten van dit tweede experiment op de activiteit van antioxidant enzymen, mineraal excretie, bloedparameters en immuunrespons. Vergeleken met de controle werd door het supplementeren van CXEO en 400 mg/kg OPE de GSH-Px en SOD activiteit in rode bloedcellen significant verhoogd bij vleeskippen op 38 d leeftijd. Daarnaast werden de concentraties aan bepaalde groeihormonen in het plasma verhoogd en vonden we een hoger serum totaal eiwit en chloride gehalte. Het gehalte aan serum LDL-cholesterol en totaal cholesterol was daarentegen lager vergeleken met de controle. Bij 400 mg/kg CXEO werd een significant hogere titer aan Bronchitis antilichamen waargenomen. Resultaten bij het supplementeren van LPE en OPE waren in

dit verband minder eenduidig. Hoogst interessant was de significant hogere concentraties aan 3,5,3'-triiodothyronine en groeihormoon wanneer 400 mg/kg LPE werd toegediend.

Een derde experiment werd uitgevoerd om meer inzicht te verwerven in de moleculaire werkingsmechanismen waarmede etherische oliën de oxidatieve status van vleeskippen onder hittestress kunnen beïnvloeden (Hoofdstuk 4). In deze proef werd het effect van 2 etherische oliën CXEO en OCEO elk afzonderlijk in 2 gehalten toegevoegd (200 en 400 mg/kg) aan het voeder, wat resulteerde in 4 voederbehandelingen en één controle. De oxidatieve status werd geëvalueerd door het bepalen van malondialdehyde, FRAP, GSH, GSSG/GSH, en de activiteit en mRNA gehalten van CAT, GSH-Px, SOD en het mRNA gehalte van heat shock proteïne 70 (HSP70). Daarnaast werd in dit experiment ook de vleeskwaliteit bestudeerd. In vergelijking met de voorgaande proef, werd in deze proef een deel van de dieren reeds bemonsterd nadat 3 d een hoge temperatuur werd aangehouden. Dit wordt beschouwd als een model voor acute hittestress. Hierna werden de resterende dieren 2 weken langer onderworpen aan een aanhoudende hoge temperatuur en bemonsterd aan het einde van deze periode om de effecten van cyclische chronische hittestress na te gaan. Op deze manier liet de proef ons toe 2 verschillende stress modellen met elkaar te vergelijken. Uit de resultaten werd duidelijk dat CXEO en OCEO de zoötechnische prestaties van de dieren niet hadden beïnvloed. Daarentegen werden uit de analyses op d 31 wel enkele belangrijke verschillen waargenomen in vergelijking met de controle. Zo werd een lager mRNA gehalte voor HSP70 in de lever en nieren gemeten bij het toedienen van 400mg/kg CXEO en OCEO. Daarnaast werden ook hogere concentraties aan mRNA voor CAT gemeten. Tevens werd in het hart een hogere GSH-Px activiteit vastgesteld wanneer CXEO of 200mg/kg OCEO werd toegediend. Ook de gehaltes aan mRNA voor SOD werden verhoogd in de nieren en het hart, respectievelijk bij 400 mg/kg OCEO en 400 mg/kg CXEO. Supplementeren van 400mg/kg CXEO

verhoogde daarenboven de SOD activiteit in zowel de lever, de nieren als het hart. Op d 42 van de proef werden ook interessante waarnemingen verricht. Zo werd een hogere CAT activiteit waargenomen bij 200 mg/kg CXEO en 400 mg/kg OCEO in het rantsoen. Het voederen van CXEO reduceerde daarnaast de FRAP waarde van het plasma. Hier werd ook, net zoals bij 200 mg/kg OCEO, een lagere MDA concentratie waargenomen. Betreffende de vleeskwaliteit werden geen verschillen waargenomen bij vers borstvlees. Toch vertoonde OCEO een tendens om het gehalte TBARS te verlagen. Bij ingevroren en ontdooid borstvlees werden daarnaast wel verhoogde a* waarden waargenomen bij OCEO en 400mg/kg CXEO t.o.v. de controle.

Finaal kan men een algemene discussie terugvinden in Hoofdstuk 5 van dit werk. Hier wordt het effect van blootstelling aan verhoogde omgevingstemperaturen op de verschillende parameters bij vleeskippen besproken, met de nadruk op de mogelijkheden en werkingsmechanismen van de bestudeerde plantenextracten. Ten slotte werd ook dieper ingegaan op de mogelijke vooruitzichten voor verder onderzoek.

Tot slot kan men stellen dat de resultaten uit dit doctoraatsonderoek aantonen dat plantenextracten en etherische oliën geen uitgesproken effect hadden op de zoötechnische prestaties. Toch werd duidelijk dat het supplementeren van OPE, LPE, of hun combinatie bepaalde bloedcomponenten en ook de morfologie van de dunne darm kunnen beïnvloeden. Het toevoegen van LPE, OPE, CXEO en OCEO bieden daarnaast potentieel om het antioxidant systeem van vleeskippen te verbeteren wanneer deze dieren langdurig worden blootgesteld aan hoge omgevingstemperaturen. Dit werd ook bevestigd door een verlaagde expressie van HSP70 bij het toevoegen van CXEO of OCEO aan het voeder. Algemeen werd ook waargenomen dat de positieve werking meer uitgesproken was bij de hogere dosis van 400 mg/kg. Niettemin werd ook vastgesteld dat verschillende andere factoren zoals de leeftijd van de dieren, het type weefsel en de duur van verhoogde

temperatuur elk op hun manier kunnen interfereren met het effect van plantenextracten en etherische oliën op verschillende parameters.

چکیده

استرس گرمایی و اثرات مضر آن بر روی عملکرد طیور در بعضی از ماههای سال توجه بسیاری از محققان را به خود جلب کرده است. ثابت شده است که درجه حرارت بالا، علی الخصوص در دوره پایانی رشد، اثرات بسیار مضری بر روی عملکرد، سلامتی و فیزیولوژی جوجه‌های گوشتیمی گذارد. تاکنون روش‌های مختلفی برای کاهش و یا از بین بردن اثرات منفی استرس گرمایی پیشنهاد شده است. از میان روش‌های موجود، دستکاری جیره و افزودن مواد آنتی‌اکسیدانی بعنوان یک راه حل مناسب و قابل انجام توصیه میشود. در این راستا، استفاده از مواد گیاهی سرشار از ترکیبات آنتی‌اکسیدانی نظیر ترکیبات فنولیکی سودمند بنظر میرسد. چنین ترکیباتی در عصاره گیاهانی نظیر لیمو، پرتقال، زردچوبه و پونه کوهی به فراوانی یافت میشوند. بنابراین و با توجه به مقدمه بیان شده، هدف از انجام این رساله شامل موارد زیر می باشد:

۱) بررسی اثرات عصاره‌های گیاهی بر روی پارامترهای عملکردی جوجه‌های گوشتی در شرایط تنش گرمایی

۲) ارزیابی توانایی عصاره‌های ذکر شده در بهبود پارامترهای مربوط به جمعیت میکروبی و دستگاه گوارش جوجه‌های گوشتی

۳) ارزیابی توانایی عصاره‌های گیاهی بر روی پارامترهای کیفیت گوشت و تنش اکسیداتیو در سطح سلولی و ملکولی از طریق روش‌های بیوتکنولوژی

در این رساله، با توجه به حساسیت بالای جوجه‌های گوشتی به درجه حرارت بالا در دوره نهایی پرورش، به همین دلیل این دوره (روزهای بین ۲۵ تا ۴۲ دوره پرورش) انتخاب گردید. درجه حرارت اعمال شده در این پژوهش ۳۴ درجه سانتیگراد و برای ۵ ساعت در روز بود. هر کدام از عصاره‌های یاد شده در ۲ سطح ۲۰۰ و ۴۰۰ میلی‌گرم به جیره جوجه‌ها افزوده شدند. ۳ روز اول تغذیه از عصاره‌ها به عنوان دوره سازگاری جوجه‌ها با عصاره‌های گیاهی در نظر گرفته شد. در آزمایش اول جهت بررسی اثر احتمالی هم افزایی عصاره‌های لیمو و پرتقال، مخلوط این دو عصاره در سطوح مختلف به

جوجه‌ها تغذیه شدند. بعد از ۳ روز، ۱۰ روز و ۲ هفته از تنش گرمایی القا شده، جوجه‌ها توزین شده و جهت بررسی کیفیت گوشت، پارامترهای فیزیولوژیک، ریخت شناسی دستگاه گوارش، جمعیت میکروبی و همچنین تنش اکسیداتیو نمونه‌برداری شدند. در پایان آزمایش تفاوت معنی‌داری در عملکرد جوجه‌ها مشاهده نشد.درمقایسه با گروه شاهد، تغذیه با ۴۰۰ میلی‌گرم عصاره پوست پرتقالو همچنین۲۰۰ و ۴۰۰ میلی‌گرم عصاره زردچوبه باعث افزایش معنی‌دار فعالیت گلوتاتیون‌پراکسیداز، و تغذیه با ۴۰۰ میلی‌گرم عصاره زردچوبه باعث افرایش فعالیت آنزیم سوپراکسید دیسموتاز گردید. افزودن ۲۰۰ میلی‌گرم عصاره پوست لیمو و۴۰۰ میلی‌گرم از عصاره‌های زردچوبه و پوست لیمو به جیره،به‌ترتیب، موجب افزایش غلظت هورمون T_3و هورمون رشد نسبت به گروه شاهد گردید، اما عصاره‌های مورد مطالعه تأثیر معنی‌داری بر غلظت هورمون T_4 نداشتند.همچنین تغذیه با۴۰۰ میلی‌گرم از عصاره زردچوبه منجر به کاهش کلی‌فرم‌های ایلئوم و سکوم نسبت به گروه شاهد شد. اثرات مثبت عصاره‌ها بر روی ریخت شناسی روده کوچک نیز مشاهده شد. تغذیه با عصاره زردچوبه و پونه کوهی به ویژه در سطوح ۴۰۰ میلی‌گرم به طور معنی‌داری باعث بهبود سیستم آنتی‌اکسیدانی پرنده شد. پروتئین‌های شوک حرارتی نیز به‌طور قابل توجهی با تغذیه عصاره‌ها کاهش یافتند. هر چند که این اثرات مثبت عصاره‌های گیاهی به طور گسترده ای بستگی به میزان تغذیه شده، سن پرنده و نوع بافت هدف متفاوت بود. به عبارت دیگر در بعضی بافت‌ها هیچ گونه اثری مشاهده نشد و در بعضی بافت‌ها اثرات مثبت معنی‌دار مشاهد شد. به عنوان نتیجه گیری کلی از نتایج مشاهده شده در این رساله، واضح است که عصاره‌های گیاهی پوست لیمو و پرتقال و عصاره زردچوبه و پونه کوهی می‌تواند بدون تأثیر منفی بر عملکرد، اثر مطلوبی بر سیستم آنتی‌اکسیدانی، پارامترهای فیزیولوژیک، ریخت‌شناسی روده کوچکو جمعیت میکروبی جوجه‌های گوشتی در شرایط تنش گرمایی داشته باشد. هر چند که اثرات سودمند عصاره‌های گیاهی غنی از ترکیبات فنولیک بر روی جوجه‌های گوشتی تحت تأثیر سن پرنده، نوع بافت هدف و طول دوره تنش گرمایی می‌تواند متغیر باشد.

REFERENCES

REFERENCES

Abdel-Hamid, N. 2007. Physiological and histopathological alterations induced by phenol exposure in Oreochromis aureus juveniles. Turkish Journal of Fisheries and Aquatic Sciences 7, 131-138.

Abe, T., Mujahid, A., Sato, K., Akiba, Y., Toyomizu, M. 2006. Possible role of avian uncoupling protein in down-regulating mitochondrial superoxide production in skeletal muscle of fasted chickens. FEBS Letters 580(20), 4815-4822.

Aebi, H.E. 1983. Catalase. In: Methods for Enzymatic Analysis. HU Bergmayer. Verlag Chemie, Weinheim, Florida. 3, 273-286.

Aengwanich, W., Suttajit, M.2010. Effect of poluphenols extracted from tamarind (*Tamarinus indica* L.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress and body weight of broilers (*Gallus domesticus*) under chronic heat stress. Animal Science Journal 81:264-270.

Ahmad, T., Khalid, T., Mushtaq, T., Mirza, M.A., Nadeem, A., Babar, M.E., Ahmad, G. 2008. Effect of potassium chloride supplementation in drinking water on broiler performance under heat stress conditions. Poultry Science 87, 1276-1280.

Ahmad, T., Mushtaq, T., Mahr, U.N., Sarwar, M., Hooge, D.M, Mirza, M.A. 2006. Effect of different non-chloride sodium sources on the performance of heat-stressed broiler chickens. British Poultry Science 47, 249-256.

Ahmad, T., Sarwar, M. 2006. Dietary electrolyte balance: implications in heat stress broilers. World's Poultry Science Journal. 62(4), 638-653.

Ait-boulahsen, A., Garlich, J.D., Edens, F.W. 1989. Effect of fasting and acute heat stress on body temperature, blood acid-base and electrolyte status in chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 94, 683-687.

Akbarian, A., Golian, A., Sheikh Ahmandi, A., Moravej, H. 2011. Effects of ginger root (*Zingiber officinale*) on egg yolk cholesterol, antioxidant status and performance of laying hens. Journal of Applied Animal Research 39, 19-21.

Akbarian, A., Golian, A., Kermanshahi, H., Raji, A. R., Farhoosh, R., De Smet, S., Michiels, J. 2013a.Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature. Spanish Journal of Agricultural Research 11, 109-119.

Akbarian, A., Golian, A., Gilani, A., Kermanshahi, H., Zhaleh, S., Akhavan, A., De Smet, S., Michiels, J. 2013b. Effect of feeding citrus peel extracts on growth performance, serum components, and intestinal morphology of broilers exposed to high ambient temperature during the finisher phase. Livestock Science 157, 490-497.

Akbarian, A., Golian, A., Kermanshahi, H., De Smet, S. & Michiels, J. 2014a. Antioxidant enzyme activities, biochemical parameters and antibody titers in blood of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and *Curcuma xanthorrhiza* essential oil. Journal of Animal Physiology and Animal Nutrition. DOI: 10.1111/jpn.12188.

Akbarian, A., Michiels, J., Golian, A., Buyse, J., Wang, Y., De Smet, S. 2014b. Gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, and meat quality of cyclically heat challenged finishing broilers fed *Origanum compactum* and *Curcuma xanthorrhiza* essential oils. Poultry Science (in press).

Aksit, M., Yalcm, S., Ozkan, S., Metin, K., Ozdemir, D. 2006. Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. Poultry Science 85,1867-1874.

Altan, O., Pabuccuoglu. A., Altan. A,, Konyalioglu, S., Bayraktar, H. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. British Poultry Science 44, 545-550.

Ananthan, J., Goldberg, A.L., Voellmy, R. 1986. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 232, 522-524.

APHA. 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Assoc, Washington, DC.

Arad, Z., Marder. J., Eylath, U. 1983. Serum electrolyte and enzyme responses to heat stress and dehydration in the fowl (Gallus domesticus). Comparative Biochemistry and Physiology Part A: Physiology 74, 449-453.

Attia, Y.A., Hassan, R.A., Tag El-din, A.E., Abou-Shehema, B.M. 2011. Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. Journal of Animal Physiology and Animal Nutrition 95, 744-755.

Aviagen. 2011. Ross 308 broiler management guide. Available in http://en.aviagen.com/ross-308/.

Avila-Ramos, A., Pro-Martínez, A., Sosa-Montes, E., Cuca-Garcia, J.M., Becerril-Pérez, C.M., Figueroa-Velasco, J.L.,Narciso-Gaytán, C. 2012. Effects of dietary oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. Poultry Science 91:505-511.

Azad, M.A.K., Kikusato, M., Sudo, S., Amo, T., toyomizu, M. 2010a. Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken. Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology 157, 266-271.

Azad, M.A.K., Kikusato, M.,Maekawa, T., Shirakawa, H., Toyomizu, M.2010b. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress.Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 155:401-406.

Bailey, M.T., Lubach, G.R.,Coe, C.L. 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. The Journal of Pediatric Gastroenterology and Nutrition 38, 414-421.

Bakker, G.C.M., Van Erk, M.J., Pellis, L., Wopereis, S., Rubingh, C.M., Cnubben, N.H.P., Kooistra, T., Van Ommen, B., Hendriks, H.F.J. 2010. An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overwieght men: a nutrigenomics approach. The American Journal of Clinical Nutrition. 91, 1044-1059.

Baldi, A., Rosen, R.T., Fukuda, E.K., Ho, C.T. 1995. Identification of nonvolatile components in lemon peel by high-performance liquid chromatography with confirmation by mass spectrometry and diode-array detection. Journal of Chromatography. A7 18, 89-97.

Balnave, D. 1974. Biological factors affecting energy expenditure. Pages 25-46 in Energy Requirement of Poultry. T. R. Morris and B. M. Freeman, ed. British Poultry Science Ltd., Edinburg.

Balnave, D. 2004. Challenges of accurately defining the nutrient requirements of heat stressed poultry. Poultry Science 83, 5-14.

Balasundram, N., Sundram, K., Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 99, 191-203.

Banan, A., Fields, J.Z., Zhang, Y. Keshavarzian, A. 2001. iNOS upregulation mediates oxidant-induced disruption of F-actin and barrier of intestinal monolayers. American Journal of Physiology Gastrointestinal and Liver Physiology 280, G1234-G1246.

Basmacioğlu, M.H., Baysal, S., Misirlioğlu, Z., Polat, M., Yilmaz, H., Turan, N. 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat-soybean meal diets. British Poultry Science 52, 67-80.

Bayram, I., Cetingul, I., Akkaya, B., Uyarlar, C. 2007. Effects of aniseed (Pimpinella anisum L.), on egg production, quality, cholesterol levels, hatching results and the antibody values in blood of laying quails (Coturnix japonica). Archiva Zootechnica 10, 73-77.

Belay, T., Wiernusz, C.J., Teeter, R.G. 1990. Mineral balance of heat distressed broilers. Animal Science Research Report Agric. Exp. Sta. Oklahoma State Univ., MP-129, 189−194.

Belay, T., Teeter, R.G. 1996. Effects of ambient temperature on broiler mineral balance partitioned into urinary and faecal loss. British Poultry Science 37, 423-433.

Benzie, I.F., Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: the FRAP assay. Analytical Biochemistry 239, 70-76.

Benzie, I.F, Strain, J.J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299, 15-27.

Biavatti, M.W., Bellaver, M.H., Volpato, L., Costa, C., Bellaver, C. 2003. Preliminary studies of alternative feed additives for broilers: Alternanthera brasiliana extract, propolis extract and linseed oil. Brazilian Journalof Poultry Science 5, 147-151.

Bogin, E. 1992. Handbook for veterinary clinical chemistry. Kodak Publishing, NY, USA.

Bogin, E., Weisman, Y., Friedman, Y. 1981. Effect of heat shock on the biochemical composition of chicken blood. Refuah Veterinarit 38, 98-104.

Bogin, E., Avidar, Y., Pech-Waffenschmidt, V., Doron, Y., Israeli, B.A., Kevkhayev, E. 1996. The relationship between heat stress, survivability and blood composition of the domestic chicken. European Journal of Clinical Chemistry and Clinical Biochemistry 34, 463-469.

Bonnet, S.I., Geraert, P.A., Lessire, M., Carre, B., Guillaumin, S. 1997. Effect of high ambient temperature on feed digestibility in broilers. Poultry Science 76(6), 857-863.

Boone, M.A. 1968. Temperature as six different locations in the fowl’s body as affected by ambient temperatures. Poultry Science 47, 1961-1962.

Boone, M.A., Hughes, B.L. 1971. Wind velocity as it affects body temperature, water consumption and feed consumption during heat stress of roosters. Poultry Science 50, 1535-1537.

Borges, S.A., Fischer da Silva, A.V., Majorka, A., Hooge, D.M., Cummings, K.R. 2004. Physiological responses of broielr chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). Poultry Science 83, 1551-1558.

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J., Spais, A.B. 2002. Effects of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. British Poultry Science 43, 223-230.

Bou, R., Codony, R., Tres, A. Decker, E.A., Guardiola, F. 2009. Dietary strategies to improve the nutritional value, oxidative stability, and sensory properties of poultry products. Critical Reviews in Food Science and Nutrition 49, 800-822.

Braddock, R.J. 1999. Handbook of citrus by-products and processing technology, John Wiley & Sons, Inc., New York, NY, USA.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56, 317-333.

Brenes, A., Roura, E. 2010: Essential oils in poultry nutrition: main effects and modes of action. Animal Feed Science and Technology 158, 1-14.

Burkholder, K.M., Thompson, K.L., Einstein, M.E., Applegate, T.J., Patterson, J.A. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. Poultry Science 87, 1734-1741.

Burtis, C.A., Ashwood, E.R. 1998. Tietz textbook of clinical chemistry. 3rd edn.W.B.Saunders Company. Philadelphia 1917 pp.

Çabuk, M., Bozkurt, M., Alicukek, A., Çatli, AU., Baser, KHC. 2006. Effect of a dietary essential oil mixture on performance of laying hens in the summer season. South African Journal of Animal Science 36, 215-221.

Cao, G., Giovanoni, M., Prior, R.L. 1996. Antioxidant capacity in different tissues of young and old rats. Proceedings of the Society for Experimental Biologyand Medicine 211, 359-365.

Castelli, W., Doyle, J., Gordon, T., Hames, C., Hjortland, M., Hulley, S., Kagan, A., Zukel, W. 2005. HDL cholesterol and other lipids in coronary heart disease. the cooperative lipoprotein phenotyping study. Circulation 55, 767-772.

Chan, C.B., MacDonal, P.E., Saleh, M.C., Johns, D.C., Marban, E., Wheeler, M.B. 1999. Overexpression of uncoupling protein 2 inhibits glucose-stimulated insulin secretion from rat islets. Diabetes 48(7), 1482-1486.

Colpo, A. 2005. LDL cholesterol: bad cholesterol, or bad science? Journal of American Physicians and Surgeons 10, 83-89.

Collin, A., Buyse, J., Van As, P., Darras, V.M., Malheiros, R.D., Moraes, V.M.B., Reyns, G.E., Taouis, M., Decuypere, E. 2003a. Cold-induced enhancement of avian uncoupling protein expression, heat production and triiodothyronine concentrations in broiler chicks. General and Comperative Endocrinology 130, 70-77.

Collin, A., Malheiros, R.D., Moraes, V.M., Van As, P., Darras, V.M., Taouis, M.., Decuypere, E., Buyse, J. 2003b. Effect of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. British Journal of Nutrition. 90, 261-269.

Craig, E.A., Gross, C.A. 1991. Is HSP70 the cellular thermometer? Trends in Biochemical Sciences 16, 135-140.

Dai, S.F., Wang, L.K., Wen, A. Y., Wang, L. X., Jin, G. M. 2009. Dietary glutamine supplementation improves growth performance, meat quality and colour stability of broilers under heat stress. British Poultry Science50, 333-340.

Dai, S.F., Gao, F., Zhang, W.H., Song, S.X., Xu, X.L. Zhou, G.H. 2011. Effects of dietary glutamine and gamma-aminobutyric acid onperformance, carcass characteristics and serum parameters in broilersunder circular heat stress. Animal Feed Science and Technology 168, 51-60.

Darras, V.M., Visser, T.J., Berghman, R., Kühn, E.R. 1992. Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: relationship with changes in plasma triiodothyronine and growth hormone levels. Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 103, 131-136.

Davis, R.H., Hassan, O. E. M.,Sykes, A. H. 1972. The adaptation of energy utilization in the laying hen to warm and cool ambient temperatures. The Journal of Agricultural Science 79, 363-369.

Debersac, P., Vernevaut, M.F., Amiot, M.J., Suschetet, M., Siess, M.H. 2001. Effects of a water-soluble extract of rosemary and its purified component rosmarinic acid on xenobiotic-metabolizing enzymes in rat liver. Food and Chemical Toxicology 39, 109-117.

Degroote, J., Michiels, J., Claeys, E., Ovyn, A.,De Smet, S. 2012. Changes in the pig small intestinal mucosal glutathione kinetics after weaning. Animal Science 90, S359-S361.

Dorhoi, A., Dobrean, V., Zahan, M., Virag, P. 2006. Modulatory effects of several herbal extracts on avian peripheral blood cell immune responses. Phytotherapy Research 20, 352-358.

Dridi, S., Temim, S.,Derouet, M., Tesseraud, S., Taouis, M. 2008. Acute cold- and chronic heat-exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309A, 381-388.

Durant, J.A., Corrier, D.E., Byrd, J.A., Stanker, L.H., Ricke, S.C. 1999. Feed deprivation affects crop environment and modulates Salmonella enteritidis colonization and invasion of leghorn hens. Applied and Environmental Microbiology65, 1919-1923.

Eraslan, G.M., Silici, S. 2007. Evaluation of propolis effects on some biochemical parameters in rats treated with sodium fluoride. Pesticide Biochemistry and Physiology 88, 273-283.

Esfahani, A., Wong, J.M., Truan, J., Villa, C.R., Mirrahimi, A., Srichaikul, K., Kendall, C.W. 2011. Health effects of mixed fruit and vegetable concentration: a systematic review of the clinical interventions. The Journal of the American College of Nutrition 30, 285-294.

Faix, S., Faixova, Z., Placha, I., Koppel, J. 2009. Effect of Cinnamomum zeylanicum essential oil on antioxidative status in broiler chickens. Acta Veterinaria 78, 411-417.

FAO. 2012. Electronic database of the Food and Agriculture Organisation. http://faostat.fao.org/

Farrell, P.M., Eyerman, E.L., Tureen,L.L. 1966. Comparison of plasma creatine phosphokinase changes in nutritional and genetic muscular dystrophy in the chicken. Annals of the New York Academy of Sciences 138, 102-110.

Fellenberg, M.A., Speisky, H. 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. World's Poultry Science Journal 62, 53-70.

Feng, J., Zhang, M., Zheng, S., Xie, P. Ma, A. 2008. Effects of high temperature on multiple parameters of broilers in vitro and in vivo. Poultry Science 87, 2133-2139.

Flanagan, S.W., Moseley, P.L. Buettner, G.R. 1998. Increased flux of free radicals in cells subjected to hyperthermia detection by electron paramagnetic resonance spin trapping. FEBS Letters 431, 285-286.

Floyd, A.R., Carney, J.M. 1992. Free radical damage to protein and DNA: Mechanisms involved and relevant observations on brain undergoing oxidative stress. Annals of Neurology32, S22-S27.

Fu, S.H., Yang, M.H., Wen, H.M., Chern, J.C. 2005. Analysis of flavonoids in propolis by capillary electrophoresis. Journal of Food and Drug Analysis 13, 43-50.

Fu, Y., Zheng, S., Lin, J., Ryerse, J., Chen, A. 2008. Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Molecular Pharmacology 73, 399-409.

Garau, M.C., Simal, S., Rosselló, C., Femenia, A. 2007. Effect of air-drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (Citrus aurantium v. Canoneta) by-Products. Food Chemistry 104, 1014-1024.

Grau. R., Hamm, R. A. 1953. Simple method for the determination of water binding in muscles. Naturwissennshaften 40, 29-30.

Garcia, V., Catala-Gregori, P., Hernandez, F., Megias, M.D., Madrid, J. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. Journal of Applied Poultry Research 16, 555-562.

Gething, M.J., Sambrook, J. 1992. Protein folding in the cell. Nature 355, 33–45.

Geraert, P.A., Padilha, J.C., Guillaumin, S. 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: biological and endocrinological variables. British Journal of Nutrition 75, 205-216.

Ghasemi, K., Ghasemi, Y., Ebrahimzadeh, M.A. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pakistan Journal of Pharmaceutical Sciences 22, 277-281.

Givisiez, P.E.N., Furlan, R.L., Malheiros, E.B., Macari, M. 2003. Incubation and rearing temperature effects on Hsp70 levels and heat stress response in broilers. Canadian Journal of Animal Science 82, 213-220.

Goglia, F, Sulachev, V.P. 2003. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. The FASEB Journal 17, 1585-1591.

Gonzales, A. M., Orlando, R.A. 2008. Curcumin and reveratrol inhibit nuclear factor kappaB-mediated cytokine expression in adipocytes. Nutrition & Metabolism 5, 17.

Gonzalez-Esquerra, R., Leeson, S. 2006. Effects of acute versus chronic heat stress on broiler response to dietary protein. Poultry Science 84, 1562-1569.

Gorman, I., Balnave, D. 1994. Effects of dietary mineral supplementation on the performance and mineral retentions of broilers at high ambient temperatures. British Poultry science 35, 563-572.

Gregory, N.G. 2010. How climatic changes could affect meat quality. Food Research International 43, 1866-1873.

Gu, X.H., Hao, Y.,Wang, X.L. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poultry Science 91, 790–799.

Gursu, M.F., Onderci, M., Gulcu, F.,Sahin, K. 2004. Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress *in vivo*. Nutrition Research 24, 157-164.

Halici, M., Imik, H., Koc, M., Gumus, R. 2012. Effects of α-lipoic acid, vitamin E and C upon the heat stress in Japanese quails. Journal of Animal Physiology and Animal Nutrition 96, 408-415.

Han, A.Y., Zhang, M.H., Zuo, X.L., Zheng, S.S., Zhao, C.F., Feng, J.H., Cheng, C. 2010. Effect of acute heat stress on calcium concentration, proliferation, cell cycle, and interleukin-2 production in splenic lymphocytes from broiler chickens. Poultry Science 89, 2063-2070.

Hernández, F., Madrid, J., García, V., Orengo, J., Megías, M.D. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science 83, 169-174.

Hernandez, P., Zomeno, L. Arino, B.,Blasco, A. 2004. Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. Meat Science 66, 525-529.

Hikosaka, K., El-Abasy, M., Koyama, Y., Motobu, M., Koge, K., Isobe, T., Kang, C.B., Hayashidani, H., Onodera, T., Wang, P.C., Matsumura, M., Hirota, Y. 2007. Immunostimulating effects of the polyphenol-rich fraction of sugar cane (*Saccharum officinarum* L.) extract in chickens. Phytotherapy Research 21, 120-125.

Hillman, P.E., Scott, N.R.., Van Tienhoven, A. 1985. Physiological responses and adaptations to hot and cold environments. In: Yousef, M.K. (ed.). Stress physiology in livestock. Vol 3, Poultry. CRC Press. Boca Raton, Florida, pp. 1-71.

Hinton, A., Buhr, R.J, Ingram, K.D. 2000. Physical, chemical, and microbiological changes in the crop of broiler chickens subjected to incremental feed withdrawal. Poultry Science 79, 212-218.

Hu, M.L. 2011. Dietary polyphenols as antioxidants and anticancer agents: more questions than answers. Chang Gung Medical Journal 34, 449-460.

Incharoen, T., Yamauchi, K., Thongwittaya, N. 2010. Intestinal villus histological alterations in broilers fed dietary dried fermented ginger. Journalof Animal Physiology and Animal Nutrition 94, e130-e137.

Ismail, A.B., Al-Busadah, K.A.,El-Bahr, S.M. 2013. Oxidative stress biomarkers and biochemical profile in broiler chicken fed zinc bacitracin and ascorbic acid under hot climate. American Journal of Biochemistry and Molecular Biology 3, 202-214.

Jamroz, D., Wertelecki, T., Houszka, M., Kamel, C. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. Journal of Animal Physiology and Animal Nutrition 90, 255-268.

Jamroz, D., Wiliczkiewicz, A., Wertelecki, T., Orda, J., Skorupinska, J. 2005. Use of active substances of plant origing in chicken diets based on maize and locally grown cereals. British Poultry Science 46, 485-493.

Jang, I.S., Ko, Y.H., Kang, S.Y., Lee, C.Y. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Animal Feed Science and Technology 134, 304-315.

Kaminishi, T., Matsuoka, T., Yanagishita, T.,Kako, K. J. 1989. Increase vs. decrease of calcium uptake by isolated heart cells induced by H2O2 vs. HOCl. American Journal of physiology 253, C598-C607.

Keller, J.S. 1980. Fasting heat production as a function of growth rate in the chicken. Archivbir Geflugelkunde 44, 168-172.

Khaligh, F., Sadeghi, G., Karimi, A., Vaziry, A. 2011. Evaluation of different medicinal plants blends in diets for broiler chickens . Journal of Medicinal Plants Research 5, 1971-1977.

Khan, W.A., Khan, A., Anjum, A.D., Rehman, Z. 2002. Effects of induced heat stress on some biochemical values in broiler chicks. International Journal of Agricultural and Biological Engineering 4, 74-75.

Khan, R.U., Naz, S., Nikousefat, Z., Selvaggi, M., Laudadio, V., Tufarelli, V. 2012. Effect of ascorbic acid in heat stressed poultry. World's Poultry Science Journal 68, 477-490.

Khassaf, M., McArdle, A., Esanu, C. Vasilaki, A.,McArdle, F., Griffiths, R.D., Brodie, D.A., Jackson, M.J. 2003. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. The Journal of Physiology 549, 645-652.

Kikusato, M., Toyomizu. M. 2013. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. Plos One 8, e64412.

Kim, S.H., Hong, K.O., Chung, W.Y., Hwang, J.K.,Park, K.K. 2004. Abrogation of cisplatin-induced hepatotoxicity in mice by xanthorrhizol is related to its effect on the regulation of gene transcription. Toxicology and Applied Pharmacology 196, 346-355.

Kim, W.R., Flamm, S.L., Di, Bisceglie, A.M., Bodenheimer, H.C. 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47, 1363-1370.

Koh, T.S., Peng, R.K., Klasing, K.C. 1996. Dietary copper level affects copper mechanism during lipopolysaccharide-induced immunological stress in chicks. Poultry Science 75, 867-872.

Kohne, H.J., Jones, J.E. 1975. Changes in plasma electrolytes, acid-base balance and other physiological parameters of adult female turkeys under conditions of acute hyperthermia. Poultry Science 54, 2034-2038.

Kolankaya, D., Selmanoglu, G., Sorkun, K., Salih, B. 2002. Protective effects of turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. Food Chemistry 78, 213-217.

Lambert, G.P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. Journal of Animal Science87, E101-108.

Lambertucci, R.H., Levada-Pires, A.C., Rossoni, L.V. Curi, R.,Pithon-Curi, T.C.2007. Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. Mechanisms of Ageing and Development 128, 267-275.

Lara, J.L., Rostagno, M.H. 2013. Impacts of heat stress on poultry production. Animal 3:356-369.

Lee, K.W., Everts, H., Kappert, H.J., Yeom, K.H., Beynen, A.C. 2003a. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. Journal of Applied Poultry Research 12, 394-399.

Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., Beynen, A.C. 2003b. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science 44, 450-457.

Lee, K.W., Everts, H., Beynen, A.C. 2004. Essential oils in broiler nutrition. International Journal of Poultry Science 3, 738-752.

Letelier, M.E., Sanchez-Jofre, S., Peredo-Silva, L., Cortes-Troncoso, J., Aracena-Parks, P. 2010. Mechanisms underlying iron and copper ions toxicity in biological systems: Pro-oxidant activity and protei-biding effects. Chemco-Biological Interactions 188, 220-227.

Lia, B.B., Smith, B., Hossain, M. 2006. Extraction of phenolics from citrus peels I. Solvent extraction method. Separation and Purification Technology 48, 182-188.

Lin, H., Decuypere, E., Buyse, J. 2006a. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology-Part A: Molecular &Integrative Physiology 144, 11-17.

Lin, H., Jiao, H. C., Buyse, J., Decuypere, E. 2006b. Strategies for preventing heat stress in poultry. World's Poultry Science Journal 62, 71-86.

Liu, X.L., Xu, Y.J., Go, M.L. 2008. Functionalized chalcones with basic functionalities have antibacterial activity against drug sensitive Staphylococcus aureus. European Journal of Medicinal Chemistry 43, 1681-1687.

Liu, F., Yin, J., Du, M., Yan, P., Xu, J., Zhu, X., Yu, J. 2009. Heat-stress-induced damage to porcine small intestinal epithelium associated with downregulation of epithelial growth factor signaling. Journal of Animal Science 87, 1941-1949.

Liu, H.W., Tong, J.M., Zhou, D.W. 2011. Utilization of chinese herbal feed additives in animal production. Chinese Agricultural Science 10, 1262-1272.

Looraine, M.C. 1982. Clinical microbiology procedures. Hand book. Volume 2, section 8: Viruses, rickettsiae, chlamydiae and mycoplasma. American Society of Microbiology, Washington, D.C. 2540 pp.

Lounsbury, K.M., Hu, Q., Ziegelstein, R.C.2000. Calcium signaling and oxidant stress in the vasculature. Free Radical Biology and Medicine 28, 1362-1369.

Luna, A., Labaque, M.C., Zygadlo, J. A., Marin, R.H. 2010. Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. Poultry Science 89, 366-370.

Luoma, P.V., Stengard, J., Korpela, H., Rautio, A., Sotaniemi, E.A., Suvanto, E., Marniemi, J. 1990. Lipid peroxides, glutathione peroxidase, high density lipoprotein subfractions and apolipoproteins in young adults. Journal of Internal Medicine 227, 287-289.

Lyte, M., Bailey, M.T. 1997. Neuroendocrine-bacterial interactions in a neurotoxin-induced model of trauma. Journal of Surgical Research70, 195-201.

Ma, D., Shan, A., Chen, Z., Du, J., Song, K., Li, J., Xu, Q. 2005. Effect of Ligustrum lucidum and Schisandra chinensis on the egg production, antioxidant status and immunity of laying hens during heat stress. Archives of Animal Nutrition 59, 439-447.

Mack, L.A., Felver-Gant, J.N., Dennis, R.L.,Cheng, H.W. 2013. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. Poultry Science 92, 285-294.

Mahmoud, K.Z., Edens, F.W. 2003. Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). Comparative Biochemistry and Physiology-Part B: Biochemistry & Molecular Biology 136, 921-934.

Mahmoud, K.Z., Edens, F.W., Eisen, E.J.,Havensteina, G.B. 2004. Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (*Gallus gallus domesticus*) subjected to cyclic heat stress. Comparative Biochemistry and Physiology-Part B: Biochemistry & Molecular Biology 137, 35-42.

Maini, S., Rastogi, S.K., Korde, J.P., Madan, A.K.Shukla, S.K. 2007. Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. The Journal of Poultry Science 44, 339-347.

Marklund, S., Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journalof Biochemistry 47, 469-474.

Masuda, T., Isobe, J., Jitoe, A., Nakatani, N. 1992. Antioxidative curcuminoids from rhizomes of *Curcuma xanthorrhiza*. Phytochemistry 31, 3645-3647

May, J.D., Deaton, J.W., Reece, F.N., Branton, S.L. 1986. Effect of acclimation and heat stress on thyroid hormone concentration. Poultry Science 65, 1211-1213.

Mccarthy, B.M., Okano, Y., Nakayasu, T., Macy, M., Watson, S.R., Harmony, J.A. 1987. Plasma lipoproteins and transferrin regulate the proliferation of a continuous t lymphocyte cell line. Journal of Lipid Research 28, 1067-1077.

Meddings, J.B., Swain, M.G. 2000. Environmental stress induced a generalized increase in gastrointestinal permeability mediated by endogenous glucocorticoids in the rat. Gastroenterology 119, 1019-1028.

Mehmet, C., Talat, G., Bestami, D., Ertas, O.N. 2005. The effect of anise oil (*Pimpinella anisum* L.) on broiler performance. International Journal of Poultry Science 4, 851-855.

Melesse, A., Maak, S., Schmidt, R., Von-Lengerken, G. 2011. Effect of long-term heat stress on some performance traits and plasma enzyme activities in naked-neck chickens and their F1 crosses with commercial layer breeds. Livestock Science 141, 227-231.

Mitchell, M.A., Carlisle, A.J. 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (*Gallus domesticus*). Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 101, 137-142.

Mitchell,M.A., Sandercock, D.A. 1995. Creatine kinase isoenzyme profiles in the plasma of the domestic fowl (*Gallus domesticus*). Research in Veterinary Science 59, 30-34.

Michiels. J., Maertens, L.Buyse, J., Lemme, A., Rademacher, M., Dierick, N.A., De Smet, S. 2012a. Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poultry Science 91, 402-412.

Michiels J., Missotten J., Fremaut, D., De Smet, S., Dierick, N. 2007. In vitro dose–response of carvacrol, thymol, eugenol and trans-cinnamaldehyde and interaction of combinations for the antimicrobial activity against the pig gut flora. Livestock Science 109, 157-160.

Michiels, J., Skrivanova, E., Missotten, J., Ovyn, A., Mrazek, J., De Smet, S., Dierick, N. 2012b. Intact brown seaweed (*Ascophyllum nodosum*) in diets of weaned piglets: Effects on performance, gut bacteria and morphology and plasma oxidative status. Journal of Animal Physiology and Animal Nutrition 96, 1101-1111.

Montagne, L., Pluske, J.R., Hampson, D.J. 2003.A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal Feed Scienceand Technology 108, 95-117.

Morimoto, R.I., Tissieres, A. Georgopoulos, C. (eds). 1990. Stress Proteins in Biology and Medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Mujahid, A., Yoshiki, Y., Akiba, Y., Toyomizu, M. 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poultry Science 84:307-314.

Mujahid, A., Sato, K., Akiba, Y., Toyomizu, M. 2006. Acute heat stress stimulates mitochondrial superoxide production in broiler sleletal muscle, possibly via downregulation of uncoupling protein content. Poultry Science 85, 1259-1265.

Mujahid, A., Pumford, N.R., Bottje, W., Nakagava, K., Miyazawa, T., Akiba, Y., Toyomizu, M. 2007a. Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. The Journal of Poultry Science 44, 439-445.

Mujahid, A., Akiba, Y. Warden, C.H., Toyomizu, M. 2007b. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. FEBS Letters 581, 3461-3467.

Mujahid, A., Akiba, Y., Toyomizu, M. 2007c. Acute heat stress induces oxidative stress and decreases adaptation in young white leghorn cockerels by downregulation of avian uncoupling protein. Poultry Science 86, 364-371.

Mujahid, A., Akiba, Y.Toyomizu, M. 2009. Olive oil-supplemented diet alleviates acute heat stress-induced mitochondrial ROS production in chicken skeletal muscle. The American Journal of Physiology-regulatory, Integrative and Comparative Physiology 290, 690-698.

Namkung, H., Li, J., Gong, M., Yu, H., Cottrill, M., de Lange, C.F.M. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Canadian Journalof Animal Science 84, 697-704.

Nassr-Allah, H. 2007. Physiological and histopathological alterations induced by phenol exposure in oreochromis aureus juveniles. Turkish Journal of Fisheries and Aquatic Sciences 7, 131-138.

Nauck, M., Warnick, G., Rifai, N. 2002. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clinical Chemistry 48, 236-254.

Nofrarías, M., Manzanilla, E.G., Pujols, J., Gibert, X., Majó, N., Segalés, J., Gasa, J. 2006. Effects of spray-dried porcine plasma and plant extracts on intestinal morphology and on leukocyte cell subsets of weaned pigs. Journal of Animal Science 84, 2735-2742.

Nordberg, J., Arner, E.S.J. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biology & Medicine 31, 1287-1312.

Nover, L. 1991. Heat Shock Response. CRC Press, Boca Raton, Florida.

NRC. 1994. Nutrient requirements of poultry, 9th rev. ed. National Research Council, National Academy Press, Washington DC, USA.

Oetting, L.L, Utiyama, C.E., Giani, P.A., Ruiz, U.D., Miyada, V.S. 2006. Efeitos de extratos vegetais e antimicrobianos sobre a digestibilidade aparente, o desempenho, a morfometria dos órgãos e a histologia intestinal de leitões recém-desmamados. Revista Brasileira de Zootecnia 35, 1389-1397.

Onderci, M., Sahin, K., Sahin, N., Gursu, M.F., Doerge, D., sarkar, F.H., Kucuk, O.2004. The effect of genistein supplementation on performance and antioxidant status of Japanese quail under heat stress. Archive of Animal Nutrition 58, 463-471.

Ozaki, Y. 1990. Antiinflammatory effect of *Curcuma xanthorrhiza* Roxb. and its active principles. Chemical and Pharmaceutical Bulletin 38(4), 1045-1048.

Oznurlu, Y., Celik, I., Telatar, T., Sur, E. 2010. Histochemical and histological evaluations of the effects of high incubation temperature on embryonic development of thymus and bursa of Fabricius in broiler chickens. BritishPoultry Science 51, 43-51.

Packer, L., Weber, S.U., Rimbach, G. 2001. Molecular aspects of α-tocotrienol antioxidant action and cell signalling. The Journal of Nutrition. 131(2), 369S-373S.

Pamok, S., Aengwanich, W.,Komutrin, T. 2009. Adaptation to oxidative stress and impact of chronic oxidative stress on immunity in heat-stressed broilers. Journal of Thermal Biology. 34, 353-357.

Pastsart, U., De Boever, M., Claeys, E., De Smet, S. 2013. Effect of muscle and post-mortem rate of pH and temperature fall on antioxidant enzyme activities in beef. Meat Science 93, 681-686.

Pech-Waffenschmidt, V. 1992. The effect of heat stress conditions on performance, physiology, and blood chemistry of laying hens of different feathering types, and nutritional considerations to improve the heat resistance. PhD dissertation, Technische Universität, Berlin, Germany.

Pech-Waffenschmidt, V., Bogin, E., Avidar, Y., Horst, P. 1995.Metabolic and biochemical changes during heat stress in relation to the feathering degree of the domestic hen. Avian Pathology 24, 33-44.

Procházková, D., Boušová, I., Wilhelmová, N. 2011. Antioxidant and prooxidant properties of flavonoids. Fitoterapia 82, 513-523.

Puvadolpirod, S., Thanxon, J.P. 2000. Model of physiological stress in chickens 1. Response parameters. Poultry Science 79, 363-369.

Quinteiro-Filho, W.M, Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M.L., Sakai, M., Sá, L.R., Ferreira, A.J., Palermo-Neto, J. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science 89, 1905-1914.

Raes, K., De Smet, S., Demeyer, D. 2001. Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Journal of Animal Science 73, 253-260.

Rajasekaran, S., Sivagnanam, K., Subramanian, S. 2005. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacological Reports 57, 90-96.

Ramnath, A., Rekha, P.S. Sujatha, S. 2008. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by brahma rasayana. Evidence-Based Complementary and Alternative Medicine 5, 77-84.

Reisinger, N., Steiner, T., Nitsch, S., Schatzamyar, G., Applegate, T.J. 2011. Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. Journal of Applied Poultry Research 20, 272-283.

Rhodes, M.J. 1996. Physiologically-active compounds in plant foods: an overview. Proceeding of the Nutrition Society 55, 371-384.

Ricardo-da-silva, J., Laureano, O., Da Cost, M. 1993. Total phenol and proanthocyanidin evaluation of lupinus species. Proceedings of the 7th International Lupin Conference, Evora, pp. 250-254.

Roche, H., Boge, G. 2000. In vivo effects of phenolic compounds on blood parameters of a marine fish (*Dicentrarchus labrax*). Comparative Biochemistry and Physiology-Part C: Toxicology & Pharmacology 125, 345-353.

Rodrigo, R., Miranda, A., Vergara, L. 2011. Modulation of endogenous antioxidant system by wine polyphenols in human disease. Clinical Chimica Acta 412, 410-424.

Romijn, C., Lokhorst, W. 1961. Climate and poultry. Heat regulation in the fowl. Tijdschr. Diergeneeskd 86, 153-172.

Rozenboim, I., Tako, E., Gal-Garber, O., Proudman, J.A., Uni, Z. 2007. The effect of heat stress on ovarian function of laying hens. Poultry Science 86, 1760–1765.

Rudrappa, S.G., Humphrey, B.D. 2007. Energy metabolism in developing chicken lymphocytes is altered during the embryonic to posthatch transition. The Journalof Nutrition 137, 427-432.

Ruiz, P.A., Braune, A., Hölzlwimmer, G., Quintanilla-Fend, L., Haller, D. 2007. Quercetin inhibits TNF-induced NF-kappaB transcription factor recruitment to proinflammatory gene promoters in murine intestinal epithelial cells. The Journal of Nutrition 137(5), 1208-1215.

Rukayadi, Y., Yong, D., Hang, J.K. 2006. In vitro anticandidal activity of Xanthorrhizol isolated from Curcuma xanthorrhiza roxb. Journal of Antimicrobial Chemotherapy 57, 1231-1234.

Sahin, K., Sahin, N., Onderci, M.,Yaralioglu, S., Kucuk, S. 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamin E, A and some mineral concentration of broilers reared under heat stress. Veterinarni Medicina 46, 140-144.

Sahin, K., Kucuk, O., Sahin, N., Gursu, F.B. 2002. Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on performance, thyroid status, ACTH and some serum metabolite and mineral concentrations in broilers. Veterinary Medicine - Czech 47, 110-116.

Sahin, K., Kucuk, O. 2003a. Zinc supplementation alleviates heat stress in laying Japanese quail. The Journal of Nutrition 133, 2808-2811.

Sahin, K., Sahin, N., Kucuk, O. 2003b. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). Nutrition Research 23, 225-238.

Sahin. K., Onderci, M.,Sahin, N.,Gursu, M.F., Kucuk, O. 2003c. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. The Journal of Nutrition 133, 1882-1886.

Sahin, N., Onderci, M., Sahin, K., Gursu, M.F.,Smith, M.O. 2004a. Ascorbic acid and melatonin reduce heat-induced performance inhibition and oxidative stress in Japanese quails. British Poultry Science 45, 116-122.

Sahin, K., Onderci, M., Gursu, M.F., Kucuk, O., Sahin, N. 2004b. Effect of melatonin supplementation on biomarkers of oxidative stress and serum vitamin and mineral concentration in heat-stressed Japanese quail. Journal of Applied Poultry Research 13, 342-348.

Sahin, N., Onderci, N., Sahin, K.,Cikim, G., Kucuk, O. 2005a. Magnesium protection is more protective than magnesium oxide in heat-stressed quail. The journal of Nutrition 135, 1732-1737.

Sahin, K., Smith, M.O.,Onderci, M., Sahin, N., Gursu, M.F.,Kucuk, O. 2005b. Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. Poultry Science 84, 882-887.

Sahin, K., Onderci, M., Sahin, N., Gulcu, F., Yildiz, N.,Avci, M., Kucuk, O. 2006a. Responses of quail to dietary vitamin E and zinc picolinate at different environment temperatures. Animal Feed Science and Technology 129, 39-48.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F., Kucuk, O. 2006b. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass cgarecteristics in heat-stressed Japanese quail. Journal of Thermal Biology 31, 307-312.

Sahin, N., Onderci, M.,Sahin, K., Kucuk, O. 2008. Supplementation with organic or inorganic selenium in heat-stressed quail. Biological Trace Element Research 122, 229-237.

Sahin, K., Orhan, C., Tuzcu, M., Ali, S., Sahin, N., Hayirli, A. 2010. Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defence system via modulating hepatic nuclear transcription factors in heat-stressed quails. Poultry Science 89, 2251-2258.

Sahin, K., Orhan, C., Tuzcu, M., Borawska, M.H., Jablonski, J., Guler, O.,Sahin, N., Hayirli, A. 2013. Berberis vulgaris root extract alleviates the adverse effects of heat stress via modulating hepatic nuclear transcription factors in quails. British Journal of Nutrition 111, 609-616.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F., Kucuk, O. 2006. Effects of lycopene supplementation of antioxidant status, oxidative stress, performance and carcass charecteristics in heat-stressed Japanese quail. Journal of Thermal Biology 31, 307-312.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A., Prasad, A.S. 2009. Role of dietary zinc in heat-stressed poultry: A review. Poultry Science 88, 2176-2183.

Sahin, K., Sahin, N., Onderci, M., Gursu, F., Cikim, G. 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. Biological Trace Element Research 89, 53-64.

Sahin, K., Orhan, C., Tuzcu, M., Borawska, M.H., Jablonski, J., Guler, O., Sahin, N., Hayirli, A. 2013. Berberis vulgaris root extract alleviates the adverse effects of heat stress via modulating hepatic nuclear transcription factors in quails. British Journal of Nutrition 110, 609-616.

Sandercock, D.A., Hunter, R.R., Nute,G.R., Mitchell,M.A., Hocking, P.M. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: implications for meat quality. Poultry Science 80, 418-425.

Sandercock, M.A., Mitchell, M.A., Macleod, M.G. 1995. Metabolic heat production in fast and slow growing broiler chickens during acute heat stress. British Poultry Science 36, 868.

SAS., 2004: Statistical Analysis Systems; 9.1th edn. SAS institute inc., Raleigh, North Carolina, USA.

Sayago-Ayerdi, S.G., Brenes, A., Goni, I. 2009. Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. Lwt-Food Science and Technology 42, 971-976.

Schewe, T., Steffen, Y., Sie, H. 2008. How do dietary flavonols improve vascular function? A position paper. Archives of Biochemistry and Biophysics 476, 102-106.

Schrauwen, P., Xia, J., Bogardus, C., Pratley, R.E., Ravussin, E. 1999. Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. Diabetes 48, 146-149.

Sehm, J., Lindermayer, H., Dummer, C., Treutter, D., Pfaffi, M.W. 2007. The influence of polyphenol rich apple pomace or red-wine pomac diet on the gut morphology in weaning piglets. Journal of Animal Physiology and Animal Nutrition. 91, 289-296.

Seven, T.P., Seven, I., Yilmaz, M.M., Ugsims, E.K. 2008. The effects of turkish propolis on growth and carcass characteristics in broilers under heat stress. Animal Feed Science and Technology 146, 137-148.

Seven, T. P., Yalmiz, S., Seven, I., Cerci, I., Azman, M.A., Yilmaz, M. 2009. Effects of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. Acta Veterinaria 78, 75-83

Shanon, D.W.F., Brown, W.O. 1969. The period of adaptation of the fasting metabolic rate of the common fowl to an increase in environmental temperature from 22°C to 28°C. British Poultry Science 10, 13-18.

Shini, S., Kaiser, P., Shini, A., Bryden, W.L. 2008. Differential alterations in ultrastructural morphology of chicken heterophils and lymphocytes induced by corticosterone and lipopolysaccharide. Veterinary Immunology and Immunopathology 122, 83-93.

Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., Johnson, R., Du, Z. 2006. Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. Journal of Applied Microbiology 100, 296-305.

Singh, R.J. 2011. Genetic resources, chromosome engineering, and crop improvement: medicinal plants, volume 6. CRC Press. 1098 pp.

Sinurat, A.P., Balnave, D., Mcdowell, G.H. 1987. Growth performance and concentrations of thyroid hormones and growth hormone in plasma of broilers at high temperatures. Australian Journal of Biological Sciences 40, 443-450.

Sinurat, A.P., Purwadaria, T., Stars, I.A.K., Ketaren, P.P., Bermawie, N., Raharjo, M., Rizal, M. 2009. The utilization of turmeric and curcuma xanthorrhiza as feed additive for broilers. J IIimu Ternak Vet 14, 90-96.

Sirvydis, V., Bobiniene, R., Gudaviciute, D., Cepuliene, R., Semaska, V., Vencius, D., Kepaliene, I. 2006. Influence of a prebiotic feed additive on some biochemical indices of blood and intestinal microbiota of broiler chickes. Zemes Ukio Mokslai 4, 57-62.

Siriwardhana, N., Kalupahana, N.S., Cekanova, M., LeMieux, M., Greer, B., Moustaid-Moussa, N. 2013. Modulation of adipose tissue inflamation by bioactive food compounds. Journal of Nutritional Biochemistry 24, 613-623.

Smith, M.W., Mitchell, M.A., Peacock, M.A. 1990. Effects of genetic selection on growth rate and intestinal structure in the domestic fowl (*Gallus domesticus*). Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 97, 57-63.

Sohail, M.U., Rahman, Z.U. Ijaz, A., Yousaf, M.S. Ashraf, K. Yaqub, T. Zaneb, H. Anwar, H., Rehman, H. 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. Poultry Science 90, 2573-2577.

Song, Z., Zhu, L., Zhao, T., Jiao, H., Lin, H. 2009. Effect of copper on plasma ceruloplasmin and antioxidant ability in broiler chickens challenged by lipopolysaccharide. Asian-Australasian Journal of Animal Science 22, 1400-1406.

St-Pierre, N.R., Cobanov, B., Schnitkey, G. 2003. Economic losses from heat stress by US livestock industries. Journal of Dairy Science 86, E52-E77.

Steel, E.J., Kucera, K.E.,Ciccone, C.A., Connealy, M.E. 2008. Exercise and diabetes influence antioxidant activity and gene expression in female rats. Transactions of the Nebraska Academy Sciences and Affiliated Societies 31, 51-60.

Sujatha, V., Korde, J.P., Rastogi, S.K., Maini, S., Ravikanth, K., Rekhe, D.S. 2010. Amelioration of heat stress induced disturbances of the antioxidant defence system in broilers. Journal of Veterinary Medicine and Animal Health 23, 18-28.

Surai, K.P., Surai, P.F., Speake, B.K., Sparks, N.H.C. 2004. Antioxidant-prooxidant balance in the intestine: food for thought. 2. Antioxidants. Current Topics in Nutraceutical Research 2, 27-46.

Surai, P.F. 2014. Polyphenol compounds in the chicken/animal diet: from the past to the future. Journal of Animal Physiology and Animal Nutrition DOI: 10.1111/jpn.12070

Suzuki, Y.J, Packer, L. 1994. Inhibition of NF-kappa B transcription factor by catechol derivatives. International Journal of Biochemistry and Molecular Biology 32(2), 299-305.

Suzuki, S., Kikusato, M., Maekawa, T., Takahashi, K., Toyomizu, M. 2009. Anacardic acid attenuates TNFα/IFNγ-stimulated ROS production in chicken hepatocytes, probably via mild uncoupling action. International Conference on Lipid Hydroperoxide Biology and Medicine p. 76.

Taga, M.S., Miller, E.E., Pratt, D.E. 1984. Chia seeds as a source of natural lipid antioxidants. Journal of the American Oil Chemists 61, 928-931.

Tan, G.Y., Yang, L., Fu, Y.Q., Feng, J.H., Zhang, M.H. 2010. Effects of different acute high ambient temperatures on function of hepatic mitochondrial respiration, antioxidative enzymes, and oxidative injury in broiler chickens. Poultry Science 89, 115-122.

Tankson, H.D., Vizzier-Thaxton, Y., Thaxton, J., May, J. Cameron, J. 2001. Stress and nutritional quality of broilers. Poultry Science 80, 1384–1389.

Tao, X., Xin, H. 2003. Temperature-humidity-velocity index for market-size broilers. ASAE Annual Meeting. Las Vegas, USA. Paper nr. 034037.

Tarladgis, B.G., Watts, B.M., Younathan, M.T., Dugan, L. A. 1960. Distillation method for the quantitative determination of malondialdehyde in rancid foods. Journal of the American Oil Chemist's Society 37, 44-48.

Thring, S.A., Hili, P., Naughton, D.P. 2011. Antioxidant and potential anti-inflammatory activity of extracts and formulations of white tea, rose, and with hazel on primary human dermal fibroblast cells. Journal of Inflammation 8, 27.

Thompson, K.L., Applegate, T.J. 2006. Feed withdrawal alters small-intestinal morphology and mucus of broilers. Poultry Science 85, 1535-1540.

Tietz, N.W. 1995. Clinical guide to laboratory tests, 3th ed. WB Saunders Company, Philadelphia, PA, USA.

Tuzcu, M., Sahin, N., Karatepe, M., Cikim, G., Kiling, U., Sahin, K. 2008. Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. British Poultry Science 49, 643-648.

Vale, M.M., Moura, D.J., Naas, I.A., Pereira, D.F. 2010. Characterization of heat waves affecting mortality rates of broilers between 29 days and market age. Brizilian Journal of Poultry Science. 12(4). 279-285.

Van der Waaij, D. 1989. The ecology of the human intestine and its consequences for overgrowth by pathogens such as Clostridium difficile. Annual Review of Microbiology 43, 69-87.

Veldkamp, T., Kwakkel, R.P., Ferket, P.R., Verstegen, M.A. 2002. Impact of ambient temperature and age on dietary lysine and energy in turkey production. World's Poultry Science Journal 58, 475-491.

Vidal-Puig, A.J., Grujic, D.,Zhang, C.Y., Hagan, T.,Boss, O., Ido, Y.,Szczepanik, A.,Wade, J., Mootha, V., Cortright, R., Muoio, D.M.,Lowell, B.B. 2000. Energy metabolism in uncoupling protein 3 gene knockout mice. The Journal of Biological Chemistry 275, 16258-16266.

Vidanarachchi, J.K., Mikkelsen, L.L., Sims, I.M., Iji, P.A., Choct, M. 2006. Selected plant extracts modulate the gut microflora in broilers. Proc 18th Aust Poultry Science Symp, Sydney (Australia), Feb 20-22. pp, 145-148.

Vissers, M.N., Zock, P.L., Katan, M.B. 2004. Bioavailability and antioxidant effects of olive oil phenols in humans: a review. European Journal of Clinical Nutrition 58, 955-965.

Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C., Brenes, A. 2011.Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poultry Science 90, 566-578.

Vozza, A., Parisi, G., De Leonardis, F., Lasorsa, F.M., Castegna, A., Amorese, D., Marmo, R., Calcagnile, V.M., Palmeiri, L., Ricquier, D., Paradies, E., Scarcia, P., Palmieri, F., Bouillaud, F., Fiermonte, G. 2014. UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. Proceedings of the National Academy of Sciences of the United States of America 21; 111(3), 960-965.

Wang, Y.C., Chuang, Y.C., Hsu, H.W. 2008a. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in taiwan. Food Chemistry 106, 277-284

Wang, L., Piao, L., Kim, S.W., Piao, X.S., Shen, X.S. Lee, S. 2008b. Effects of Forsythia suspense extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. Poultry Science 87, 1287-1294.

Wang, R.R.., Pan,X.J., Peng, Z.Q. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of pectoralis major in broilers. Poultry Science 88, 1078-1084.

Ward, M.A., Peterson, R.A. 1973. The effect of heat exposure on plasma uric acid, lactate dehydrogenase, chloride, total protein and zinc of the broiler. Poultry Science 52, 1671-1673.

Westwood, J.T., Clos, J., Wu, C. 1991. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. Nature 353, 822–827.

Willemsen, H., Swennen, Q., Everaet, N., Geraert, P.A.,Mercier, Y., Stinckens, A., Decuypere, E. Buyse, J.2011. Effects of dietary supplementation of methionine and its hydroxyl analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperature. Poultry Science 90, 2311-2320.

Woisky, R.G., Salatino, A. 1998. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of Apicultural Research 37, 99-105.

Xu, H., Lohr, J., Greiner, M. 1997. The selection of ELISA cut-off points for testing antibody to Newcastle disease by two-graph receiver operating characteristic (Tg-Roc) analysis. Journal of Immunological Methods 208, 61-64.

Yahav, S. 2009. Alleviating heat stress in domestic fowls: different strategies. World's Poultry Science Journal 65(4), 719-732.

Yahav, S., Shamay, A.,Horev, G., Bar-Ilan, D., Genina, O., Friedman-Einat, M. 1997. Effect of acquisition of improved thermotolerance on the induction of heat shock proteins in broiler chickens. Poultry Science 76, 1428-1434.

Yahav, S., Mcmurty, J. 2001. Thermotolerance acquisition in broiler chickens by temperature conditioning early in life - the effect of timing and ambient temperature. Poultry Science 80, 1662-1666.

Yahav, S., Straschnow, A., Luger, D., Shinder, D., Tanny, J., Cohen, S. 2004. Ventilation, sensible heat loss, broiler energy, and water balance under harsh environmental conditions. Poultry Science 83, 253-258.

Yahav, S., Shinder, D., Tanny, J.Cohen, S. 2005. Sensible heat loss: The broiler's paradox. World's Poultry Science Journal 61, 419-434

Yalçin, S., Bruggeman, V., Buyse, J., Decuypere, E., Cabuk, M., Siegel, P.B. 2009. Acclimation to heat during incubation: 4. blood hormones and metabolites in broilers exposed to daily high temperatures. Poultry Science 88, 2006-2013.

Yamauchi, K., Yamamoto, K., Ishiki, Y. 1995. Morphological alterations of the intestinal villi and absorptive epithelial cells in each intestinal part in fasted chickens. The Journal ofPoultry Science 32, 241-251.

Yamauchi, K., Kamisoyama, H., Isshiki, Y. 1996. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. British Poultry Science 37, 909-921.

Yamauchi, K., Buwjoom, T., Koge, K., Ebashi, T. 2006. Histological intestinal recovery in chickens refed dietary sugar cane extract. Poultry Science 85, 645-651.

Yang, L., Tan, G.Y., Fu, Y.Q., Feng, J.H., Zhang, M.H. 2010. Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ROS production and lipid peroxidation in broiler chickens. Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology 151, 204-208.

Yanishlieva, N.V., Marinova, E.M., Gordon, M.H., Raneva, V.G. 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry 64, 59-66.

Yeh, C.T., Ching, L.C., Yen, G.C. 2009. Inducing gene expression of cardiac antioxidant enzymes by dietary phenolic acids in rats. The Journal of Nutritional Biochemistry 20, 163-171.

Young, D.S., 1995. Effects of drugs on clinical laboratory tests, 4th ed. AACC Press. Washington, DC, USA.

Zaidi, S., Al-Qirim, T.M., Banu, N. 2005. Effects of antioxidant vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver. Drugs in R&D 6, 157-165.

Zhou, L.Z., Johnson, A.P., Rando, T.A. 2001. NF kappa B and AP-1 mediate transcriptional responses to oxidative stress in skeletal muscle cells. Free Radical Biology and Medicine 31, 1405-1416.

Zulkifli, I., Norma, M.T.C., Israf, D.A., Omar, A.R. 2002. The effect of early-age food restriction on heat shock protein 70 response in heat-stressed female broiler chickens. BritishPoultry Science 43, 141-145.

CURRICULUM VITAE

Abdollah Akbarian was born on 22 September 1981 in Fasa, Iran. He obtained his Bachelor and Master of Science degrees in Animal Science (with Poultry Nutrition as a major) at Kurdistan and Shiraz University (Iran), respectively. In September 2009 he started his PhD at Ferdowsi University of Mashhad (Iran). After the completion of Introductory PhD Courses and examination (as an Honour Student), he received a-6-month grant from the Ministry of Science, Research, and Technology of Iran to continue his research work as an 'exchange student' at Ghent University. Upon finishing this period, he was awarded a fellowship grant from Ghent University (Belgium) to pursue and continue his PhD as a Joint Doctorate. Moreover, he was supported a grant from running projects and LANUPRO to continue and finalize his PhD.His previous research has resulted in 2 I.R. patents. He has been awarded several national and international grants for presenting at various congresses and workshops worldwide. He is author or co-author of several peer-reviewed publications. He can be reached by email at: aakbary60@yahoo.com.

PUBLICATIONS

Peer reviewed A1-publications

Akbarian, A., Michiels, J., Golian, A., Buyse, J., Wang, Y., De Smet, S. (2014) Gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, and meat quality of cyclically heat challenged finishing broilers fed *Origanum compactum* and *Curcuma xanthorrhiza* essential oils. Poultry Science (*in press*).

Akbarian, A., Golian, A., Kermanshahi, H., De Smet, S., Michiels, J. (2014) Antioxidant enzyme activities, plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and *Curcuma xanthorrhiza* essential oil. Journal of Animal Physiology and Animal Nutrition. DOI: 10.1111/jpn.12188.

Zhaleh, S., Golian, A., Mirghelenj, S. A., Akhavan, A., Akbarian, A. (2014) Effects of feeding various levels of full fat soybean extruded at high temperature on performance, serum components and intestinal morphology of broiler chickens. Animal Production Science. DOI: 10.1071/AN13457.

Michiels, J., Tagliabue, M. M., Akbarian, A., Ovyn, A., De Smet, S. (2014) Oxidative status, meat quality and fatty acid profile of broiler chickens reared under free-range and feed-restricted conditions as compared to conventional indoors. Avian Biology Research (*in press*).

Akbarian, A., Golian, A., Gilani, A., Kermanshahi, H., Zhaleh, S., Akhavan, A., De Smet, S., Michiels, J. (2013) Effect of feeding citrus peel extracts on growth performance, serum components, and intestinal morphology of broilers reared at high ambient temperature during the finisher phase. Livestock Science,**157**, 490-497.

Akbarian, A., Golian, A., Kermanshahi, H., Raji, A.R., Farhoosh, R., De Smet, S., Michiels, J. (2013) Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature. Spanish Journal of Agricultural Research,**11** (1), 109-119.

Missotten, J. A., Michiels, J., Dierick, N., Ovyn, A., Akbarian, A., De Smet, S. (2013) Effect of fermented liquid feed on performance, gut bacteria and histo-morphology and tibia bone characteristics in broilers. British Poultry Science,**54** (5), 627-634.

Akbarian, A., Kazerani, H. R., Mohri, M., Raji, A. R., Jamshidi, A., Golian, A. (2014) Exogenous melatonin improves growth performance, intestinal microbiota, and morphology in temporarily feed restricted broilers.Livestock Science (revised version submitted).

Shirzadi, H., Moravej, H., Shivazad, M., Akbarian, A. (2012) Effects of exogenous enzymes addition to wheat-based diets on growth and meat performance of male (Ross 308) broiler chickens. Animal Nutrition and Feed Technology,**12** (3), 325-334.

Akbarian, A., Golian, A., Sheikh Ahmadi, A., Moravej, H. (2011) Effect of ginger root (*Zingiber officinale*) on egg-yolk cholesterol, antioxidant status and performance of laying hens. Journal of Applied Animal Research,**39** (1), 19-21.

Sheikh Ahmadi, A., Golian, A., Akbarian, A., Ghaffari, M., Shirzadi, H., Mirzaee, M. (2010) Effect of extruded cotton seed and canola seed on unsaturated fatty acid composition in plasma, erythrocyte and liver of lambs. South African Journal of Animal Science,**40** (4), 311-318.

A2-publications

Zhaleh, S., Golian, A., Hassanabadi, A., Mirghelenj, S. A., Akhavan, A., Akbarian, A. (2014) Intestinal microflora and antioxidative status of broiler chickens fed extruded soybeans. International Journal of Applied Animal Sciences (*in press*).

Akbarian, A., Golian, A., Kermanshahi, H., Gilani, A., Moradi, S. (2012) Influence of turmeric rhizome and black pepper on blood constituents and performance of broiler chickens. African Journal of Biotechnology,**11** (34), 8606-8611.

Khayyat, M., Tehranifar, A., Akbarian, A., Shayesteh Nia, Sh., Khabari, S. (2009) Effects of Calcium Forms on Electrolyte Leakage, Total Nitrogen, Yield and Biomass Production by Strawberry Plants under NaCl Salinity. Journal of Central European Agriculture,**10** (3), 297-302.

Rowghani, E., Arab, M., Akbarian, A. (2007) Effects of a Probiotic and Other Feed Additives on Performance and Immune Response of Broiler Chicks. International Journal of Poultry Science,**6** (4), 261-265.

A4-publications

Goilan, A., Sedghi, M., Akbarian, A. (2011) The use of precipitated microcrystaltic waste of Mashhad power plant as a new calcium source for laying hens. Iranian Animal Science Researches 21 (2): 129-138.

CONFERENCES, WORKSHOPS, SEMINARS

2011

- The 5th Combined Workshop: Fundamental Physiology of the European Working Group of

Physiology and Perinatal Development in Poultry. August 31 - September 3. Wageningen, The Netherlands.

2012

- **Akbarian, A., Golian, A., Kermanshahi, H., Farhoosh, R., De Smet, S., Michiels, J.** Blood antioxidant and metabolic parameters in broilers fed lemon peel extract, orange peel extract and Curcuma xanthorrhiza essential oil, and subjected to heat stress. The 15thAAAP Animal Science Congress: 26-30 November. Banhkok, Thailand. *Oral presentation*

- **Akbarian, A., Golian, A., Kermanshahi, H., Farhoosh, R., De Smet, S., Michiels, J.** The effects of citrus peel extracts and Curcuma xanthorrizha essential oil on growth performance and intestinal morphology of broiler chickens during chronic heat stress. The 37thAnimal Nutrition Research Forum, April 18th. Wageningen, The Netherlands. *Oral presentation*

- **Tagliabue, MM., Akbarian, A., Michiels, J., Ovyn, A., De Smet, S.** The antioxidant system in broilers is altered under free-range conditions and fed restrictedly. XXIV World's Poultry Congress. 5-9 August. Salvador, Bahia, Brazil. *Poster presentation*

- **Akbarian, A., Golian, A., Kermanshahi, H., Farhoosh, R., Raji, AR., De Smet, S., Michiels, J.** Blood antioxidant and metabolic parameters in broilers fed lemon peel extract, orange peel extract and Curcumaxanthorrhizaessentialoil,andsubjectedtoheatstress. The 17thPhD symposium on Applied Biological Sciences; February 10th. Leuven, Belgium. *Poster presentation*

- **Tagliabue, MM., Akbarian, A., Michiels, J., Ovyn, A., De Smet, S.** Oxidative status in free-range broilers compared to conventional indoors reared chickens. The 17th PhD symposium on Applied Biological Sciences; February 10th. Leuven, Belgium.

- **Akbarian, A., Golian, A., Gilani, A., Kermanshahi, H., De Smet, S., Michiels, J., Zhaleh, S., Akhavan, A.** Evaluation of dietary citrus peel extracts to relieve the impact of heat stress in ross broiler chickens. The 3rd International Veterinary Poultry Congress: 22-23 February. Tehran, Iran. *Poster presentation*

- **Tagliabue, MM., Akbarian, A., Michiels, J., Ovyn, A., De Smet, S.** Oxidative status in free-range broilers compared to conventional indoors reared chickens. The 37th Animal Nutrition Research Forum, April 18th. Wageningen, The Netherlands. *Poster presentation*

- **Akbarian, A., Golian, A., Kermanshahi, H., Gilani, A., Moradi, S.** Effects of turmeric rhizome powder and black pepper on blood constituents and performance of male broiler chickens. The 100th annual meeting Poultry Science Association. 16-19 July. USA. *Poster presentation*

- International Symposium on 'Developments in Phosphorus nutrition in pigs and poultry'. June 14th. Centre for Animal Nutrition, Wageningen, the Netherlands.

2013

- **Akbarian, A., Michiels, J., Golian, A., De Smet, S.** Responses of broilers reared under hot conditions to dietary essential oils. The 17th European Society of Veterinary and Comparative Nutrition Congress: 19-21 September. Ghent, Belgium. *Oral presentation*

- **Akbarian, A., Michiels, J., Golian, A., Buyse, J., Wang, Y., De Smet, S.** Use of plant

extracts to reduce heat stress in broilers: involvement of HSP70 and antioxidant enzymes. The 38th Animal Nutrition Research Forum. May 21st. Roeselare, Belgium. *Oral presentation*

- **Akbarian, A., Michiels, J., Golian, A., Buyse, J., Wang, Y., De Smet, S.** Effects of plant-derived oils on HSP70 and antioxidant enzyme gene expression in broilers under high ambient temperatures. The 19th European Symposium on Poultry Nutrition: 26−29 August. Potsdam, Germany. *Poster presentation*

- **Akbarian, A., Michiels, J., Golian, A., De Smet, S.** Meat quality, oxidative status and performance of broiler chickens fed *Origanum compactum* and *Curcuma xanthorrhiza* essential oils and exposed to high ambient temperature. The 18th PhD symposium on Applied Biological Sciences; February 8th. Ghent, Belgium. *Poster presentation*

2014

- **Akbarian, A., Michiels, J., Golian, A., De Smet, S.** Fourteen days cyclic heat challenge and feeding *Origanum compactum* and *Curcuma xanthorrhiza* essential oils: effects on antioxidant system of broilers. The 19th PhD symposium on Applied Biological Sciences; February 7th. Liege, Belgium. *Oral presentation*

Conference proceedings

Akbarian, A., Golian, A., Gilani, A., Kermanshahi, H., Zhaleh, S., Akhavan, A., De Smet, S., Michiels, J. (2012). Evaluation of dietary citrus peel extracts to relieve the impact of heat stress in Ross broiler chickens. The 3rd International Veterinary Poultry Congress: February 22-23, Tehran, Iran.

Akbarian, A., Golian, A., Tahmasbi, M., Ghaffari, MH., Mirzaee, M. (2009). Effect of extruded cotton seed and canola seed on the composition of unsaturated fatty acids in plasma, erythrocytes and liver of mehraban male lambs. J. Dairy Sci. Vol. 93, E-Suppl. 1. P: 177-178.

Ghaffari, MH., Ghorbani, GR., Rahmani, HR., Yari, M., Ghafari, AH., Akbarian A., Mirzaee, M. (2009). Influence of two drying off methods on udder health in Holstein cows given short dry periods. XIth International Symposium on Ruminant Physiology, Clermont-Ferrand, France, P: 812-813.

Tagliabue, MM., Akbarian, A., Michiels, J., Ovyn, A., De Smet, S. (2012). The antioxidant system in broilers is altered under free-range conditions and fed restrictedly. World´s Poultry Science Journal, Supplement 1. Salvador - Bahia – Brazil.

Akbarian, A., Golian, A. H. Kermanshahi, A.R Raji, R. Farhoosh, S. De Smet, J. Michiels. (2012). Microbial changes in the ileal and caecal digesta of broilers fed lemon peel and orange peel extracts and *Curcuma xanthorrhiza* essential oil, and subjected to chronic heat stress. International Symposium on Alternatives to Antibiotics, Challenges and Solutions in Animal Production. Paris-France, September 25-28; Nr. 1.36; p 38.

Janssens, G.P.J., De Graeve, M., Tagliabue, M., Geda, F., Pauwels, J., Van Hecke, T., Akbarian, A., Michiels, J. (2012). Association between intestinal allometry, nutrient utilisation and growth in broiler chickens. In Proceedings of the 16th ESVCN Congress,

Bydgoszcz, Poland, nr. 08T.

National conferences (Iran)

Akbarian, A., Golian, A and Saleh, H. (2010). An overview on the effects of medical plants on animals immunity. National symposium on medical plants and their economic potential. Islamic Azad University of Birjand. Birjand, Iran. June 1-2.

Saleh, H., Golian, A and Akbarian, A. (2010). Application and benefits of medical plants in laying hens. National symposium on medical plants and their economic potential. Islamic Azad University of Birjand. Birjand, Iran. June 1-2.

Golian, A., Sedghi, M and Akbarian, A. (2010). The use of precipitated microcrystaltic waste of mashhad power plant as a new calcium source for laying hen. Proceedings of the 4th Iranian Congress on Animal Science. September 20-21.

Akbarian, A., Saleh, H., Tahmasebi, AM, Shirzadi, H and Ghaffari, SA. (2010). Effects of supplementary extruded canola and cotton seed on carcass characteristics and conjugated linoleic acid (CLA) content of muscle in Mehraban male lambs. Proceedings of the 4th Iranian Congress on Animal Science. September 20-21.

Akbarian, A., Sheikhahmadi, A., Golian, A, Shirzadi, H and Zhaleh, S. (2010). Effects of ginger root (*Zingiber officinale*) on Egg-Yolk Cholesterol, antioxidant status and performance of Laying Hen. Proceedings of the 4th Iranian Congress on Animal Science. September 20-21.

Shirzadi, H., Moravej, H., Shivazad, M., Akbarian, A., Maghsoudi, SM., Haghighat, M. (2010). Influence of Commercial Enzymes of Safizyme and Rovabio Excel in Wheat/Barley-Based Diets on Performance and Some Characteristics of Gastrointestinal Tract of Broiler Chicks. Proceedings of the 4th Iranian Congress on Animal Science. September 20-21.

Shirzadi, H., Moravej, H., Shivazad, M., Akbarian, A., Goldansaz, SA and Hedayat, N. (2010). Effect of Commercial Enzymes of Rovabio Excel and Safizyme on Water to Feed Ratio and Some Characteristics of Gastrointestinal Tract of Broiler Chicks Fed Wheat-Based Diets. Proceedings of the 4th Iranian Congress on Animal Science. September 20-21.

PATENTS

Abdollah Akbarian. Industrial intelligent device for identification and evaluation of diseases resulting from humidity increase such as *Coccidiosis* and *Aflatoxine* etc. The Judiciary Real Estates and Deeds Registration Organization. Industrial Ownership and Companies Registration General DEPT, Iran. Serial No. A/85-014784 - Declaration Registration Book No. 38610077 - Invention Registration Book No. 46464 - Date of Submission and Protection: 23/12/2007 - Date of Invention Registration: 23/02/2008.

Abdollah Akbarian, Mahdi Khayat and Morteza hosseini Ghaffari. Stair-Type cage system ground bed free for rearing broiler chickens. The Judiciary Real Estates and Deeds Registration Organization, Industrial Ownership and Companies Registration General DEPT, Iran. Serial No. A/87-004511 -Declaration Registration Book No. 38708458 - Invention Registration Book No. 54914 - Date of Submission and Protection: 02/11/2008 - Date of Invention Registration: 24/11/2008.

ACKNOWLEDGMENT

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"Thousands of candles can be lighted from a single candle, and the life of the candle will not be shortened. Happiness never decreases by being shared." (Buddha)

Ever since I started my M.Sc. studies in my home country, I hoped of one day joining an internationally renowned research lab abroad. Now that I am about to finish my PhD in April 2014 at Ghent University, I believe I have obtained what I dreamt for.I must confess all these achievements have been because of my supervisor in Iran, Prof. Dr. Abolghasem Golian, whose positive recomendation to Prof. Dr. ir. Stefaan De Smet allowed me to move abroad and start a new session of my life in Belgium at LANUPRO.

First and foremost, I would like to express my deep appreciation and thanks to my promoters Prof. Dr. Abolghasem Golian, Prof. Dr. ir. Stefaan De Smet and Dr. ir. Joris Michiels for all the enthusiastic guidance, encouragement, and invaluable advices throughout this PhD. I have learned from them an entirely new way of thinking, and that is something I will carry with me for the rest of my life. They have shown me what it means to work hard every single day. Appreciation and heartly thanks for their understanding, patience and suggestions during this research. Everything is because of them and I COULD NEVER REPAY THEM.

I would also like to thank the members of the reading and examination committee, Prof. Dr. ir. Monica Höfte (chairman), Prof. Dr. Daisy Vanrompay (secretary), Prof. Dr. ir. Johan Buyse, Dr. Anne Collin, Prof. Dr. Richard Ducatelle, Dr. Luc Maertens, and Prof. Dr. ir. Dirk Fremaut for the thorough reading of my manuscript and the critical comments and suggestions towards improving my thesis.

I would also like to extend my gratitudes to all my colleaques at Ferdowsi university of Mashhad and LANUPRO, Saeid Yaghfoori, Ali Gilani, Seyyed Javad Hosseini, Javad Agah,Hassan Saleh, Taher Mirakzehi, Mohammad Sedghi, Safa Zhaleh, Avisa Akhavan,

Samira Hassan pour, Ann, Joris, Jeroen, Sabine, Thomas, Tessa, Jana, Marta, Geoffrey, Nympha, Hong, Alberto, Wasseem, Wei, Sisitorn, Joaquin, Fredrik, Marlene, Daisy, Erik, Charlotte, Annick, Allex, for their great helpfulness and friendship. These are the people I have lived with and the people I have worked with. They are the people I play badminton and football with and the people I go swimming with. Working with them made past few years in the lab rather enjoyable. I also would like to thank our Iranian community in Ghent and its members Mohammad Masoudi, Ali Esmaeilpour, Seyfollah Soleimani, Ebrahin Hosseini, Amir Fardis, Mahdi Shmas, Hadi Mosallanejad, Meysam Rezaei, Siamak Ghaffaripour, Hossein Azadi, Reza Poureslami, Reza Barekatian and other friends that I migh have forgotten to include them in the list.

My lovely family – mom, Hamideh, mother in law, Jamileh, dad, Hassan, father in law, Ali Asghar, brothers and sisters, words cannot express how much I love you all and how grateful I am for your love, encouragement and support, without which I most certainly would not be where I am today. While living and working in Belgium, I have been distanced from them. And yet, there has not been a single day in which I have felt alone these past four years, because their love and support can be felt hundreds of miles away, without words, and without fail.

My very special appreciation goes to my beloved wife, Mozhgan and my cute son, Padra for their kind patience through my tenure which has been as a Marathon Race for me. I believe this thesis would not have been possible to finish without your patience, sacrifice, help and support. At the last step of my PhD, we were far from each other but of course very closely in heart. Padra just started to speak, who knows how much I loved to be with him at that time. I cannot describe my feelings how hard it was while we were not together for a long time...

Abdollah Akbarian (Pooya)

Melle, 12 May 2014

'The only way to do great work is to love what you do'

Steve Jobs