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Effects of rearing system and vitamin E on the performance and meat quality of Kabir broiler chickens.

Biochemical parameters in the blood and meat quality of white hybrid XL turkeys.

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ABSTRACT

Animal's welfare is increasingly viewed as a factor affecting the quality of animal products while being an important tool of marketing strategy. A survey of consumers, showed that they prefer to purchase food products obtained from livestock animals raised in production systems that are considered more animal welfare friendly, such as free-range systems. The environment' elements to which free-range poultry birds are exposed affect the performance of the birds and quality of their meat. Available literature shows that the chickens reared under free-range breeding systems have total protein content higher than the chickens bred in confinement breeding system. Birds reared outdoor can have also significantly higher microelements. Moreover, the reduced content of fat and elevated polyunsaturated fatty acids content in muscles of free-range broilers were observed. However, high degree of unsaturation of intramuscular lipids results in shorter shelf-life. Thus, it is necessary to highlight the role of antioxidants, such as vitamin E. Mentioned vitamin plays a major role as a chain-breaking antioxidant of the membranes. Meat quality can be affected also by other factors. Among internal factors that substantially affect certain meat quality traits, poultry muscle type plays an pivotal role. The chemical composition of muscle tissue of major primal cuts is an important element of broiler meat quality. Moreover, the fundamental role in the maximization of animals productivity plays constant monitoring of their health state- one of the criteria for welfare assessment. Essential to controlling the health status of animals is testing of physiological indicators (e.g. biochemical parameters of blood serum).

Aim of the first experiment was to evaluate the effects of rearing system (outdoor *versus* indoor) and of DL-α-tocopheryl acetate single intramuscular injection on carcass traits and meat quality of slow-growing broilers (Kabir).

This study was carried out on the farm located in the countryside of Bonefro (Italy). For the experiment were used sixty Kabir male chicks. Animals were separated into two groups: indoor and outdoor. At 84 d of age, half of examined chickens received, into the right-side breast fillet, single intramuscular injection with of DL-α-tocopheryl acetate (Vit E group). Kabir chickens assigned to the control group received injection of physiological saline. Chickens were slaughtered at 94^{th} day of age. Animals were individually weighted just before slaughter for the final body weight

determination. The weights of carcass, breast muscle, legs, wings and back+neck were measured and their percentage was calculated based on carcass weight. Muscle pH, water-holding capacity (WHC) and color were measured at 24h *post-mortem*. Intramuscular collagen (IMC) content, fatty acid (FA) composition, total lipid level, and lipid stability were evaluated in breast muscle. Birds reared indoor were heavier that those reared outdoor ($P < 0.05$). Also value of the breast weight ($P < 0.01$) was higher for birds reared indoor in comparison to the group with outdoor access. Moreover, the birds in the free-range treatment showed lower ($P < 0.01$) wings weight and yield than birds in the indoor treatment. Presented study revealed no vitamin E effect on growth and most carcass traits. Chickens from control group were characterized by higher ($P < 0.01$) value of wing yield than those from Vit E group. Regarding, the physicochemical properties of meat, access to outdoor reduced ($P < 0.01$) the values of L* (lightness) and increased ($P < 0.05$) the value of yellowness (b*). While, vitamin E reduced ($P < 0.01$) the value of a* (redness) parameter and increased ($0.01 > P < 0.05$) the value of L* and b*. The effect of rearing system and vitamin E on fatty acids profile was described as well. Outdoor access have no effect on total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Quantitatively, the palmitic acid (C 16:0) was the most concentrated SFA. While, among MUFA the most abundant was oleic acid (C 18:1 n- 9). The linoleic acid (C 18:2 n-6) was the most concentrated PUFA. Taking into account PUFA, the content of eicosatrienoic acid (C 20:3 n-3) was influenced by the outdoor access, resulting in lower level ($P < 0.05$) in meat from free-range chickens. The Σn-3 PUFA, n-6/n-3 and n-3/n-6 ratios were also affected by rearing system. Considering vitamin E effect, birds of control group were characterized by lower ($P < 0.05$) concentration of myristic acid (C 14:0) than birds from Vit E group. Levels of total PUFA, the eicosatrienoic acid (C 20:3 n-3), arachidonic acid (C 20:4 n-6) and eicosapentaenoic acid (C 20:5 n-3) were higher ($P < 0.05$) in breast muscles of birds from control group than that in muscles of birds injected with vitamin E. Vitamin E significantly ($0.05 > P < 0.01$) reduced Σn-6 PUFA, Σn-3 PUFA and PUFA/SFA ratio. Moreover, the level of TBARS was higher ($P < 0.01$) for outdoor chickens in comparison to indoor birds.

This research contributes to extend existing knowledge on free-range broiler chickens by providing new data regarding their performance and meat quality trait in comparison to birds reared indoor. Presented study have confirmed the effect of the increased physical activity of birds, and the natural pigments present in the plant

material on slaughter and poultry meat quality traits. Moreover, the current research showed the effect of intramuscular injection of the vitamin E on meat quality traits (color and fatty acid profile) of Kabir broiler chickens. While, insignificant effect of examined vitamin on growth and carcass traits of Kabir chickens may be connected with the inadequate amount and/or mode of administration of dl-α-tocopheryl acetate.

The main goal of second experiment was to evaluate the effects of muscle type on chemical composition and nutritional properties of turkey meat. A secondary objective was to assess some blood serum biochemical parameters of turkeys. Ten broad-breasted female white turkeys hybrid XL were used in trial. During slaughter the blood of birds was collected from jugular vein and after all serum was separated. The concentrations of serum parameters: glucose (Glu), triglycerides (Tg), total cholesterol (Chol), total protein (TP), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in blood serum of turkeys were analysed. After slaughter of turkeys, breast and leg muscles of eight individuals chosen randomly, were collected for future analysis. Dry matter, crude protein, fat, ash, metabolizable energy, intramuscular collagen content, macro- and microelements level, fatty acid composition, amino acids profile were evaluated in breast and leg muscle of turkeys.

The Glu concentration in turkeys was 13.06±0.45 mM/L. While, the mean serum Tg content in birds of presented research was 0.55±0.07 mM/L. The serum Chol and TP level in examined turkeys was 3.29±0.38 mM/L and 39.99±4.1 g/L, respectively. In our study, serum Ca concentration in turkeys was 2.86±0.11 mM/L. While, the mean serum P level was 2.00±0.12 mM/L. In the present research, serum Mg content in turkeys was 0.76±0.09 mM/L. The level of Na and K in turkey blood serum was 150.93±1.71 mM/L and 5.24±0.30 mM/L, respectively. Regarding, the activity of selected enzymes, the activity of aspartate aminotransferase and alanine aminotransferase in turkeys was 7.32±2.59 μkat/L and 0.25±0.03 μkat/L, respectively. Among evaluated in our study enzymes was also alkaline phosphatase, that activity in turkey serum was 33.12±4.33 μkat/L. The effect of muscle type on chemical composition of turkey meat was observed. Crude protein content was higher in turkeys breast muscles than in leg muscles ($P < 0.01$), while in case of crude fat level the opposite tendency ($P < 0.01$) was reported. Energy level was markedly ($P < 0.05$) influenced by the muscle type; value of this trait was higher for leg muscles than that of breast muscles. Leg muscle was distinguished by markedly higher ($P < 0.01$) content of intramuscular collagen in

comparison to breast muscle. The mean mineral composition was also affected by the muscle type. Phosphorus content was higher ($P < 0.01$) in leg muscle in comparison to breast muscle. The opposite tendency was observed in case of magnesium concentration ($P < 0.01$). Moreover, in presented study the leg muscle was distinguished from breast muscle by significantly higher ($P < 0.01$) content of copper, iron and zinc (+51.5%, 143.6% and 178.7%, respectively). The fatty acids profile of both turkey muscles was evaluated. In our research, the fatty acid profile exhibits a dominance of two classes: the MUFA and the SFA; the third position belongs to the PUFA. Lauric (C 12:0) and myristic (C 14:0) acids contents were markedly higher in leg muscles than that in breast muscles ($P < 0.01$; $P < 0.05$, respectively). Muscle type affected the total MUFA amount that was higher for breast muscle compare to leg muscle ($P < 0.05$). Moreover, breast muscle was characterized by higher ($P < 0.05$) palmitoleic acid content (C 16:1) than the other investigated muscle. Total PUFA amount was higher ($P < 0.05$) in leg muscle than in breast muscle. The muscle type markedly affected the linoleic acid (C 18:2 n-6) content that was higher ($P < 0.05$) in leg muscle when compared to that in breast muscle. Quantitatively, the palmitic acid (C 16:0) was the most concentrated SFA (24.95-25.12%). The most abundant MUFA was the oleic acid (C 18:1 n-9), while in case of PUFA it was the linoleic acid (C 18:2 n-6). In the current research, levels of Σn-6 PUFA was higher in leg muscles in comparison to breast muscles ($P < 0.05$). The n-6/n-3 ratio was significantly lower in white meat than that in dark meat of thighs ($P < 0.05$). The muscle type influenced some of non-essential (NEAA) and essential amino acids (EAA). Levels of asparagine and tyrosine contents were higher ($P < 0.01$) in breast muscle than that in leg muscle. Moreover, turkey breast muscle was characterized by higher content of alanine in comparison to leg muscle ($P < 0.05$). In the current research, breast muscle compared with leg muscle showed higher content of valine, leucine, threonine, lysine ($P < 0.05$) and of histidine and methionine ($P < 0.01$).

Results of the second experiment completing existing knowledge regarding level of biochemical parameters of blood serum in poultry species. Moreover, the results of this research confirmed that muscle type markedly influences the chemical composition and the nutritional value of turkey meat.

Concluding, results of both experiments indicated the potential impact of some extrinsic (the rearing system, intramuscular injection of vitamin E) and intrinsic (the muscle type) factors on performance and poultry meat quality traits. Although the

treatment effects noted in this study were subtle, further research is warranted to elucidate the effect of vitamin E on chicken growth.

RIASSUNTO

Il benessere degli animali è visto sempre più spesso come un fattore in grado di influire sulla qualità dei prodotti di origine animale. Le opinioni dei consumatori indicano una preferenza per l'acquisto di alimenti provenienti da animali allevati in sistemi di produzione ritenuti capaci di garantire un maggior benessere (ad es. allevamento all'aperto). La bibliografia disponibile indica che i polli allevati all'aperto (sistema "free range") producono carne con un maggior contenuto di proteine e minerali, rispetto a quella proveniente da allevamenti intensivi; nonché, una minore quantità di grassi e un maggior contenuto di acidi grassi polinsaturi. Tuttavia, l'elevato grado di insaturazione degli acidi grassi potrebbe avere un effetto negativo sulla shelf life della carne. Per questo, è indispensabile sottolineare il ruolo degli antiossidanti, come la vitamina E. La qualità della carne dipende anche da altri fattori. Tra gli elementi che influiscono sulle caratteristiche qualitative della carne di pollame, il tipo di muscoli riveste un ruolo di primo piano. Per ottimizzare la produttività di un allevamento, è assai importante monitorarne lo stato di salute degli animali. Uno dei criteri per il controllo dello stato di salute degli animali consiste nella determinazione dei parametri chimico clinici del sangue.

L'obiettivo del primo studio è consistito nella valutazione degli effetti del sistema di allevamento e della somministrazione intramuscolare di vitamina E sulle caratteristiche della carcassa e della qualità della carne di polli di razza Kabir, genotipi a crescita lenta. Lo studio è stato effettuato presso un'azienda agro-zootecnica situata in agro di Bonefro (Italia). Per l'esperimento sono stati utilizzati sessanta pulcini Kabir di sesso maschile. Gli animali sono stati divisi in due gruppi sperimentali di egual numero: allevati a terra con possibilità di libero movimento all'aperto (O); allevati a terra senza possibilità di libero movimento all'aperto (I). All'età di 84 giorni, metà dei polli di ciascun gruppo hanno ricevuto, a livello del filetto destro del muscolo pettorale, un'iniezione di vitamina E (50 U.I di α-dl-tocoferil acetate, Vitalene® E, Fatro, Bologna). Gli animali appartenenti al gruppo di controllo hanno ricevuto un'iniezione di soluzione fisiologica. I polli prima della macellazione (94° giorno d'età) sono stati individualmente pesati, previo digiuno di 12 ore. Alla macellazione è stata pesata la carcassa e calcolata la resa alla macellazione. Inoltre, la carcassa è stata sezionata in tagli (petto, cosce, ali, parte posteriore) e calcolate le rispettive incidenze percentuali. A

24 h dall'abbattimento è stato misurato il pH, il colore (L*, a*, b*) e la capacita di ritenzione idrica del muscolo pettorale. Inoltre, sul muscolo pettorale sono state effettuate le seguenti analisi: contenuto del collagene totale e del grasso, profilo acidico e degradazione ossidativa del grasso. Gli animali non allevati in regime di allevamento all'aperto sono risultati più pesanti ($P < 0.05$) e con un muscolo pettorale più grosso ($P < 0.01$) rispetto a quelli cresciuti con l'accesso all'esterno. I polli Kabir allevati all'aperto hanno evidenziato un peso inferiore ($P < 0.01$) delle ali e della loro incidenza percentuale ($P < 0.01$) rispetto ai capi allevati convenzionalmente. La somministrazione della vitamina, anche se ha marcatamente ridotto ($P < 0.01$) l'incidenza del taglio delle ali, nel complesso non ha avuto effetti particolarmente significativi sulle caratteristiche della carcassa. La possibilità di movimento all'aperto ha ridotto ($P < 0.01$) il valore del parametro luminosità (L*) e ha aumentato ($P < 0.05$) quello dell'indice del giallo (b*). Allo stesso tempo, la vitamina E ha ridotto ($P < 0.01$) il valore dell'indice del rosso (a*) ed ha aumentato ($0.05 > P < 0.01$) quello dei parametri L* e b*. Il pH_{24} non è stato influenzato significativamente ne dal sistema di allevamento ne dal trattamento con vitamina E; i valori riscontrati sono compresi in un range di normalità della carne di pollo.

Significative interazioni ($0.05 > P < 0.01$) tra la tecnica di allevamento e il trattamento intramuscolare di Vit. E sono risultate per alcune caratteristiche della carcassa e della carne.

La concentrazione del collagene intramuscolare non ha risentito significativamente di entrambi i fattori sperimentali studiati.

L'allevamento all'aperto non ha influito sulla quota totale di acidi grassi saturi (SFA), monoinsaturi (MUFA) e polinsaturi (PUFA). Dal punto di vista quantitativo, l'acido palmitico (C 16: 0) è stato l'acido saturo più concentrato. Tra gli acidi MUFA, il più abbondante è stato quello oleico (C 18:1 n-9). L'acido linoleico (C 18: 2n-6) è stato il più concentrato tra i PUFA. Per quanto concerne i PUFA, il contenuto di acido eicosatrienoico (C 20:3 n-3) è risultato più basso ($P < 0.05$) nei polli con accesso all'esterno. Anche il contenuto totale n-3 PUFA e gli indici nutrizionali, n-6/n-3 e n-3/n-6, hanno subito l'influenza del sistema di allevamento ($P < 0.05$); Anche la vitamina E ha influenzato il profilo degli acidi grassi. La concentrazione dell'acido miristico (C 14:0) è risultata più elevata ($P < 0.05$) nel gruppo Vit. E rispetto al gruppo non trattato. Al contraio, la concentrazione degli acidi eicosatrienoico (C 20: 3 n-3), arachidonico (C 20: 4 n-6) e eicosapentaenoico (C 20:5 n-3), nonché la quantità totale dei PUFA, n-6

PUFA e n-3 PUFA ed il rapporto PUFA/SFA sono risultate significativamente ($0.05 > P < 0.01$) più elevate nella carne del gruppo non trattato rispetto al gruppo Vit. E. Inoltre, il livello di TBARS è stato superiore ($P < 0.01$) negli animali allevati a terra. L'ossidazione dei lipidi della carne (TBARS), dopo un mese di congelamento della stessa, è risultato più elevato ($P < 0.01$) nella carne degli animali con accesso all'esterno rispetto a quelli senza accesso. Tuttavia, va evidenziato che i valori riscontrati dei TBARS (mg MDA/kg) sono risultati molto contenuti. Lo studio effettuato perme di arricchire le conoscenze sull'allevamento di broiler a terra, fornendo nuovi dati relativi al rendimento e alla qualità della carne. I risultati ottenuti hanno evidenziato, inoltre, l'influenza dell'iniezione intramuscolare di vitamina E sulle caratteristiche qualitative della carne (colore della carne e profilo degli acidi grassi) dei polli Kabir.

L'obiettivo del secondo studio è stato quello di valutare alcuni parametri chimico clinici del sangue di tacchini, nonché l'effetto muscolo sulla composizione chimica e nutrizionale della carne. Per questo studio sono stati utilizzati 10 pulcini di tacchino bianco grande (Hybrid XL). Durante l'abbattimento, è stato prelevato dalla vena giugulare il sangue, dopodiché si è provveduto a separare il siero per le analisi riguardanti: glucosio (Glu), trigliceridi (TG), colesterolo totale (Chol), proteine totali (TP), calcio (Ca), fosforo (P), magnesio (Mg), sodio (Na), potassio (K), aspartato aminotransferasi (AST), alanina aminotransferasi (ALT), fosfatasi alcalina (ALP). Dopo l'abbattimento, da otto carcasse sono stati prelevati campioni muscolari dal petto e dalle cosce per le analisi che hanno riguardato: la sostanza secca, le proteine grezze, il grasso, l'energia metabolica, il collagene totale, i micro- e macroelementi, il profilo degli acidi grassi e degli amminoacidi.

I valori medi della concentrazione dei parametri chimico clinici del sangue dei tacchini sono di seguito riportati:

Glu =13.06±0.45 mM/L;
Tg = 0.55±0.07 mM/L;
Chol = 3.29±0.38 mM/L;
TP = 39.99±4.1 g/L;

Na = 150.93±1.71 mM/L;
Ca = 2.86±0.11 mM/L;
P = 2.00±0.12 mM/L;
Mg = 0.76±0.09 mM/L;
K = 5.24±0.30 mM/L.

AST = 7.32±2.59 μkat/l;
ALT = 0.25±0.03 μkat/l;
ALP = 33.12±4.33 μkat/L;

La composizione chimica della carne ha risentito dell'effetto muscolo. Il contenuto di proteina è risultato più elevato ($P < 0.001$) nel petto rispetto alla coscia. Il contenuto in grasso, invece, è risultato più elevato ($P < 0.001$) nella coscia e di conseguenza, sempre nella stessa, anche la quantità di energia ($P < 0.05$). Inoltre, i muscoli della coscia hanno evidenziato un maggior contenuto di collagene totale ($P < 0.01$). Relativamente al contenuto dei minerali, il fosforo è risultato più elevato ($P < 0.01$) nella coscia, mentre il magnesio più elevato ($P < 0.01$) nel petto. Inoltre, i muscoli delle zampe si sono contraddistinti per un maggior ($P < 0.001$) contenuto di rame, ferro e zinco (+ 51,5%, 143,6% e 178,7%, rispettivamente).

Anche la composizione lipidica della carne è stata influenzata dal tipo di muscolo. La quantità totale di MUFA è stata più elevata ($P < 0.05$) nei muscoli pettorali rispetto a quelli della coscia. Il petto, inoltre, ha evidenziato un maggior livello ($P < 0.05$) di acido palmitoleico. La concentrazione totale dei PUFA, all'opposto, è stata più elevata ($P < 0.05$) nei muscoli della coscia. Diversamente, la quantità totale degli SFA non è stata influenzata dal tipo di muscolo. Relativamente alla concentrazione dei singoli acidi grassi, solo l'acido linoleico (C 18: 2n-6) è risultato più elevato ($P < 0.05$) nella coscia rispetto al petto. Dal punto di vista quantitativo, l'acido palmitico (C 16: 0) è stato l'acido saturo più concentrato (24.95-25.12%), tra i MUFA l'acido oleico (33.14-34.29%), mentre nel caso dei PUFA l'acido arachidonico (0.62-0.78%). La concentrazione totale degli Σn-6 PUFA è stata più elevata ($P < 0.05$) nei muscoli della coscia rispetto a quelli pettorali. Il petto ha inoltre mostrato un valore più basso degli indici n-6/n-3 ($P < 0.05$) e PUFA/SFA ($P = 0.059$).

Per quanto concerne il contenuto degli amminoacidi, il petto ha mostrato un maggior contenuto di asparagina e tirosina ($P < 0.01$) nonché di alanina ($P < 0.05$). I muscoli pettorali, rispetto a quelli delle zampe, hanno mostrato un maggior contenuto di amminoacidi essenziali, quali valina, leucina, treonina, lisina ($P < 0.05$), istidina e metionina ($P < 0.01$).

I risultati di questo studio integrano le conoscenze già esistenti sul livello dei parametri biochimici presenti nel siero ematico dei tacchini. Ha confermato, inoltre, l'influenza del tipo di muscolo sulla composizione chimica e pertanto sul valore nutrizionale della carne di tacchino.

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4-HNE 4-hydroxynonenal

AA Amino acids

AI Atherogenic index

Ala Alanine

ALA α-linolenic acid

ALP Alkaline phosphatase

ALT Alanine aminotransferase

AP Acidification potential

Arg Arginine

Asp Asparagines

AST Aspartate aminotransferase

ATP Adenosine triphosphate

BHA Butylated hydroxyanisol

BHT Butylated hydroxytoluene

Ca Calcium

CF Crude fibre

Chol Cholesterol

Cl Chlorine

CP Crude protein

Cys Cysteine

deoxyMb Deoxymyoglobin

DFD Dark-Firm-Dry

DHA Docosahexanoic fatty acid

DM Dry matter

EAA Essential amino acids

EFAs Essential fatty acids

EP Eutrophication potential

EPA Eicosapentaencic fatty acid

EU European Union

F Crude fat

FA Fatty acid

FAO Food and Agriculture Organization of the United Nations

FCR Feed Convertion Ratio

Fe Iron

FID Flame ionisation detector

GC Gas chromatography

GLM General Linear Model

Glu Glutamine

Gly Glycine

GSH-Px Glutathione peroxidase

GWP Global worming potential

HDL High-density lipoprotein

heFe Haem iron

His Histidine

HLP Hydroxylysylpyridinoline

HMGR 3-hydroxy-3-methyl glutarylCoA reductase enzyme (

HPLC High-performance (high-pressure) liquid chromatography

Ile Isoleucine

IMC Intramuscular collagen

IPP Isopentenyl pyrophosphate

K Potassium

LA Linoleic acid

LDL Low-density lipoprotein

Leu Leucine

LPL Lipoprotein lipase

Lys Lysine

MDA Malondialdehyde

MEN Metabolisable energy

Met Methionine

metMb Metmyoglobin

Mg Magnesium

MRP Maillard reaction products

MUFAs Monounsaturated

Na Sodium

NFE Nitrogen free extract

nheFe Non-haem iron

oxyMb Oxymyoglobin

P Phosphorus

PG Propyl gallate

PGE2 Prostaglandin E2

Phe Phenylalanine

Pro Proline

PSE Pale, Soft, Exudative

PUFAs Polyunsaturated fatty acids

ROS Reactive oxygen species

RS Rearing system

Se Selenium

SE Standard error

Ser Serine

SFAs Saturated fatty acids

SOD SuperOxide Dismutase

T Treatment

T.s. Total sugars

TBA Thiobarbituric acid

TBARS Thiobarbituric Acid Reactive Substances

TBHQ Tertiary butyl hydroquinone

Tg Triglycerides

Thr Threonine

TI Thrombogenic index

TSAA Total sulfur amino acids

Tyr Tyrosine

US United States

Val Valine

VLDL Very low density lipoproteins

WHC Water-Holding Capacity

WHO World Health Organization

WOF Warmed-over flavor

Zn Zinc

PART I: INTRODUCTION (Literature review)

CHAPTER I

POULTRY MEAT PRODUCTION, CONSUMPTION AND TRADE

Among different types of meat, poultry meat plays a key role and become a mass consumer product throughout the world: in every region, in countries with very different level of development, and diverse forms (Magdelaine et al., 2008). Moreover, poultry sector has become highly dynamic, particularly in developing countries that are evolving in response to rapidly increasing demand for animal products (Mengesha, 2013). The fact that poultry meat production has maintained its recent steady upward trend is influenced by very good conversion of feed (low FCR) per kg gain, which results from the rapid initial growth of chickens, the adaptation of diet composition to meet chicken needs, the improvement of environmental conditions, and the intensification of production to reduce production costs (Kokoszyński et al., 2013).

1.1. Production of poultry meat

Global poultry meat production increased from almost 54.2 million tonnes in 1995 to 107.0 million tonnes in 2013 (Scanes, 2007; FAO, 2014a), and is expected to increase by over 181 million tonnes to 2050 (Alexandratos and Bruinsma, 2012). Worldwide no other agricultural product reached such a remarkable relative growth rate. Among countries leading in poultry meat production are United State, China, Brazil, Russian Federation. For the special attention deserve also the EU- one of the world's top producers in poultry meat and a net exporter of poultry products. The leading European countries in poultry meat production are Poland (13.7 %), France (12.7 %), closely followed by UK (12.4 %), Germany (11.4 %) and Spain (11.1 %). These five countries ensure 61.3% of the EU production of poultry meat (http://ec.europa.eu/agriculture/poultry/index_en.htm).

1.1.1. Chicken broiler

The most common sources of poultry meat are popular domestic Galliformes, such as: chickens and turkeys (respectively: 87% and 6% of total poultry production). However, other commercially available poultry meats come from ducks, geese, pigeons, quails, pheasants, ostriches and emus. As was above-noted, chicken meat has maintained its place as world's most popular meat, usually ahead of all red meats, and its popularity is expected to continue, according to a recent international agricultural reports. The United States is the world's largest producer of broiler meat, followed by China, Brazil, and the EU-27. Broiler production in the United States in 2015 was 17.971 million tones. In China, production is decreased last year to 13.40 million tones in 2015 due to tighter margins on higher feed costs and lower prices. In the EU-27 in 2015 slaughtered broiler chickens produced around 10.620 million tonnes of chicken meat (Index Mundi, 2016). In the European Union, the largest producer of chicken meat is UK (USDA, 2013a). Brazil broiler production of broiler meat in 2015 was 13.146 million tones (Index Mundi, 2016).

1.1.2. Turkey

After chicken broiler, the important position in poultry meat production occupy turkeys. Leadership in production of turkey meat belong to North America. In the United States and Canada, turkey meat is the second most important poultry meat consumed; in other countries turkey meat is less important. Interest in turkey meat in North America still growing; U.S. turkey meat production in August 2014 was 484 million pounds. The number of turkeys slaughtered was 20.1 million; the average live weight at slaughter was 30 pounds (1.1% higher than year before) (USDA, 2014a). The EU has second position in turkey meat production; among the top European producing turkey meat countries are France, Germany, Italy and Poland (Table 1.1) (avec, 2014). Except American and European market on attention deserve also African market, especially the North African which increased dramatically due to increased financial investment and a shift in affluence (Aviagen, 2015). Meanwhile in Asia, in contrast to chicken meat, turkey meat production has minor importance. Consumption of turkey meat has no tradition on this continent (Windhorst, 2011).

Table 1.1. Turkey production in the EU (‘000 tons carcass weight) during present decade (avec, 2014).

Country	2010	2011	2012	2013
Austria (10)*	24	25	26	27
Belgium/Luxemburg (17)	4	3	3	3
Bulgaria	0	0	0	0
Croatia (15)	6	5	6	6
Cyprus (18)	1	1	1	1
Czech Republic (18)	4	4	8	1
Denmark	0	0	0	0
Finland (14)	9	8	8	7
France (1)	409	406	415	386
Germany (2)	434	398	392	385
Greece (17)	3	3	3	3
Hungary (7)	100	101	95	89
Ireland (13)	8	10	9	8
Italy (3)	279	276	315	314
Malta	0	0	0	0
Netherlands (9)	28	28	28	28
Poland (4)	280	280	290	285
Portugal (8)	39	38	39	39
Romania (12)	2	5	10	10
Slovakia (11)	14	14	14	14
Slovenia (14)	6	7	7	7
Spain (6)	111	104	111	179
Sweden (16)	4	4	4	4
United Kingdom (5)	162	171	196	187

*Ranking of the country for the variable considered for the year 2013

1.1.3. Waterfowl (duck and geese)

On a globe scale, consumption of waterfowl products is plying a minor role. Duck and goose meat consumption is lower compare to chicken or turkey, most probably because of several factors such as taboo on waterfowl meat consumption and lack of technical know-how on duck and goose husbandry. Among people who have

never tried meat of waterfowl or these who rarely eat it, there appears to be two concerns. The first concern seems to be lack of knowledge on how to properly prepare this kind of meat while the other is somewhat higher fat content of waterfowl, which is true of whole duck or goose but not of leg meat and skinless breast meat (Omojola et al., 2014).

Duck production for meat products is a growing food industry on a global scale. Between 2000 and 2013, in terms of the numbers of ducks slaughtered worldwide, the total went up from 1,969 million to 2,886 million (ThePoultrySite Digital Report, 2015). Regarding geese, in 2011, goose and guinea fowl meat (the available data from FAO on goose meat production includes guinea fowl) production was 2.563 MMT (million metric tons) (USDA, 2013b). Furthermore, it can be said that Asia is clearly taking the lead in duck and geese production. Especially in countries of Eastern and Southern Asia, significant amounts of meat and eggs are obtained from ducks and geese which are important for the economy of these regions. China led world production of both duck meat and goose and guinea fowl meat in 2011, producing 66% and 94% of the total, respectively (USDA, 2013b). It has been noted, that almost 30% of poultry meat in China is from ducks and geese (Stipkovits and Szathmary, 2012). However, there exists a real opportunity for the waterfowl industry to expand in other areas of the world, particularly the Americas and Africa, because of the hardy nature of the bird and its ability to support rural populations in developing countries. After Asia, second biggest waterfowl meat producer is EU. In Europe, France is by far the largest producer of duck meat, accounting for more than half of the total EU production (0.5 million tonnes) (Eurostat, 2015). Currently in Europe, the highest duck production have been reported in France and Hungary. Meanwhile, among leading goose and guinea fowl meat producing countries in EU are Poland, Italy and France. In Africa, Egypt is the biggest producer of waterfowl meat (duck meat: 63,000 metric tons, goose and guinea fowl meat: 21,000 metric tons) (USDA, 2013b).

1.2. Poultry meat consumption

The poultry sector is the most dynamic meat sector during the last decade, showing the greatest growth of all meat sectors as reflected in world consumption. As proportion of consumed meat, chicken is eaten much more, and beef much less, now than in 1961 (chicken 25% *versus* 11% and beef 21% *versus* 34%, respectively), so in this case can be talk about a trend of replacing beef with poultry. Pig meat as a

proportion has stayed the same, over the time period, and sheep and other meats, consumed only in small proportions overall, have decreased their share slightly, when calculated on a *per capita* basis (Kanerva, 2013). The consumption of poultry meat increased from 43 kg per person per year in 2000 to 48 kg per person per year in 2015 (broiler chicken 41 kg) (USDA, 2016). The most evident growth of poultry meat consumption is observed in East and Southeast Asia and in Latin America, particularly in China and Brazil. Moreover, have been estimated that consumption of poultry meat in developing countries will increase until 2030 by 3.5% *per annu* (Narrod et al., 2007).

The fact that poultry meat consumption has increased greatly over the past decades has been driven by a number of converging parameters: (1) demographic growth; (2) growth of disposable income, which favors consumption of income- elastic foods such as meat; (3) price competitiveness of poultry meat relative to pork and beef due to higher productivity gains in production process; (4) widespread consumer acceptance of poultry meat products (Henry and Rothwell, 1995). The significant effect on poultry meat consumption has acceptation by most cultures and religions. Researchers numerous times evaluated the effect of these factors on meat consumption. In a study by Reicks (2006) it was established that the most three important factors influencing the purchase of meat products are taste attributes, price, and product consistency. While, Damisa and Hassan (2009) noted that among factors influencing the consumption of poultry meat are: income, price, household size and education. In the study conducted by Antwi-Boateng et al. (2013) was noted that the three most important factors which consumers consider in the purchase of meat are taste, health and price of meat. In this study authors observed also that demographic variables such as gender, age, family income levels, religion significantly affect the attitude towards meat purchase. Meat marketers and producers should take these factors into consideration in producing and selling meat products.

1.3. Trade of poultry meat

Poultry is considered as the main product traded, representing 43% of the total, followed by bovine, pig and ovine meat, respectively (FAO, 2014a). Over the past ten years, the players of poultry industry have changed. Windhorst (2006) considered that the centre of poultry meat production shifted from North and Central America to South and East Asia, and assumed that, in a few years, production volume in South America will surpass that of Europe.

Poultry meat is traded as raw meat; additionally, it must be highlighted that poultry parts are traded rather than whole carcasses (Josling et al., 2001). In low-income countries, imports of cheap low-quality cuts such as wings, lower legs, necks and giblets sold by the piece, make chicken meat more accessible to the average consumer. This coincides with changing eating habits in developed countries, where consumers tend to buy chicken breast and thigh meat and, to a lesser extent, drumsticks (Da Silva, 2013). Second to raw poultry meat, prepared poultry is increasingly taking a larger share in the international trade of poultry products. A large part of the trade in prepared poultry meat takes the form of convenience food such as fried, steamed, or roasted chicken meat. The product is usually packaged "ready-to-eat", and shipped frozen. Consumers' demand for this type of prepared food is rapidly increasing especially in developed countries (Nicita, 2008). Moreover, poultry trade is hardly a free market, as it is subjected to substantial tariffs often provided on a preferential basis, and non-tariff measures such as veterinary certification, licensing, product characteristic requirements and quotas. These policy instruments are quite effective in shaping bilateral trade flows as they effectively limit imports or favor determined countries (Nicita, 2008).

The main global leading exporters of poultry meat products are Brazil, the United States, the EU and China, which together account for almost three-quarters of global trade (FAO, 2014a). Brazil is the world's leading chicken exporter (USDA, 2014b). In 2015, Brazilian poultry meat exports broke all records to reach 4.304 million tonnes, an increase of 5% on 2014 (Clements, 2016). Among main export destinations of poultry meat from Brazil are Saudi Arabia, EU, Japan, United Arab Emirates, Hong Kong, China, Kuwait, Iraq (Santin, 2013).

The United States- the world's largest poultry producer is the second-largest exporter of poultry meat. Seven of the top foreign markets for U.S. broiler meat, on average during 1997-2012, were Mexico, Russia, Angola, Canada, Cuba, Hong Kong, and China. However, while sales of U.S. broilers to most of these larger markets rose during this period, U.S. exports to some countries fell. For example, Russia was the leading destination for U.S. broiler meat exports for most of the past decade, but exports to Russia dropped sharply after 2008. In 2012, 17% of U.S. broiler meat exports went to Mexico, while almost 8% were shipped to Russia, 5.5% to Angola, 5.2% to Canada, and less than 5% to each of the remaining major markets. Several countries that were once minor destinations for U.S. broiler meat exports have grown in importance as trade partners. For example, exports to Angola rose to 181.9 thousand metric tons in 2012

from 3 tmt in 1997. In 2002, Cuba became a major broiler market. From 2002 to 2012, the U.S broiler exports to Cuba increased from 52.3 tmt to 150.9 tmt (Davis et al., 2013). Important is to mention also, that in 2014, U.S. losed one of the main recipient of poultry meat. In August of 2014, Russian President Vladimir Putin signed a decree to establish restrictions on imports of agricultural products, include poultry meat, from countries that have imposed economic sanctions on Russia as a result of the ongoing situation in Ukraine (Newman and Bunge, 2014).

Third the biggest poultry meat exporter is EU. In the EU half of exports of poultry meat are shared between five destinations relatively equally (Saudi Arabia, Benin, South Africa, Hong Kong and Russia) while the other half goes to a long list of countries. The EU poultry meat exports increased from 2009 to 2012 by 40.8% (http://ec.europa.eu/agriculture/poultry/index_en.htm). The export of broiler meat from EU in 2013 was 1,094 thousand metric tons, while import 670 thousands metric tons (ready-to-cook equivalent) (USDA, 2013c). The average value of exports is 1.40 EUR/kg. The EU imports high value products mainly from Brazil (70% of total EU poultry meat imports) and Thailand (20%), poultry breasts and other high value added products, such as cooked preparations etc. The average value of imports is 2.65 EUR/kg (http://ec.europa.eu/agriculture/poultry/index_en.htm).

CHAPTER II

POULTRY MEAT QUALITY

Meat among other foods of animal origin, make a valuable contribution to human nutrition (Balş, 2009). It is the primary product of the livestock industry and constitutes the economic value. Meat and meat products have an important role in many Western and non-Western countries from a social and cultural perspective, and they are a central constituent of our meals and diet (Font-i-Furnols and Guerrero, 2014). Nowadays, meat is a good example for a complex structured food whose role often is controversially discussed in the context with health. The word "meat" comes from Old English word "mete", which referred to food in general. This term is related to "mad" in Danish, "mat" in Swedish and Norwegian, and "mature" in Icelandic and Faroese, which also mean "food" (Ahmad and Badpa, 2014). Meat is defined as the edible *post-mortem* component originating from life animals (Kauffman, 2012). Meat flesh is defined also as skeletal muscle to distinguish it from other parts of a carcass of meat such as offal, bone and bone marrow. Meat flesh includes any attached fat, connective tissue, rind, nerves, blood vessels and blood, and skin (if poultry) (Ahmad and Badpa, 2014). The definition of meat does not include eggs or fish.

Total meat can be broken down into red, white meat and processed (McAfee et al., 2010; Wang et al., 2012). Meat can be classified as red or white depending on the concentration of myoglobin in muscle fibers. When myoglobin is exposed to oxygen, reddish oxymyoglobin develops making myoglobin-rich meat appear red. Red meat includes the meat of most adult mammals and some fowl (e.g. duck) (Ahmad and Badpa, 2014). Red meat provides essential nutrients, containing high quality protein and essential micronutrients such as vitamins A, B6, B12, D and E, iron, zinc and selenium, contributing to consumers' health throughout life (Wezemael et al., 2010). This kind of meat is the richest source of alpha-lipoic acid, an extraordinary antioxidant. While, white meat or light meat refers to the light-colored meat of poultry as contrasted with red meat like beef or dark meat like horse. Processed meat as category is a continuum of products ranging from products with minimum of 30% meat to products that are all meat flesh. The definition for processing meat encompasses the processes of smoking, drying, salting, curing, fermenting, pickling, cooking and forming. Examples of

processed meat containing between 30% and 66% meat would include some sausages and some Frankfurt's, whereas processed meat that contain more than 66% meat would include products like ham or prosciutto (Ahmad and Badpa, 2014).

Meat quality, both red and white, has always been important to the consumer, and it is an especially critical issue for the meat industry in the current century. Fresh meat quality is a complex concept determined by preferences of consumer (Joo et al., 2013). Consumers are the last step in the production chain, and having their expectations met is an important part of their satisfaction and shopping behavior. It is therefore important to understand the factors affecting consumer behaviour (Font-i-Furnols and Guerrero, 2014). Consumers preference for meat are affected by race, ethnicity, social background, geography, family composition and household income (Gossard and York, 2003; Ripoll et al., 2015). Taste, flavor, visual appearance, texture (sensory factors), and price, label, brand, availability (marketing factors) may tend to dictate preference for a product. The price preferences have been linked to consumer age and gender. Usually lower prices are preferred and are probably especially important for a segment of consumers with low purchasing power or these for whom meat characteristics or type is not an important issue. In fact, high price is one reason that can explain, for instance, the low consumption of lamb in some countries where it is highly priced. Some people cannot afford to buy this type of meat very often and its consumption is only occasional, being replaced by other more affordable type of meat in most meals. Important role play also psychological factors; consumers as rational beings are affected by many external inputs that can modulate their cognitive, emotional, volitional and even automatic actions. The role of psychological influences on people's behavior has been widely analyzed and described in the scientific literature, especially in relation to the selection and purchase of different products, services or experiences (Font-i-Furnols and Guerrero, 2014). Considering the psychological factors, on special attention deserve the lifestyle of consumers. The lifestyle comprises five interrelated aspects: ways to buy, quality cues used in the evaluation of foods, cooking methods, consumption situations and buying motives (Ripoll et al., 2015). Moreover, consumers have become increasingly concerned about food-borne risks and personal health. As a consequence, consumer demand for safe and healthful foods has been increasing (Wezemael et al., 2010). During the last decade of the twentieth century, a clear relation was identified between diet and health, especially focusing on obesity and the relation between saturated fats from animal products. Chicken is considered

healthier than red meat; because of lower levels of fat and cholesterol chicken plays an important role in the diet of the general population, especially among groups such as the elderly, adolescents, pregnant women and people following low-energy diets (Ripoll et al., 2015). Furthermore, consumers have been increasingly expressing ethical and environmental concerns related to meat consumption (Wezemael et al., 2010). Meat is one of the food products with the greatest environmental impact due to the inefficiency of animals in converting feed to meat. It is assumed that 75-90% of the energy consumed by livestock is needed for body maintenance or lost in manure and by-products such as skin and bones (Djekic, 2015).

The quality attributes of food products, including poultry meat, have been attracting an increasing interest in recent years. The quality is the set of features of an entity that give that entity the ability to satisfy the expressed and implicit needs of its user or consumer. The meat quality concept is used to add the overall meat traits including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional and culinary properties. The consumers judge meat quality from its appearance, texture, juiciness, water holding capacity, firmness, tenderness, odor and flavor. These meat features are among the most important and perceptible that influences the initial and final quality judgment by consumers (reviewed by Tougan et al., 2013a).

2.1. Chemical composition

Chemical composition is one of the fundamental attributes that decide about meat quality. Food composition data, including these regarding meat, are important to a spectrum of users ranging from international organizations and private individuals, to food assistance programs, epidemiologists correlate patterns of disease with dietary components (Rand et al., 1991). Moreover, chemical composition influences meat nutritional value that becoming an increasingly important factor in food choices, especially in a context of increasing demand for more convenience food. Nutritional quality is tied to the ability of the meat to feed the consumer in proteins, lipids, carbohydrates, as well as many other essential compounds, such as vitamins, minerals, trace elements (Tougan et al., 2013a). These elements play key role especially considering the fact that meat is an important source for some micronutrients, that are either not present in plant derived food or have a poor bioavailability (Biesalski, 2005). The chemical properties of poultry meat (or muscle) have been studied intensely.

However, few papers have reported that the chemical composition of poultry meat varies depending on the numerous factors, including the muscular type; e.g. poultry meat from meat breast part have highly protein amount than the other parts from carcass with large fat deposits (Apetroaei et al., 2012a). Chemical composition of meat depends also on the composition of poultry feed, for example supplementation diet with vitamin E (Guo et al., 2001; Skřivan et al., 2010; Miezeliene et al., 2011). Moreover, the composition of poultry meat differs in different commercially available brands (Kumar and Rani, 2014).

2.1.1. Amino acids

According to Boisen et al. (2000) the ideal protein can be defined as the perfect ratio among individual amino acids (AA) and nitrogen required for optimal performance. Amino acids are defined as organic substances containing both amino and acid groups. Except for glycine, all AA have an asymmetric carbon and exhibit optical activity. The absolute configuration of AA (L- or D-isomers) is defined with reference to glyceraldehydes. Except for proline, all protein AA have a primary amino group and a carboxyl group linked to the α-carbon atom. In β-AA, an amino group links to the β-carbon atom (Wu, 2009). A series of amino acids joined by peptide bonds form a polypeptide chain, and each amino acid unit in a polypeptide is called a residue (Liu and Liu, 2016). The constituent amino acids differ only in the chemical nature of the side-chain group at the carbon atom. The physicochemical properties, such as charge, solubility, and chemical reactivity of the amino acids are dependent on the chemical nature of the side-chain group. Amino acids with aliphatic (alanine Ala, isoleucine Ile, leucine Leu, methionine Met, proline Pro, and valine Val) and aromatic (phenylalanine Phe, tryptophan Trp, and tyrosine Tyr) side chains are nonpolar, they exhibit limited solubility in water. Amino acids with charged (arginine Arg, lysine Lys, histidine His, glutamic acid Glu and aspartate Asp) and uncharged (serine Ser, threonine Thr, asparagine Asn, glutamine Gln, cysteine Cys) side chains are quite soluble in water (Damodaran, 1997). Moreover, amino acids can be degraded into carbon skeleton and ammonia. Glucogenic amino acids (including Ala, Cys, Gly, Ser, Thr, Trp, Asn, Asp, Phe, Ile, Met, Val, Arg, Glu, Gln, His, and Pro) are glucose precursors and can be degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate. Ketogenic amino acids (Leu and Lys) can be converted to fatty acids or ketone bodies and degraded to acetyl-CoA or acetoacetate (Fei, 2004).

The ideal protein concept is defined as the amino acid profile which meets the animals' requirement for protein accretion and maintenance (Primot et al., 2008). In poultry, 22 amino acids are needed to form body protein, some of which can be synthesized by the bird (non-essential amino acids, NEAA: Cys, Asp, Ser, Glu, Pro, Gly, Ala, Tyr, His, Arg), whereas others can not be made at all or in sufficient quantities to meet metabolic needs (essential amino acids, EAA: Thr, Val, Met, Leu, Ile, Phe, Lys) (Applegate, 2014). Proteins with a high content of essential amino acids are the most important components of poultry meat. Essential amino acids must be supplied by the diet, and a sufficient amount of non-essential amino acids must also be supplied to prevent the conversion of essential amino acids into non-essential amino acid (Applegate, 2014). In addition, the type of EAA in food varies, causing a variation in digestion quality and, ultimately, differences in protein value among food sources (Aronal et al., 2012).

Among above mentioned amino acids is lysine that is the reference amino acid because it is the 1st or 2nd limiting amino acid respectively in pigs and broilers and is mainly used for muscle protein accretion (Primot et al., 2008). Content of Lys in the breast muscle is relatively higher than other AA. Lysine represents approximately 7% of the protein in breast meat. However, is necessary to highlight that increasing the Lys concentration in the feeds of broilers as well as of slower growing laying hens leads to greater proportions of Lys in the total body protein, although a greater effect is observed in broilers (Vieira and Angel, 2012). Interestingly, increasing the level of lysine in the diet of broilers (beyond the requirement for growth) reduced drip loss of breast muscle during storage by increasing its ultimate pH (Berri et al., 2008). On the other side, it has been reported that dietary Lys inadequacy reduce breast meat yield compared with other muscles. Therefore, defining dietary AA needs for optimal growth and meat yield is of utmost importance (Nasr and Kheir, 2012). On attention deserves also methionine, which together with lysine, as two essential precursors of L-carnitine, can play important roles in lipid and energy metabolism in poultry (Bouyeh, 2013). Methionine is one of the sulfur-containing amino acids and has a strong effect on the activity of GSH-Px, glutathione reductase and glutathione transferase (Blaszczyk et al., 2010). Moreover, Met is a precursor of cysteine. The next role of methionine is as a key intermediate in methyl group transfer (Attia et al., 2005). Methionine cannot be produced by birds in adequate amounts to support maximum growth. Broilers have a high Met requirement that cannot be obtained from the corn and soybean fraction of

diets; therefore, birds require an additional ingredient source of Met (Moritz et al., 2005). Among the essential amino acids, threonine occupy particular position as well. The nutrient Thr must be considered in dietary formulations for commercial broilers because its excess is costly and its deficiency will decrease the efficiency of total sulfur amino acids (TSAA) and Lys use. In addition, Thr is typically the third limiting amino acid behind TSAA and Lys in commercial broiler diets composed of corn or sorghum, soybean meal, and meat meal (Kidd, 2000). Threonine is particularly important for mucin synthesis and maintenance of gut barrier integrity. Mentioned mucins are major glycoproteins protecting the epithelium from injury (Star et al., 2012).

The amino acid pattern of body protein may be affected by numerous factors, including genotype. For example, Fatufe et al. (2004) observed significant differences between the genotypes in the majority of the amino acids and concluded that the amino acid profile of the deposited protein was genotype-dependent. In their study, the amino acid composition of protein gain from 8 to 21 days of age; the aforementioned authors found significantly higher levels of lysine, methionine, alanine, aspartic acid, glutamic acid and glycine in broilers than in laying-type chickens. Moreover, the amino acids requirements and the amino acid profile in body depend on nutrition. It was recognized that the amino acid requirements of the bird are proportional to the crude protein content of the diet. The ratios of amino acids in muscle and other tissues in the body of the bird are constant. Birds consuming lower protein levels synthesize smaller amounts of protein and so need less of each amino acid, and vice versa (Pesti, 2009).

2.1.2. Proteins

Proteins are considered by many scientists to be the most multifunctional component of food. The role of meat, especially white meat, as a protein source is unequivocal. Have been reported that poultry meat and egg production is the most environmentally efficient animal protein production system. Poultry is by far the largest group of livestock species contributing about 30% of all animal protein consumed in the world (reviewed by Mengesha, 2013). Compare to other types of meat this obtained from poultry is more easily digestible due to high content of high-value protein. According to the standard recommended by the Codex Alimentarius Commission FAO/WHO, biological value of poultry meat protein is similar to the value of milk proteins (Nowak and Trziszka, 2010).

The unit of skeletal muscle is the muscle fiber. Among many components of muscle fiber, protein is the most important one. Proteins play a pivotal role, first of all because they are building blocks of muscle cellular structures and, in addition, they constitute components of enzymes. Thus, they play both static and dynamic functions (Pospiech et al., 2007). Moreover, the typical traits of numerous poultry products (e.g. yield, quality, and sensory features) are dependent on the successful manipulation of protein functional properties during processing (Smith, 2010). The functional properties of proteins are defined as their physical or chemical properties that affect food during their preparation, processing, storage, and consumption (Culbertson, 2005). These properties of poultry proteins must be understood for effective utilization of new ingredients, development of new products, modification of existing products, reduction of waste, and control of energy consumption during processing. The functional properties of poultry proteins can be classified into these involving following interactions:

(1) protein – water, include solubility, extractability, water retention and viscosity;

(2) protein – fat, include fat holding and emulsification;

(3) protein – protein, include gelation (Smith, 2010).

Muscle proteins comprise 15-22% of the total muscle weight (about 60-88% of mass) and can be broadly divided into three groups based on their solubility characteristics:

1. the sarcoplasmic proteins that are soluble in water or dilute salt solutions (about 30-34% of total protein content);
2. the myofibrillar proteins the contractile proteins that are soluble in concentrated salt solutions (approximately 50-55% of total protein content);
3. stromal proteins, the proteins of connective tissue and other formed structures that are insoluble in both (about 10-15% of total protein content) (Xiang, 1997; Olaoye, 2011; Monteiro, 2012).

The sarcoplasmic and myofibrillar fraction proteins are intracellular whereas the stroma proteins are found outside the muscle cell (Greaser, 2009).

2.1.2.1.The sarcoplasmic and myofibrillar proteins

The sarcoplasmic proteins are proteins of the sarcoplasma, soluble in water or at low ionic strength mediums, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. Approximately 100 different proteins are known to be present in the sarcoplasmic fraction and they are globular proteins of relatively low molecular weight ranging from 17,000 (myoglobin) to 92,500 (phosphorylase b) (Tornberg, 2005). This fraction contains the oxidative enzymes including the cytochromes, the flavin nucleotides, the various heme pigments and the mitochondrial oxidative enzymes. The sarcoplasmic fraction also contains the glycolytic enzymes, which control both aerobic and anaerobic glycolysis, thereby functioning in the conversion of glycogen to lactic acid and aerobic oxidation of pyruvate. Moreover, the sarcoplasmic classification also contains lysosymal enzymes and nucleoproteins, which function in hydrolytic degradation of waste material and regulate protein synthesis and deposition, respectively. It can be readily seen that the sarcoplasmic fraction covers a widely divergent group of proteins that control a widely differing group of tissue functions (Pearson and Gillett, 1996).

Myofibrillar proteins are made up of 12-14 major proteins including myosin and actin (Babij and Kee, 1994). Myosin is the prototype of a molecular motor- a protein that converts chemical energy in the form of ATP to mechanical energy, thus generating force and movement. While, actin filaments, usually in association with myosin, are responsible for many types of cell movements. The binding between myosin and actin molecules occurs through cross-links between the two proteins, during the process of muscle contraction, forming the actin-myosin complex (Clark et al., 2002). Moreover, interactions of actin and myosin are responsible also for a variety of movements of nonmuscle cells, including cell division (Cooper, 2000). Furthermore, the myofibrillar fraction includes tropomyosin, troponin, the actinins (α and β forms) and other minor regulatory proteins, which play important roles in muscle and meat (Pearson and Gillett, 1996). Myofibrillar proteins comprise more than 55% of the total protein content of the muscular tissue. These proteins are responsible for water retention. In muscles with low content of connective tissue, myofibrillar proteins contribute significantly to meat tenderness and toughness (Zayas, 1997b).

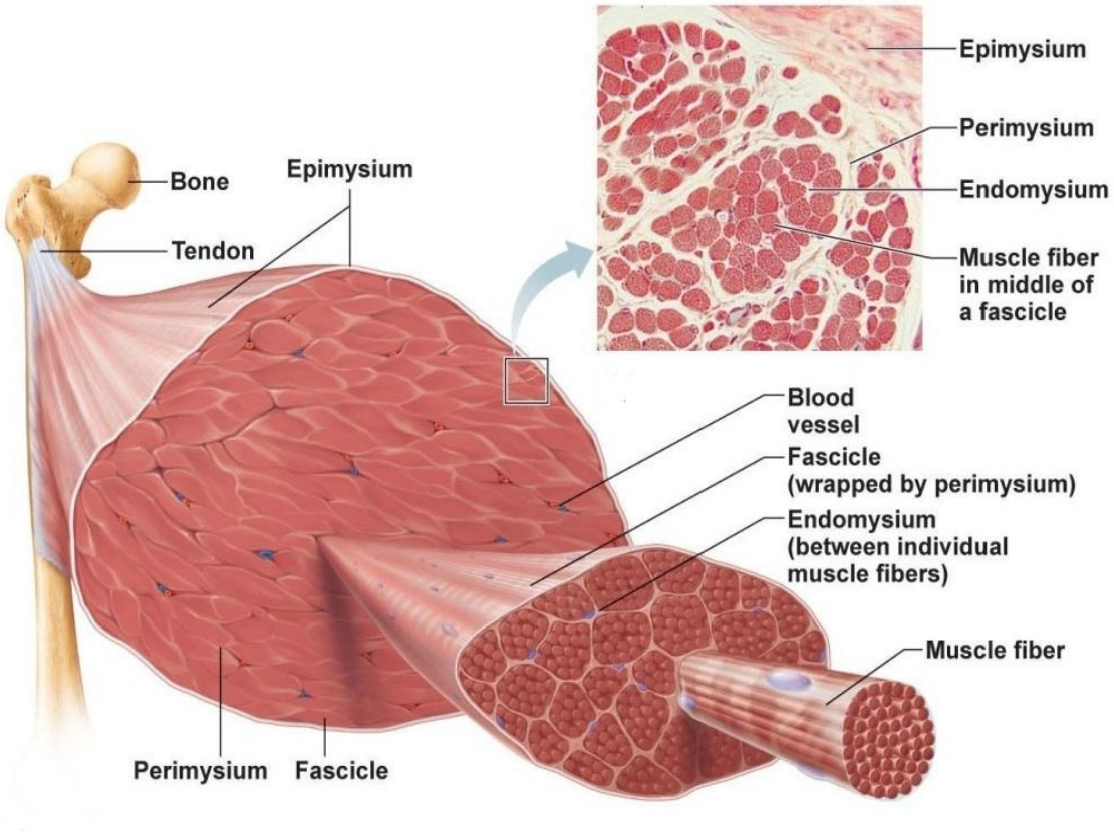
2.1.2.2. Stroma proteins (collagen, elastin)

Stroma proteins are usually measured as the insoluble proteins remaining after exhaustive extraction of all soluble muscle proteins. Stroma proteins influence meat quality directly:

a) lower tenderness of meat and the effect depends on the amount of stroma proteins and the degree of cross-linking among stroma proteins;
b) because of their insoluble nature, they decrease the emulsifying capacity of meat;
c) because of their low content of charged and hydrophilic amino acids, stroma proteins lower water holding capacity of meat;
d) decrease the nutritive value of meat (Zayas, 1997a).

Of insoluble proteins, collagen is composed about 0.5 proportions, elastin is about 0.03, and the remaining 0.47 is a mixture of various proteins such as reticulin (Thu, 2006). Collagen makes up the majority of the stroma proteins and is considered the most abundant protein in the body, comprising up to one third of the total body protein (Alvarado and Owens, 2005). However, from a nutritional aspect, collagen is of lower biological value than other meat proteins. Thus, variation in the content of collagen and elastin (second stroma protein) influences the biological value of meat proteins (Vognarová et al., 1968). It is found in muscle as 1-9% of the dry, fat-free mass where it exists as networks of fibres. In the living muscle these fibres resist over-extension which may cause damage to the tissue. The force of contraction is transmitted to the tendons through sheets of intramuscular connective tissue which enclose the individual muscle fibres. Three collagenous structures can be distinguished morphologically into epimysium, perimysium and endomysium (Figure 2.1) (Etherington and Sims, 1981). The epimysium is often thick and tough and resistant to both shear and solubilization. However, it is easily (and usually) separated from cuts of meat and is generally not considered to be a factor in meat quality. The perimysium is thought to play the major role in determining meat texture differences that are related to connective tissue (McCormick, 1999). While, the endomysium covers individual muscle fibers and fills out spaces among them (Kurose et al., 2006). Moreover, the epi-, peri-, and endomysial sheaths determine the architecture of muscle and protect muscle from overstretch (McCormick, 1999).

Figure 2.1. Composition of muscle indicating epimysium, perimysium and endomysium (source: http://www.neoreh.pl/blog/rola-powiezi-w-ukladzie-ruchu/).



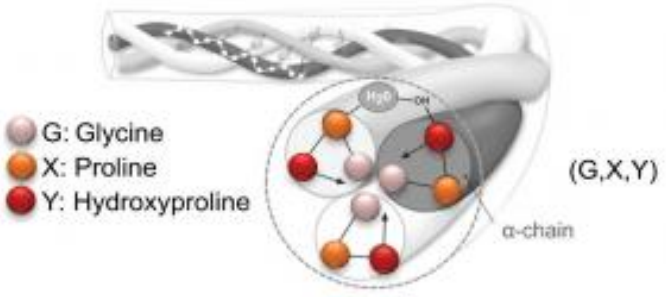
Vertebrates have at least 27 collagen types with 42 distinct polypeptide chains, more than 20 additional proteins with collagen-like domains and approximately 20 isoenzymes of various collagen -modifying enzymes. Collagen type I is predominantely present as a heterotrimeric molecule, composed of two α1(I) chains and one α2(I) chain, $[\alpha1(I)]_2$ α2(I), while the homotrimeric form $[\alpha1(I)]_3$ has been shown to occur at low levels in normal adult skin. Type I collagen is frequently accompanied by several other collagen types such as type III and type V collagen. Type II collagen is present in cartilage and the vitreous humor in association with type XI collagen. Type IV collagen is only found in basement membranes, where it is the major structural component (Pataridis et al., 2009).

The basic structural unit of collagen is tropocollagen, that is 300 Å long and 14 Å thick and has a molecular weight of about 300,000. It consists of three polypeptide chains, each having over 1000 amino acids residues. The most prominent amino acid is glycine, which accounts for about one-third of all amino acids in collagen. Proline and hydroxyproline (22%) and alanine (11%) are the next most abundant amino acids. In

addition to hydroxyproline, collagen contains another amino acid, hydroxylysine, which rarely, if ever, occur in other proteins. The typical amino acid sequence in a collagen polypeptide chain is Gly-X-Y, where proline may occupy positions X or Y, but hydroxyproline may occupy position Y only.

The secondary structure of tropocollagen is helical on two levels: each polypeptide chains has a left-handed helical sense, with 3.3 amino acids per turn and a total linear distance of 9.6 Å per turn. This gives a pitch of 9.6/3.3- 2.91 Å for each amino acid. Three such helical polypeptide chains are assembled into tropocollagen cables consisting of three intertwined polypeptide chains, forming a superhelix with right=handed sense. The superhelix, and each subhelical component, has a total of about 29 turns (104 Å per turn). Each turn consists of 36 amino acids, giving a total of about 1044 amino acids per strand and a total of about 3100 amino acids per tropocollagen molecule. Moreover, the subhelices are affanged in the superhelix in such way that the glycine residues are on the interior of the superhelix and in contact with each other. The X and Y residues face the environment and are therefore able to accommodate any bulky side chains (Figure 2.2) (Bezkorovainy and Rafelson, 1995).

Figure 2.2. Structure of collagen triple helix (source: http://www.collagen-colway.com/everything-you-wanted-to-know-about-native-collagen/).



Collagen fibrils and the fibrous matrices they form are stabilized by covalent crosslinks. The first step in crosslink formation is the conversion (by lysyl oxidase) of the epsilon amino group of selected lysine or hydroxylysine residues to the corresponding aldehyde (allysine or hydroxyallysine). Crosslinks then form by spontaneous reaction of an allysine or hydroxyallysine with an unmodified lysine or hydroxylysine residue on an adjacent polypeptide chain. The initial crosslinks formed are dysfunctional and are usually described as reducible crosslinks because they possess Schiff base double bonds. With maturation divalent crosslinks disappear from many

tissues and may be replaced by mature, non-reducible crosslinks (McCormick and Thomas, 1998). It's necessary to highlight that the progression of crosslinking occurs significantly faster in avian skeletal muscle that in the mammals examined. Regarding, mechanisms regulating crosslink formation, there are two enzymes of collagen metabolism that may play a regulatory role in crosslinking are lysyl hydroxylase and lysyl oxidase. Lysyl hydroxylase catalyzes the post-translational hydroxylation of selected lysyl residues prior to collagen helix formation. While, lysyl oxidase, which requires copper as a co-factor, is the only known enzyme involved in the actual formation of crosslinks (McCormick, 1999).

The content of collagen has been the ultimate goals in numerous studies. It was observed that collagen content of dark colored muscles (red meat) was higher than in light colored muscles (white meat). Moreover, breast muscles of broiler chickens characterized by highest collagen content at the ages of two weeks and five weeks, respectively. In an available literature the effect of different factors on collagen concentration in muscles has been examined (reviewed by Mobini, 2015). Kerr et al (2001) reported that the collagen of fast growing animals is less matured than that of slow growing animals at the same slaughter weight.

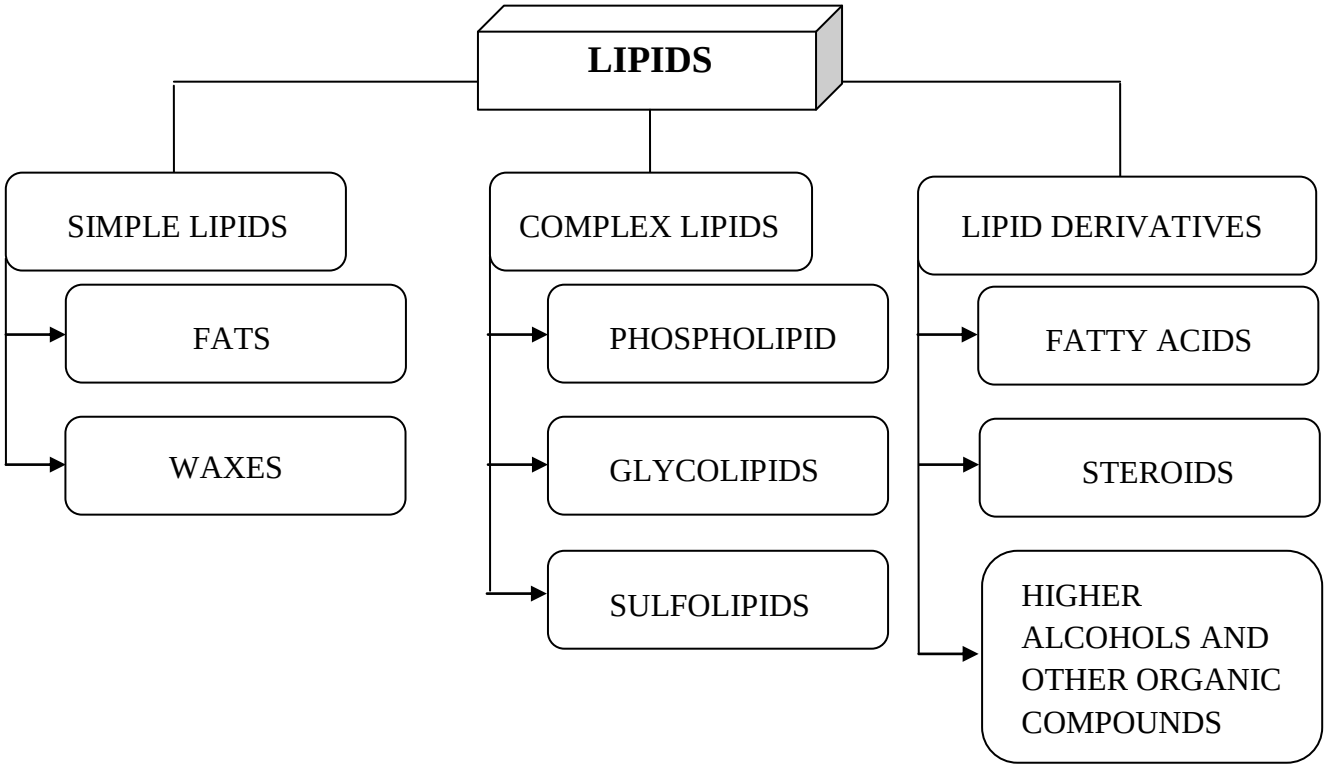
Elastin is the extracellular matrix protein responsible for the resilience of tissues such as skin, arteries and lung. It is an insoluble, hydrophobic and extensively cross-linked protein forming fibers which are present in variable amounts depending on the tissue. Although it has been involved in numerous biological activities, elastin's function is restricted to elasticity (Debelle and Alix, 1999). Elastin is composed of tropoelastin-the soluble precursor of elastin and as such it plays a dominant role in elastogenesis (Vrhovski and Weiss, 1998). It has a molecular weight of about 72,000 and contains 800-850 amino acid residues (Bezkorovainy and Rafelson, 1995). The expression of tropoelastin is under a complex control mechanism, with many isoforms existing. Two major types of domains are found in tropoelastin: (1) hydrophobic domains rich in non-polar amino acids especially Gly, Val, Pro and Ala, and often occurring in repeats of three to six peptides; (2) hydrophilic domains typically rich in Lys and Ala involved in cross-linking. These domains often consist of stretches of Lys separated by two or three Ala residues (Vrhovski and Weiss, 1998). Moreover, the hydrophobic domains are rich in nonpolar residues and are involved in the alignment of tropoelastin; while the hydrophilic domains are mostly composed of lysine and alanine and participate in cross-linking (Yang et al., 2015).

The process of elastin formation includes the coacervation of about 15-nm soluble tropoelastin molecules into micron-sized spherules and lysyl oxidase-mediated cross-linking of these spherules. Following complex cross-linking, the insoluble elastin fibres are resistant to most proteases and, thus, only sensitive to a limited number of elastases (enzymes capable of solubilizing fibrous elastin) (Yang et al., 2015). They may belong to the class of serine proteinases, cysteine proteinases and metalloproteinases. Mammalian elastases occur mainly in the pancreas and the phagocytes. Among non-mammalian elastases there is a great variety of bacterial metallo and serine elastases. The elastolytic activity varies from one elastase to another and is usually not correlated with the catalytic efficiency of these proteinases (Bieth, 2001).

2.1.3. Lipids

Lipids differ markedly from the other groups of biomolecules considered in this chapter. By definition, lipids are water-insoluble biomolecules that are highly soluble in organic solvents such as chloroform. Lipids have a variety of biological roles: they serve as fuel molecules, highly concentrated energy stores, signal molecules, and components of membranes (Berg et al., 2002a). Lipid compounds may be categorized based on their chemical structure and biological functions. The most common classification is presented below (Figure 2.3).

Figure 2.3. Classification of lipids (adopted from Dąbrowska et al., 2015).



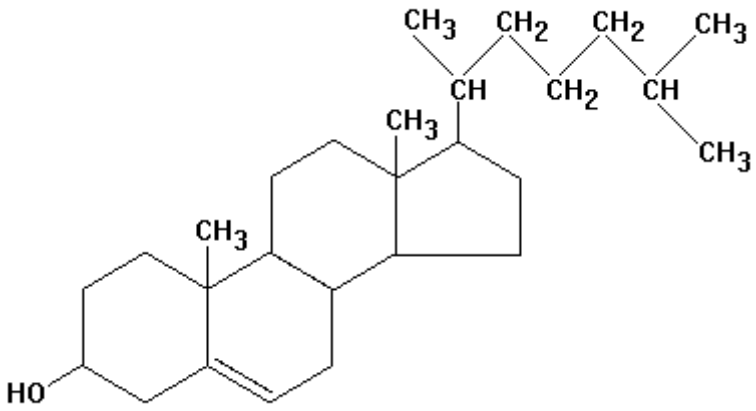
Taking into consideration lipids as the muscles component its necessary to highlight that among livestock species chickens are recognized as an efficient source of lean meat. However, long-term intense selection for increased juvenile growth in broiler chickens has led to increased fat deposition in the chicken abdomen (Guo et al., 2011a). Excessive fat deposition is an unfavorable trait for producers and consumers because it is considered to be wasted dietary energy and a waste product with low economic value, which also reduces the carcass yield and affects consumer acceptance. Have been observed that modern broiler strains contain 15% to 20% fat and >85% of this fat is not physiologically required for body function (Fouad and El-Senousey, 2014). Moreover, adipose tissue in meat is desirable, to some extent, to give a finished appearance to a carcass (Musa et al., 2007). In avian species, the amount of fat that accumulates in the body depends on the available plasma lipid substrate, which originates from the diet or *de novo* lipogenesis in the liver (Fouad and El-Senousey, 2014). The biological mechanisms that regulate the synthesis and degradation of lipids and lipid transport in plasma are of great significance to animal in zootechnical production (Musa et al., 2007).

2.1.3.1.Cholesterol

Cholesterol is an unsaturated alcohol of formula $C_{27}H_{45}OH$ that is a waxy substance made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. Cholesterol is needed in the body to insulate nerves, make cell membranes and produce certain hormones, and it is an important lipid in some membranes (Ma, 2006). Cholesterol was first isolated from gallstones close to the turn of the 19th century and soon "*exerted a hypnotic fascination for scientists from the most diverse domains of science and medicine*", noted cholesterol researchers Michael Brown and Joseph Goldstein in their 1985 Nobel prize acceptance speech (Patlak, 2005).

The molecular structure of cholesterol includes a tetracyclic fused ring skeleton, with a single hydroxyl group at carbon 3, a double bond between carbons 5 and 6, and an iso-octyl hydrocarbon side chain at carbon (Figure 2.4). An important notion on the three-dimensional structure of cholesterol is that the 3β-OH group, the two methyl groups and the side chain are all located on the same side of the ring skeleton (β-configuration). The hydroxyl group in cholesterol is very important, because it gives the otherwise hydrophobic compound its amphiphilic character and therefore orients the molecule in membranes. Further, the hydroxyl group can also mediate the hydrogen bonding of cholesterol with water and possibly with other lipid components of cellular membranes (Ohvo-Rekilä et al., 2002).

Figure 2.4. The chemical structure of cholesterol.



Cholesterol can be divided into the good one and the bad one. High-density lipoprotein (HDL) is called "good cholesterol" that is good for the cardiovascular

system and low-density lipoprotein (LDL) is called “bad cholesterol” that is bad for the cardiovascular system. These are the form in which cholesterol travels in the blood. LDLs have little protein and high levels of cholesterol and HDL has a lot of protein and very little cholesterol. LDL is the main source of artery clogging plaque. HDL actually works to clear cholesterol from the blood (Ma, 2006).

Cholesterol synthesis is essential for normal development and maintenance of tissues that cannot obtain cholesterol from plasma lipoproteins, such as brain (Liscum, 2002). The process of cholesterol synthesis has five major steps: (1) Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA); (2) HMG-CoA is converted to mevalonate; (3) Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2; (4) IPP is converted to squalene; (5) Squalene is converted to cholesterol (Thomas, 2012). Cellular cholesterol levels are modulated by a cycle of cholesterol esterification by acyl-CoA : cholesterol acyltransferase (ACAT) and hydrolysis of the cholesteryl esters, and by cholesterol metabolism to bile acids and oxysterols. It’s important to highlight that the regulation of cholesterol biosynthetic enzymes takes place at the level of gene transcription, mRNA stability, translation, enzyme phosphorylation and enzyme degradation (Liscum, 2002).

The most important function of cholesterol is perhaps its ability to modulate the physicochemical properties of cellular membranes. Moreover, cholesterol can also manipulate the behavior and functions of proteins residing in the membrane. Cholesterol has also an important role in eukaryotes as the precursor of steroid hormones in steroidogenic cells and of bile acids in hepatocytes. Except above mentioned functions, cholesterol acts as a precursor of the active form of vitamin D. Additionally, cholesterol can participate in cellular signaling both indirectly by modulating the physical properties of the plasma membrane thereby affecting the activity of receptors and enzymes residing in it, or directly as a regulator of enzymes in the cholesterol metabolic pathways (Ohvo-Rekilä et al., 2002).

Cholesterol is present in muscle as well as adipose tissues because it is an essential component of cell membranes and can be stored as cholesterol esters in lipid droplets. The differences in muscles cholesterol content among livestock species are explained by variations in absorption and biosynthesis of cholesterol, lipoprotein metabolism, diet, muscle fiber type distribution, genetic variation, subcutaneous and intramuscular fat, body weight and cell size. In case of poultry significant factor

affecting cholesterol content is type of retail cut because of the difference between dark and white chicken meat and the presence of skin in many retail cuts. Poultry skin has the greatest cholesterol concentration compared with poultry meat or poultry fat. The difference in cholesterol content between white and dark poultry meat is more pronounced than that between white (predominantly glycolytic) and red (predominantly oxidative) muscles in beef and pork. In general, raw poultry meat has approximately 27 to 100 mg cholesterol/100 g and cooked poultry meat contains around 59 to 154 mg/100 g (Chizzolini et al., 1999; Bragagnolo, 2009; Milićević, 2014).

2.1.3.2. Fatty acids

Among the most biologically significant properties of lipids are their hydrophobic properties. These properties are mainly due to a particular component of lipids: fatty acids (Berg et al., 2002a). Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism – major metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators. Fatty acids are also important for thermal and electrical insulation, and for mechanical protection (Rustan and Drevon, 2005). Fatty acids are divided into two categories based on structural and chemical properties: (1) saturated and (2) unsaturated. Saturated fatty acids do not contain any double bonds or other functional groups along the chain. Unsaturated fatty acids contain at least one pair of carbon atoms linked by a double bond enabling the addition of other atoms to these carbons. Distinction between the two is simply that saturated fatty acids are usually solid at room temperature whereas unsaturated fatty acids are liquid. Unsaturated fatty acids can be further divided into monounsaturated (MUFA) which contains only one double bond and polyunsaturated fatty acids (PUFA), which contain more than one double bond (Panickar and Bhathena, 2010). In PUFA the first double bond may be found between the third and the fourth carbon atom from the ω carbon; these are called omega-3 (ω-3 or n-3) fatty acids. If the first double bond is between the sixth and seventh carbon atom, then they are called omega-6 (ω-6 or n-6) fatty acids. The double bonds in PUFA are separated from each other by a methylene grouping (Rustan and Drevon, 2005). Omega-3 and omega-6 are termed “essential fatty acids” (EFA). Such EFA are obtained from diet since they cannot be manufactured by cells (double bonds can be introduced into all positions of the fatty acid chain except the n-3 and n-6 positions) (Panickar and Bhathena, 2010). Among PUFA for the special attention deserve linoleic acid [C 18:2 (n-6 omega)] and

α-linolenic acid [C 18:3 (n-3 omega)]. These two essential fatty acids are sources for the production of important longer chain PUFA such as prostaglandins; dynamic but short lived compounds that control blood vessels and other body functions (Gogus and Smith, 2010). Taking into consideration functions of fatty acids, results of numerous studies showed that fatty acids influence many aspects of meat quality. This property is connected with the fact that they have very different melting points. It has been observed that melting point of FA increases with chain length (Berg et al., 2002b) and it declines with increases of the unsaturation of FA (Wood et al., 2003). In the 18C fatty acid series, stearic acid (C 18:0) melts at 69.6^{o}C, oleic acid (C 18:1) at 13.4^{o}C, 18:2 at 5^{o}C and 18:3 at 11^{o}C. Variation in the structure of the molecule are also important. For example, trans fatty acids melt at a higher temperature than their cis-isomers and branched chain fatty acids have lower melting points than the straight chain fatty acids with the same number of carbon atoms. Variation in fatty acid composition has an important effect on firmness or softness of the fat in meat (Wood et al., 2003).

Poultry meat has been considered as one of the main sources of PUFA for human diets, in particular n-3 PUFA (Ponte et al., 2008c). Additionally, it has been shown, that the content of poultry meat in n-3 fatty acids, particularly in α-linolenic acid, can be readily improved by increasing the levels of n-3 PUFA in poultry diets through the incorporation of oily fish by-products (Ponte et al., 2008c). Fish oil is the most common long-chain n-3 PUFA supplement used but is unsustainable and reduces the oxidative stability of the meat. This marine supplement represents a rich source of polyunsaturated fatty acids and especially the more highly unsaturated fatty acids such as DHA (docosahexanoic fatty acid), EPA (eicosapentaencic fatty acid). In the study conducted and described by Mirghelenj et al. (2009) was observed that the DHA content in breast and thigh meat of bird fed diet with 0-2% fish oil increased from 0.046 and 0.086 mg/g to 0.166 and 0.27 mg/g, respectively. However, when fish oils are used in the feed formulations of birds to improve the nutritional value of the products there is some decrease in sensory quality reported as fishy off-flavors. This can be alleviated by using marine algae. It was reported that although the overall acceptability of the meat from broilers fed marine algae was reduced (compared with the control), it was more acceptable than meat from birds fed fish oil, suggesting that the oxidative stability of the meat from birds fed algae rather than fish oil was greater (Mooney et al., 1998). While, in the study described by Rymer et al. (2010) was concluded that that algal biomass is as effective as fish oil at enriching broiler diets with PUFA content. On special attention

deserve also vegetable oils (Zduńczyk and Jankowski, 2013). The study conducted by Kamran Azad et al. (2009) showed that inclusion of full-fat flaxseed and canola seed significantly increased in meat the concentration of omega-3 fatty acids and decreased the content of the arachidonic acid and n-6:n-3 PUFA ratio.

Decreasing the level of saturated fatty acids and/or increasing monounsaturated fatty acids and polyunsaturated fatty acids content in poultry meat can be beneficial for human health (Mašek et al., 2013). Saturated fatty acids in meat as a source of fat in the human diet are associated with several diseases of modern civilization such as obesity or cancer (Zymon et al., 2007). Additionally, dietary SFA cause an increase in serum total and LDL cholesterol and therefore increase the risk of heart disease. Considering the human health it's important to mention about the ratio of n-6 to n-3 PUFA. The n-6 to n-3 ratio of diets during human evolution was estimated to be close to 1:1 (Vahmani et al., 2015). However, a typical Western diet is characterized by a very high n-6/n-3 PUFA ratio of 10:1 to 30:1 or even 50:1. This ratios results from very high levels of n-6 PUFA and relatively low consumption of n-3 PUFA (Zduńczyk and Jankowski, 2013). It has been observed that the great amounts n-6 PUFA in the diet promotes the production of eicosanoids that result in allergic and inflammatory responses such as increase in platelet aggregation, blood viscosity, vasospasm and vasoconstriction as well as reduced bleeding time. Furthermore, an increased n-6 to n-3 ratio could promote or exacerbate atherogenesis (Vahmani et al., 2015). Moreover, numerous studies showed that in secondary prevention of cardiovascular diseases, a ratio of 4:1 was associated with a 70% decrease in total mortality. A lower n-6/n-3 PUFA ratio decreased the risk of breast cancer in women and suppressed inflammations in patients with rheumatoid arthritis. The ratio of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer (Zduńczyk and Jankowski, 2013).

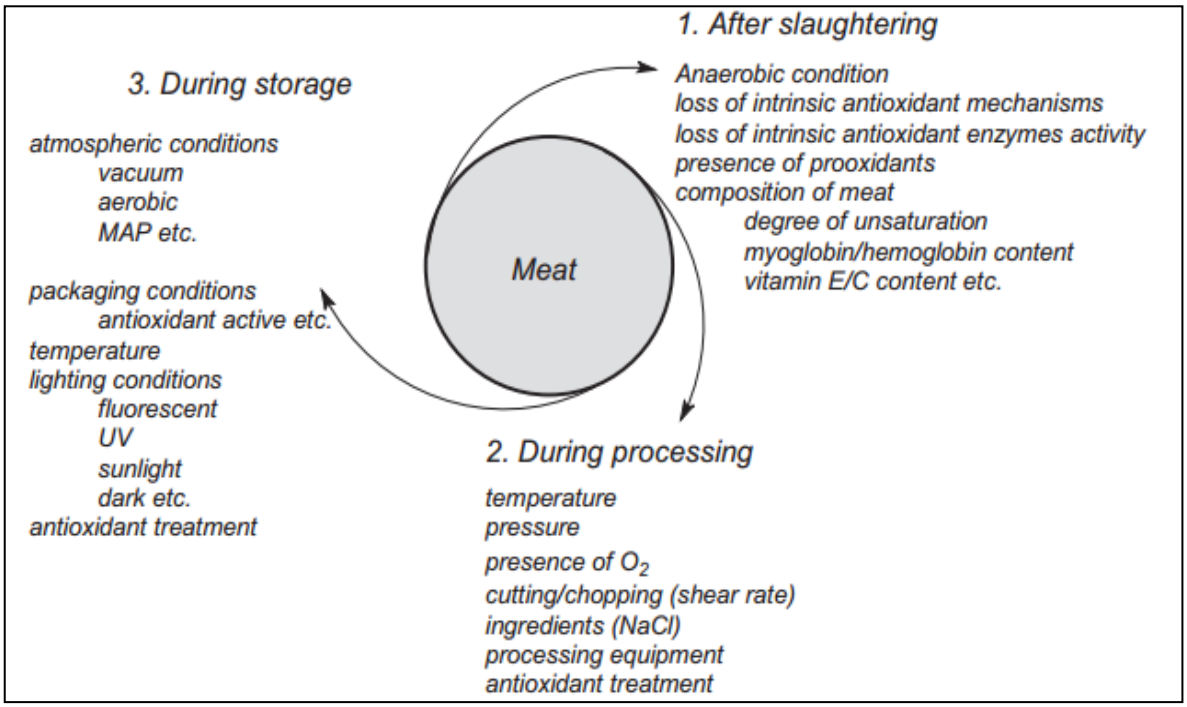
2.1.3.3. Lipid oxidation

On the other hand, increasing the degree of unsaturation of muscle membranes reduces the oxidative stability of the muscle. The relative oxidation rates of fatty acids containing 1, 2, 3, 4, 5 or 6 double-bonds are 0.025, 1, 2, 4, 6 and 8, respectively. Dietary fats differ in terms of unsaturation level what has effect on the discussed lipid stability. According to Hugo et al. (2009), who have investigated the effects of different dietary lipid sources on lipid oxidation of broiler muscles, meat samples from different dietary oil treatments vary in oxidative stability. In their study birds fed fish oli showed

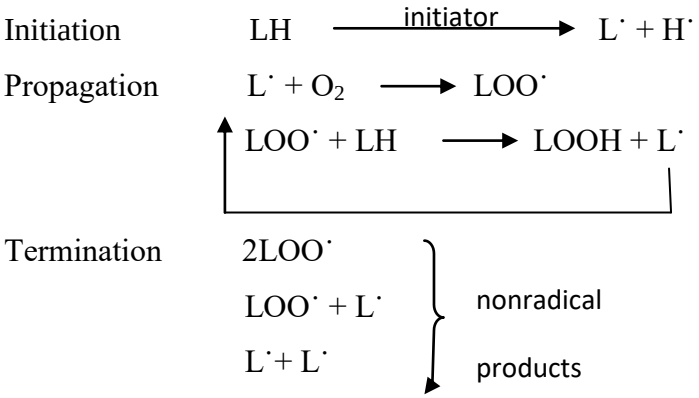
significantly more oxidation in both thigh and breast meat than birds from any of the other treatments during storage.

Oxidation is a complex, natural process in meat and its derivatives, and its occurrence is potentiated in the presence of oxidative agents (Bigolin et al., 2013) (Figure 2.5). Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions, most of which involve some type of free radical or oxygen species.

Figure 2.5. Factors affecting the oxidative stability of meat at various stages (Adopted from Kumar et al., 2015).



Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids (Shahidi and Zhong, 2005). Classical studies established that the mechanism of autoxidation of lipids involves the three stages (Kanner and Rosenthal, 1992):



Firstly, the reaction rate increases significantly with increasing oxygen pressure and temperature. The factors which cause a reduction in oxidation rate, include reduced temperature, reduced oxygen pressure, inert gas environment and the presence of antioxidants. As previously mentioned, lipid oxidation is a very complex process. It is initiated by the formation of free lipid radicals (L˙). This step requires a relatively high activation energy, which may be provided e.g. with heat energy or singlet oxygen (Dąbrowska et al., 2015). In the propagation phase, lipid radical (L˙) rapidly reacts with oxygen to form a lipid peroxy radical (LOO˙) which abstracts a hydrogen from another lipid molecule generating a new (that continues the chain reaction) and lipid hydroperoxide (LOOH) (Ayala et al., 2014). The last step of lipid peroxidation is termination process in which the LOOs react with each other and/or self destruction to form non-radical products. Although LOOH is stable at physiological temperature, it can be decomposed by heating at high temperature or by exposure to transitional metal ions (Min and Ahn, 2005).

The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH) (Ayala et al., 2014). Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odourless and relatively unstable compounds. They decompose to form volatile aromatic compounds, which are perceived as off-flavors and as a warning that food is no longer edible (Wąsowicz et al., 2014). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, propanal, hexanal, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) have been extensively studied (Ayala et al., 2014). MDA is an end-product generated by decomposition of arachidonic acid and larger PUFA, through enzymatic or nonenzymatic processes (Ayala et al., 2014). It is a organic compound with the formula $CH_2(CHO)_2$ (Kshitiz et al., 2015). The monitoring of MDA levels in different biological

systems can be used as an important indicator of lipid oxidation (Singh et al., 2014). Moreover, because MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations, and due to MDA's high reactivity and toxicity underlying the fact that this molecule is very relevant to biomedical research community. It has been noted that MDA is an important contributor to DNA damage and mutation (Ayala et al., 2014).The amount of MDA generated during lipid peroxidation can be measured with TBARS (Thiobarbituric Acid Reactive Substances) method. It is the most widely used method for determination of lipid peroxidation, especially due to its simplicity and cheapness. As the name of this method implies, it is based on the ability of malondialdehyde, which is one of the secondary products of lipid peroxidation, to react with thiobarbituric acid (TBA) (Sochor et al., 2012).

From technological point of view is important to highlight that, lipid oxidation affects shelf life. It is one of the most important parameters affecting the quality of poultry meat after its distribution to the market (Kozačinski et al., 2012). Generally, shelf life is understood as "the time period for the product to become unacceptable from sensory, nutritional or safety perspectives". Taking into consideration aforementioned sensory perspective is worth to add few information about the warmed-over flavor (WOF). This term is used to define the rapid increase in oxidation in cooked meat products, which is characterised by the rancid flavor developed during storage under refrigeration. The cooking temperature, time, and final internal temperature of the meat can influence the development of WOF. The effects of such cooking parameters are related to differences in the formation of Maillard reaction products (MRP) in the meat, which may include antioxidants suitable for preventing the development of WOF in cooked meat (Lage et al., 2012).

2.1.4. Vitamins and mineral elements

Micronutrients differ from macronutrients in key characteristics. Water, proteins, carbohydrates and fat are consumed in large amount, whereas vitamins and minerals are ingested in much smaller amounts (milligrams to micrograms per day). These differences in magnitude reflect turnover rates in the body and specific functions. Macronutrients provide source of energy, needed to fuel the body, maintain cellular hydration and provide the body structure to perform work. Micronutrients enable the use macronutrients for all physiologic processes. Despite their relative paucity in the diet and the body, vitamins and minerals are key regulators of health (Lukaski, 2004).

Micronutrient deficiencies, which are commonplace in many developing countries, can have major adverse health consequences, contributing to impairments in growth, immune function, mental and physical development and reproductive outcomes that cannot always be reversed by nutrition interventions (van Huis et al., 2013).

Vitamins, a heterogeneous group of substances, are vital nutrients that must be obtained from the diet (Soriano-Santos, 2009). Poultry meat is a source of vitamins, such as vitamin A, E, K, C, and vitamins of B complex group (Table 2.1). The content of these compounds in the skeletal muscle of birds is about 1-1.5% (Kłoczko, 2004). Vitamins listed above as the first three belong to the fat-soluble vitamins. These fat-soluble vitamins have no direct role in energy metabolism; they function in roles supportive of energy use. β-carotene, a precursor of vitamin A, and vitamin E act as antioxidants in reducing muscle damage and enhancing recovery from physical activity (Lukaski, 2004). Last but not least among fat-soluble vitamins present in poultry meat is vitamin K. Vitamin K acts as a cofactor for the carboxylation of certain glutamic acid residues in specific vitamin K-dependent proteins, to form γ-carboxyglutamic acid (Gla) (Elder et al., 2006). As was mentioned, poultry meat is also rich in vitamins of B complex and vitamin C (ascorbic acid), that are classified as water-soluble vitamins. The B vitamins (thiamin, riboflavin, niacin, pyridoxine, folate, biotin, pantothenic acid, and choline) regulate energy metabolism by modulating the synthesis and degradation of carbohydrate, fat, protein and bioactive compounds. Vitamin B12 is required for hemoglobin synthesis and vitamin C acts as an antioxidant (Lukaski, 2004).

Table 2.1. Vitamin and mineral content in meat of selected poultry species (adopted from Ioniţă et al., 2011).

Item	Unit	Broiler chicken meat	Duck meat
	Vitamins		
Vitamin A	UI	140	168
Vitamin C	mg	1.6	2.8
Vitamin E	mg	0.3	0.7
Vitamin B2	mg	0.1	0.2
Folic acid	mcg	6	13
Vitamin B12	mg	0.3	0.3
Vitamin K	mcg	1.5	5.5
Vitamin B1	mcg	0.1	0.2
Vitamin B3	mg	6.8	0.2
Vitamin B6	mg	0.4	0.2
Vitamin B5	mg	0.9	1
Vitamin B4	mg	59.7	31
	Mineral Elements		
Calcium	mg	11	11
Iron	mg	0.9	2.4
Magnesium	mg	20	15
Phosphorus	mg	147	139
Potassium	mg	189	209
Sodium	mg	70	63
Zinc	mg	-	1.4
Copper	mg	-	0.2
Manganese	mg	-	0.38
Selenium	mcg	14.4	12.4

Moreover, meat and meat products are good sources of most minerals- inorganic elements (Table 2.1), other than carbon, hydrogen, oxygen, and nitrogen, that remain behind in the ash when food is incinerated. They are usually divided into two groups: macrominerals and microminerals (or trace elements). Minerals are classified as either essential or nonessential, depending on whether or not they are required for human

nutrition and have metabolic roles in the body. Nonessential elements are also categorized as either toxic or nontoxic (Soriano-Santos, 2009). Muscle tissue is an important source of phosphorus and potassium, but is low in calcium and is a moderate source of magnesium. Offal, particularly liver is also a good source of essential macronutrients, mainly potassium and phosphorus followed by sodium, magnesium and calcium. The levels of sodium in raw meat are not significant. However, meat products may contain high levels of this mineral because it is added as part of curing and preserving or as a flavor-enhancing ingredient.

Meat, including liver, is an important source of essential microminerals such as iron, zinc, copper, manganese, selenium, cobalt, iodine and molybdenum; some of them exclusively are present in meat or have a higher bioavailability than these from plant sources. Iron, selenium, zinc are the most important not only because of the significant concentrations in which they are found but also due to their high bioavailability. Iron is an essential mineral involved in gas exchange at the tissue and cellular levels via the oxygenation of haemoglobin in red cells and myoglobin in skeletal muscle. This mineral is present in meat and meat products as non-haem iron (nheFe) and haem iron (heFe) being the most efficiently absorbed form from the diet (Ortega-Barrales and de Córdova, 2015). In the study by Lombardi-Boccia et al. (2002) have been found that the percentage of heFe in chicken, turkey, beef, lamb, pork, rabbit and ostrich meats were 38, 42, 87, 75, 62, 56 and 72%, respectively. Therefore, beef and lamb are among the most iron-rich meat source. Regarding selenium, is important to highlight that it is an antioxidant mineral, and is known to influence the production of feathers and the maintenance of cellular integrity in tissues in avian species. Different forms of selenium are available for supplementation in animal and poultry feed: inorganic sodium selenite or selenate, and yeast-derived selenium. The latter is incorporated into small peptides and amino acids, such as selenomethionine, selenocysteine, and selenocystine. The organic form from yeast is similar to the Se compounds found in grains and forages. Animals and poultry have naturally adapted to take up this form from all sections of the gastrointestinal tract by using the amino acid transport mechanism. Organic Se accumulates in tissues such as the liver, brain, and muscle. However, in birds, feathers contain the highest amounts of Se compared with any other tissue, and can be used as an indicator of Se availability because they are the first tissue to accrete Se, if it is available. It has been documented improvements in feathering as a result of Se supplementation, particularly from the use of organic forms (Perić et al., 2009).

Moreover, is worth to mention that Se-rich meat is more juicy, crispy, and better looking. For animal fodder enrichment, Se is used in combination with other antioxidants, such as tocopherol (vitamin E) (Suchý et al., 2004). While zinc has been used as a growth stimulator of farm animals for several decades, and is important as an activator of significant enzymes and hormones. Interactions exist among zinc and other elements (Herzig et al., 2009). Furthermore, zinc has been known to be an essential nutrient for animals for many years. It was shown that Zn acts as an antioxidant which reduces the cell membrane damage due to free radicals, which in succession changes the immunological status of the animal (Karamouz et al., 2011). In Western societies, more than 70% of zinc consumed is provided by animal products, especially meat. Liver and other organ meats are particularly rich in this element. Zinc content in mixed dark and white chicken meat falls within a range of 8.5 to 9.0 mg/kg of the edible portion, and 100 g provides, roughly 6% of the daily value. Other foods that contain high levels are seeds and nuts, as well as whole-grain cereals. However, these and other plant foods also contain phytate, which can decrease the bioavailability of the element (Soriano-Santos, 2009).

2.2. Physicochemical properties

2.2.1. pH

According to many studies the ultimate pH is one of the most important indices of meat quality. Together with meat color, pH should be used in a standard evaluation of meat (Węglarz, 2010). The pH of muscle/meat is a measurement of acidity. In the chicken, normal pH values at 15 min *post-mortem* (pH_{15}) are around 6.2 to 6.5, whereas normal ultimate pH (pHu) values are around 5.8. The acidification process depends on the rate of the glycolysis (Duclos et al., 2007). Moreover, muscle glycogen concentration at the time of slaughter is one of the most important factors affecting meat quality, including pH (Šimek et al., 2003), but measurement of this polysaccharide concentration in the muscle is complicated. Cutting or touching the muscle activates the muscle metabolism, specifically glycogen breakdown. Thus, measured muscle-biopsy glycogen concentration is always lower than actual muscle glycogen concentration. Glycolytic potential provides a more accurate measure of glycogen level in the living muscle. After slaughter, the muscle converts glycogen into lactic acid and energy. Lactate formation reduces pH. However it is worth to complete that muscles with the same lactate concentration may have a different pHu. The reason why the same lactate

concentration results in a different pHu is not clear. Possibly, different muscles have different buffering capacity and/or differ in the concentration of strong ions such as Mg^{+2}, Ca^{+2}, and Cl^{-} (van Laack, 2000). Taking into consideration buffering capacity, it is important to mention that light muscles (breast muscles) usually have a notably better buffering capacity than dark muscles (leg muscles). The principal difference in the buffering capacity of different types of muscles is that white fibres have a higher content of histidine compounds than red ones. Besides buffering capacity of meat, the pH value is also influenced by other acids, especially free amino acids. The total amount of all acids is expressed as titratable acidity of meat (Šimek et al., 2003).

The rate and the extent of pH decrease during rigor mortis are influenced by intrinsic factors such as species, breed, muscle and animal variability, as well as extrinsic factors such as environmental temperature. It has been observed that heat stress of broilers increases the rate of *post-mortem* glycolysis that may produce pale meat color with low pH. While, muscles derived from cold-stressed birds showed pH about 6.06 and lower shear values resulting into meat with good quality characteristics (Babji et al., 1982). Moreover, it has been demonstrated that diet can affect the glycolytic potential of breast muscles and the pH of meat. Guardia et al. (2014) studied the impact of inclusion of different dietary levels of lysine and other amino acids on the pH of breast meat and found that lower pH values were observed in broilers fed lysine-deficient diets containing a high amount of other amino acids. The pH value also depends on post-slaughter factors, i.e. meat thermal processing, term of this procedure (pre- or post-rigor mortis). It decreases by about 0.15 when the meat is warmed from 20 to 38^{o}C and it increases by about 0.2 when the meat is cooled from 20 to 0^{o}C (Swatland, 1994). Young et al. (1993) reported that pH of pre-rigor muscle declines during cooking, whereas that of post-rigor muscles increases during cooking. These data suggest that cooking poultry meat before completion of the rigor process can lead to sufficient pH change such that the temperature required for myoglobin denaturation increases above that normally achieved, and the myoglobin is inadvertently protected.

Currently, pH is normally measured electrochemically in the carcass using either glass electrodes or solid state electrodes (Andersen et al., 1999). However, pH electrodes, no matter how carefully are used, sometimes may give erroneous reading when they are pushed into a piece of meat (Swatland, 1994). Among the possible alternative methods that are available for measuring pH in meat are the optical methods. Contrary to electrochemical methods which are based on the establishment of a

chemical equilibrium at the electrode surface, optical/spectroscopic methods are distinguished by giving an immediate response. Moreover, the spectrometer working in the visual or near infrared spectral region will register differences in absorbance patterns due to changes in e.g. inter-molecular forces or structural changes in the meat at different pH levels (Andersen et al., 1999).

2.2.2. Water-Holding Capacity

Water is most important as constituent of almost all foods. However, taking into consideration meat production it is inevitable that water will be lost from the carcass. This is a key concern for meat producers as this water content is said to contribute to the juiciness and tenderness of meat products, which impacts on consumer opinion, thus affecting demand and saleable value (Mason, 2016).

Lean muscle contains approximately 75% water, that is held as a lubricant, as well as a medium to transport metabolites in the fibre (Puolanne and Halonen, 2010). The majority of water in muscle is placed within the structure of the muscle and muscle cells (Huff-Lonergan and Lonergan, 2005). Moreover, water in can exist in three forms: (a) bound; (b) immobilized and (c) free water (Mason, 2016). Bound (structural) water includes water directly attached to the protein molecules that is no longer available as a solvent. In muscle food, it usually amounts to 5-10 g water per 100 g of protein (Barbut, 2015a). Meanwhile, immobilized (also referred to as entrapped) water makes up to 80% of the water in muscle and is held within the myofibrillar structure, between the myofibrils, and between the myofibrils and the sarcolemma. Due to changes in muscle structure and pH that occur during the transformation of muscle to meat, immobilized water can escape from the muscle along with the free water as drip loss (Bowker and Zhuang, 2013). Free water, on the other hand, is held loosely in the capillary space between and within proteins and, unlike bound and immobilized water, is easily lost (Mason, 2016). Moreover, this form of water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found (Huff-Lonergan and Lonergan, 2005).

An important property of fresh meat is the ability to retain inherent water, so called water-holding capacity (WHC). It is a very important quality attribute which has an influence on product yield, but is also important in terms of eating quality (Cheng and Sun, 2008). Thus knowledge of factors of influence on the WHC of meat has considerable economic interest. Moreover, investigations into the causes of changes in

WHC of meat teach us about alterations in muscle proteins, especially the myofibrillar ones, which play the most important role not only in the function of the muscle but also in its WHC (Honkiel, 2004). During the growth and development of meat animals, genotype and animal diet are important due to their direct influence on muscle characteristics. Aforementioned diet, has direct effect on intramuscular fat level in the animal body. This information is important, especially considering the fact that the muscle having a high content of intramuscular fat tend to have a high water-holding capacity. The reasons for this effect are unknown; possibly the intramuscular fat loosens up the microstructure, thus allowing more water to be entrained (Lawrie, 1985). Meanwhile, in the pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC (Cheng and Sun, 2008). As was already explained, the stress affects the pH of meat, that is an important determent of WHC. Denaturation of the sarcoplasmic proteins is worsened the faster the rate of pH fall. A fast rate of pH fall will increase the tendency of actomyosin to contract as it forms and thus express to the exterior fluid which has become dissociated from the proteins. When a fast rate of pH fall *post-mortem* is due to elevated temperatures the enhanced loss of WHC observed is partly due to increased denaturation of the muscle proteins, and partly to enhanced movement of water into extracellular spaces (Lawrie, 1985). In the post-slaughter period chilling, ageing, injecting non-meat ingredients, as well as tumbling have important influences on WHC. Furthermore, cooking and cooling procedures for the final meat products can also affect the WHC of the product, in particular the cooking and the cooling methods, the heating and the cooling rate, the cooking temperature, and the endpoint temperature (Cheng and Sun, 2008). Regarding the impact of post-slaughter procedures, Lesiak et al. (1996) studied the effect of *post-mortem* temperature (0, 12, and 30^{o}C) and time on the water-holding capacity of hot boned turkey breast and thigh muscle. They found that higher temperature and longer storage time induced greater drip losses in breast. Longer storage time induced greater drip losses but least drip loss occurred at 12^{o}C in thigh muscle.

The WHC of meat can be detected within a few minutes or an hour. Water-holding assessment comprises: filter paper press methods (e.g. method of Grau and Hamm, 1953), centrifugation methods (e.g. method of Honkiel and Hamm, 1987), and suction loss methods (e.g. method of Fischer et al., 1976). With these methods the amount of water released is far higher than with methods without external force as the

pressure applied enforces the release of water from the intra- and extracellular space of the muscle structure. In drip loss measurements only extracellular water exudes from the meat. Therefore, a factor must be known to evaluate the actual drip loss of the meat (Honkiel, 2004).

2.3. Sensory aspects

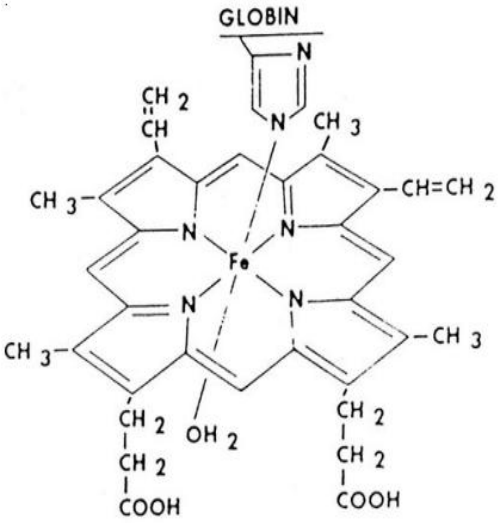
Meat quality can be expressed by determining a number of its properties of which, for the consumer and manufacturer of meat products, the most important ones include: color, juiciness, taste, smell and texture (Pospiech et al., 2007).

2.3.1. Color of meat

Poultry meat color is a critical food quality attribute. Color is important for both the consumer's initial selection of a raw meat product in the marketplace and for the consumer's final evaluation and ultimate acceptance of the cooked product upon consumption (Fletcher, 1999). Consumers will often reject products in which the color varies from the expected normal appearance (Qiao et al., 2001). Thus, it is very important to improve color stability because it will increase the shelf life of meat and meat products by increasing the time that meat will be visually accepted by consumers at the point of purchase (Font-i-Furnols and Guerrero, 2014).

Primarily responsible for the meat color is the sarcoplasmic heme protein (myoglobin) which from chemistry point of view is species specific (Surendranath and Poulson, 2013). Myoglobin (Figure 2.6) is the pigment most responsible for the color of meat, though hemoglobin (the oxygen-binding protein in blood, which has considerable homology) may also be present in small quantities (King and Whyte, 2006).

Figure 2.6. Chemistry of myoglobin (adopted from Chaijan, 2008).



Myoglobin is a water-soluble protein containing 8 α-helices (A–H) linked by short nonhelical sections (Mancini and Hunt, 2005). A prosthetic heme group of myoglobin containing a centrally located iron atom is positioned in the protein's hydrophobic core. Of the six bonds associated with this iron atom, four connect iron to the heme ring, the 5th attaches to the proximal histidine-93, and the 6th site is available to reversibly bind ligands including diatomic oxygen, carbon monoxide, water, and nitric oxide (AMSA, 2012). Mentioned heme group gives myoglobin and its derivatives their distinctive color. The structure and chemistry of the iron atom have an impact on the reactions and color changes that myoglobin undergoes. The oxidation of ferrous-oxymyoglobin (Fe^{2+}) to ferric-metmyoglobin (Fe^{3+}) is responsible for discoloration of meat during storage. Ferrous iron (Fe^{2+}) can react with molecular oxygen to produce superoxide anion (O_2^-) with concomitant oxidation to ferric iron (Fe^{3+}). Hydrogen peroxide (H_2O_2), which may be produced by dismutation of O_2^-, can react with Fe^{2+} to produce hydroxyl radical (OH). This reaction termed the Fenton reaction is the principal mechanism for myoglobin oxidation (Chaijan, 2008).

Myoglobin exists in three redox forms, each producing a distinctive color. In living tissue, the physiologically active oxymyoglobin (oxyMb) and deoxymyoglobin (deoxyMb) forms are maintained through the activity of metmyoglobin (metMb) reductase enzymes (Warriss, 2000). Mentioned forms of myoglobin produce different colors, resulting in purple (deoxymyoglobin), red (oxymyoglobin), and brown (metmyoglobin) meat (Hunt and Zenger, 2003). Deoxymyoglobin occurs when no ligand is present at the 6th coordination site and the heme iron is ferrous (Fe^{2+}). This

results in the purplish-red or purplish-pink color typically associated with vacuum packaged product and muscle immediately after cutting. Oxygenation occurs when myoglobin is exposed to oxygen (Mancini and Hunt, 2005). Oxygenation of deoxymyoglobin forms a bright-red color via the formation of oxymyoglobin, which has diatomic oxygen attached to the 6^{th} coordination site of ferrous iron (Fe^{2+}). The oxygen ligand also interacts with the distal histidine-64, producing a more compact protein structure than deoxymyoglobin. Carboxymyoglobin formation occurs when carbon monoxide attaches to the vacant 6^{th} position of deoxymyoglobin. Atmospheres containing O_2 will result in the conversion of carboxymyoglobin to either oxymyoglobin or metmyoglobin (AMSA, 2012).

Taking into consideration color of meat is important to mention about meat color variations and their related problems that occur in the poultry industry. Color defects of raw and cooked poultry meat have been a problem for the poultry producers for many years (Qiao et al., 2001). Among most important meat defects connected with color deterioration are PSE and DFD. PSE is the acronym for Pale, Soft, and Exudative, which indicate that the meat is pale or yellowish, flaccid or soft, and exudative or wet. The first studies on PSE-like condition were carried out with pigs, and then expanded to turkey. In practice, it results from poor and stressful handling of animals ante-mortem, causing an acceleration of rigor mortis (Garcia et al., 2010). Acute or short term stress that can lead to PSE include the use of electric goads, fighting among animal just before sticking, beating of animals prior to slaughter and overcrowding in the lairage (Adzitey and Nurul, 2011). PSE meat is characterized by low moisture retention, soft texture, and light appearance (Barbut et al., 2005). Moreover, PSE is also connected with pH usually lower than 5.8 combined with high muscle temperature - usually higher than 35°C - at the beginning of rigor mortis. This is due to the rapid metabolic transformation of glycogen into lactic acid, which results in achieving ultimate pH before carcass cools, causing protein denaturation, and consequently, meat becomes pale, soft, and exudative and have its functional qualities compromised (Garcia et al., 2010). Meanwhile, from animals exposed to pre-slaughter stress, is obtaining meat with DFD (Dark-Firm-Dry) defect. DFD meats can occur when animals are exposed to chronic or long term stress before slaughtering (e.g. transportation animals over long distances, long hours of food deprivation, and overcrowding of animals in the transport car over a long period of time). This kind of stress leads to the depletion of stored glycogen, thus less glycogen is available *post-mortem* affecting the normal process of acidification and leaving the pH

of meat at high level. The high value of pH results in relatively little denaturation of proteins, water is tightly bound and little or no exudates is formed. The muscles of animals after chronic pre-slaughter stress absorbed light making the meat appear darker (Adzitey and Nurul, 2011).

Color of meat depends on numerous factors. First of all, as was mentioned in case of meat defects, one of the strongest agent that influences meat color is stress, both acute and chronic. Stress is the most frequently identified factor in the pre-slaughter handling of animals. It negatively affects meat quality, which results in economic losses (Węglarz, 2010). Secondary, it has been observed that the composition of muscle fibers influences meat color via the amount and the chemical state of myoglobin. The high myoglobin content of type I and type IIA fibers (oxidative fibers) results in a positive relationship between the proportion of these fibers and red color intensity. In contrast, a high proportion of glycolytic fibers results in the production of white meat, as found in chickens (Listrat et al., 2016). Available literature regarding birds has shown that myoglobin and hemoglobin levels were lowest in the glycolytic muscles, *Pectineus* and *P. superficialis*, and highest in the oxidative *Adductor* muscle and the heart (Kranen et al., 1999). Furthermore, the color of meat can be controlled by birds diet. Most of researchers agreed that the high b* values observed in meat from slow-growing and organic broilers, might be derived from the consumption of green grass. It was demonstrated that herbal intake, in addition to consumption of feed mixture, causing a rise in the yellowness of meat due to the high carotenoid pigment content (Küçükyılmaz et al., 2012). Among feed additives that can influence meat color are probiotics. Karaoğlu et al. (2006) have observed that the use of probiotic in broiler diets had significant effect on the carcass color traits, thus it can positively affect acceptability of consumer.

Moreover, Le Bihan-Duval et al. (1999) have demonstrated that color parameters (L* a* b*) of meat are characterized by the greatest inheritance out of all traits determining meat quality and, thus, are dependent on birds genotype. The effect of sex on meat color has been observed in the study described by Damaziak et al. (2013). They reported that the meat of hens was more light then males, which was indicated by lower values of a* (redness) parameter and higher values of L* (lightness) and b* (yelloness) parameters. According to Sirri et al. (2009) this may be attributed to differences in the metabolism of muscle fibers, which is affected by sexual hormones,

because these authors demonstrated significantly higher values of b* and lower values of a* in muscles of caponized male chickens compared to testosterone-producing males.

The color of meat is influenced also by heat. While, heme proteins, mostly myoglobin, constitute only small part of the wet weight of meats, the response of these pigments to heat largely determines the color of cooked meat. Heating causes denaturation of the globin, which then precipitates with other meat proteins. Denaturation of myoglobin begins between 55°C and 65°C in meat (King and Whyte, 2006). It's important to mention that the denaturation temperature for difference redox forms of myoglobin is not constant; therefore, the color of cooked product interiors is not necessarily a reliable indicator that meat has been cooked sufficiently to ensure safety. Myoglobin's denaturation temperature depends on the protein's redox status (AMSA, 2012). Deoxymyoglobin is the least sensitive to heat denaturation, followed by oxyMb, then metMb, though the latter 2 have fairly similar heat sensitivities (King and Whyte, 2006). Color of muscle is also affected by pH, as pH controls the chemical state of myoglobin. It has been reported that the isoelectric point (PI) of myoglobin is near 7.0. Apparently, myoglobin is less heat sensitive near its PI than at remote pH values (Young et al., 1996). Also time *post-mortem* can influence meat color. Young et al. (1996) observed that meat aging decreased the degree of redness and increased myoglobin denaturation in the cooked product. Except the heat and pH, the meat color depends on anatomical location of the muscle (muscle type).

The color of meat can be measured visually, chemically, or instrumentally. Visual assessments usually involve a panel of two or more trained people who assign values according to a preset descriptive scale. Chemical assessments typically measure the concentration of heme proteins as extracted from a sample. Several methods have been described to measure the content of hemoglobin and myoglobin in muscle tissue. The methods are based on the physical, biochemical, and immunological properties of the proteins and the heme groups. Hemoglobin and myoglobin can be measured by methods specifically quantifying each protein or, after separation of the proteins, by quantifying heme (Kranen et al., 1999). The third groups of method aimed to measure the meat color are instrumental methods. A typical instrumental technique uses a color meter to define color in terms of the values L∗ (lightness), a∗ (redness), and b∗ (yellowness), commonly referred to as the Hunter values (AMSA, 2012). The Hunter L, a, b color scale can be used on any object whose color may be measured. It is not used as frequently today as it was in the past because the CIE L*a*b* scale, which was

released in 1976 by Commission Internationale de l'Eclairage, has gained popularity. Despite this fact, the use of both color scales with practice can easily lead to understanding and communication of color values. Moreover, both hunter L, a, b and CIE 1976 L*a*b* (CIELAB) are scales based on the Opponent-Color Theory. This theory assumes that the receptors in the human eye perceive color as the following pairs of opposites:

- L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light,
- a scale: Red vs. green where a positive number indicates red and a negative number indicates green,
- b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue (HunterLab, 2012).

Further optical values can be calculated from these measurements, such as chroma (saturation index; $(a^{*2} + b^{*2})^{½}$) and hue angle ($\tan^{-1}$ (b∗/a∗)) (King and Whyte, 2006). Mentioned saturation index refers to how vivid or dull the color is. Hue is the color description as we communicate it in language (red, yellow, green, blue, etc.). Hue is developed by the specific wavelengths reflected from a meat surface back to the detector (AMSA, 2012).

2.3.2. Palatability of meat

Meat palatability describes the overall eating experience achieved when consuming a meat product. Palatability is affected by three following factors: tenderness, juiciness and flavor. All these characteristic belong to the sensory attributes of meat and meat products, thus play key role at moment of consumption of food product.

2.3.2.1. Tenderness

Tenderness is one of or the most discussed features in meat; it is one of most important meat texture attributes which affects the perception of meat, by the customers. Tenderness of meat may be simply defined as the ease of teeth to cut meat fibers during mastication. The word tenderness is often used in reciprocation of its antonym, toughness (Xiong et al., 1999). Taking into consideration tenderness of meat is important to highlight that *post-mortem* changes are similar in different species, but the time-scale differs considerably. Rigor lasts more than 24 hours in cattle and sheep,

but only 6 hours in chicken breast muscle. Minimum toughness is reached after 8 days in cattle, whereas 18 hours are enough in the chicken (Lee et al., 2008).

The three factors that determine meat tenderness are background toughness, the toughening phase and the tenderization phase. The toughening and tenderization phases take place during the *post-mortem* storage period, but background toughness exists at the time of slaughter and does not change during the storage period. The background toughness of meat is defined as the resistance to shearing of the unshortened muscle and variation in the background toughness is due to the connective tissue component of muscle. While the toughening phase is similar in all carcasses under similar processing conditions, the tenderization phase is highly variable (Luciano et al., 2007).

Tenderness is governed by *post-mortem* biochemical processes, particularly proteolysis. There are several proteolytic systems that could have a potential role in *post-mortem* proteolysis and meat tenderisation, including the cathepsins, the multicatalytic protease (or proteasome) and calpains. However, it is generally believed that the calpain system plays a major role in this *post-mortem* degradation. Calpains are probably the most extensively studied protease family with regard to meat science and it is widely accepted that calpain-mediated proteolysis does play a major role in the process of meat tenderization (Parr et al., 2007). Calpains are a superfamily of 14 cysteine proteases, but the system of calpains in a skeletal muscle consists of at least 3 proteases: calpain I (μ), calpain II (m) and calpain 3 (p94), as well as calpastatin-being a calpain inhibitor. The level of calpains depends on the species of animals, their breed, and the type of muscle and its activity (Nowak, 2011). In muscle *post-mortem*, only μ-calpain is autolyzed as calcium is released and therefore seems to be involved in the tenderization mechanisms, unlike m-calpain, which remains intact for several days after slaughter because it requires high calcium concentrations. It has been noted that the distribution of these 2 calpains in chicken varies from one tissue to another. These chicken calpains are also more calcium-sensitive than mammalian calpains and may, therefore, play a special role in the *post-mortem* process in chicken muscle (Lee et al., 2008). However, it must be added that also other novel proteolytic systems can affect meat tenderness. Recently, several reviewers have reexamined the role of proteolytic systems in *post-mortem* proteolysis and meat tenderisation and have proposed that the caspase family of proteases could be activated *post-mortem* and influence the rate of tenderisation. Caspases are a family of intracellular cysteine aspartatespecific proteases. Firstly caspases are endogenous to skeletal muscle fibres and secondly it appears that

they have the ability to degrade myofibrillar proteins. Moreover, caspases are present in a range of muscle types and their activity could be found at and around the point of slaughter (Parr et al., 2007).

Objective measures of meat tenderness have commonly used Warner-Bratzler shear method, that was developed in 1932 by Lyman J. Bratzler and since the 1950's it is widely used to determine the tenderness of meat products, fish and cakes (Guzek et al., 2013). The Warner-Bratzler device consists of a rigid frame supporting a shear bar. Interchangeable blades fit into the frame. For meat testing, a triangular slotted blade is used. Samples of meat are cooked, cooled and then 6 core samples taken, parallel to the longitudinal orientation of the muscle fibers. The maximum shear force is the highest peak of this curve. Although the Warner-Bratzler method has been in use for some time, many institutions used variations on the test protocol, so there was variation in results from institution to institution (Ross and Keeping, 2008).

2.3.2.2. Flavor

Flavor of meat is one of the most important factors in determining the acceptability of food. Overall, flavor is a combination of taste and smell, which are perceived by the taste buds and olfactory receptors in the nose, respectively. Flavor and taste perception mechanisms are complex and are still not fully understood. It is known, however, that they are affected by numerous factors such as the quantity and ratio of different flavor compounds, fat content, and temperature. Taste is perceived by sensors on the tongue that are capable of detecting four major tastes: salty, sweet, acid, bitter. Other sensations such as "umami" (a Japanese term meaning deliciousness), astringency, metallic and pain ("hot" and "cold") are also known (Barbut, 2015b).

Raw meat has little aroma and only blood-like taste, meat develops its aroma flavor characteristics during cooking as the result of complex interaction of precursors derived from both the lean and fat compositions of meat generating volatile flavor compounds that contribute to meat flavor. There is a large number of these compounds contributing to the flavor characteristics of cooked meat (Ba et al., 2012). Among the flavor and aroma compounds found in meat are hydrocarbons, aldehydes, ketones, alcohols, furans, thriphenes, pyrrols, pyridines, pyrazines, oxazols, thiazols, sulfurous compounds, and many others that have been identified. As mentioned above, significant changes take place in the flavor of meat during cooking. The main reactions involved during cooking that are responsible for flavor developemnt are Maillard reaction,

thermal degradation of lipids and Maillard-lipid interactions (Jayasena et al., 2013). The Maillard reaction is one of the most important processes that takes place in food processing and storage. It is a non-enzymatic interaction between reducing sugar and amino acid, peptide or protein, resulting in a variety of by-products, intermediates and brown products, which contribute markedly to the aroma, taste and color, as well as to the antioxidant potential of stored and processed foods (Phisut and Jiraporn, 2013).

The flavor of chicken meat was studied by Gasser and Grosch (1990) who identified 2-methyl-3-furanthiol, 2-furfurylthiol, methionol, 2,4,5-trimethyl-thiazole, nonanol, 2-trans-nonenal. The most important compound in chicken flavor is 2-methyl-3-furanthiol, generated from the Maillard reaction and lipid oxidation. Formation of 2-methyl-3-furanthiol in chicken broth via Maillard reaction involves interaction between ribose and sulphur-containing amino acids (cysteine or cystine) or peptide (glutathione). (Jayasena et al., 2013).

Available literature has shown that there is a wild range of pre- and *post-mortem* factors affect the flavor of chicken meat. The effect of chicken origin has been evaluated numerous times, among others by Lee et al. (2012). They assessed the components related to flavor and taste in commercial broiler and Korean native chicken meat. Their findings revealed that the thigh meat from Korean native chickens showed higher contents of arachidonic acid and docosahexaenoic acid compared to commercial broilers. In addition, flavor contributing amino acids, including aspartic acid, threonine, serine, glycine, alanine, tyrosine, lysine, and arginine, were significantly higher in breast meat from Korean native chickens. Also in the study described by Horsted et al. (2010) was assessed the influence of breed on meat flavor. In their research the sensory profiling of male broiler breast meat were carried out to evaluate the effect of two very different broiler breeds (JA757 and New Hampshire), two different feed types (broiler and grower feed) and age at slaughter (82 and 110 d). They found a very distinct difference in sensory profile between the two breeds. The effect of sex on chicken meat flavor was demonstrated by many researchers, however the results were not consistent. Meat from male birds received higher scores for flavor as opposed to that from female birds (Ramaswamy and Richards, 1982; Farmer, 1999). Although, it was also recorded that the breast and leg meat of female birds were preferred to these of male birds. Other studies have found that the sensory quality of broiler meat depended on diet ingredients and the nutrient content of the feed. For instance, Jang et al. (2008) indicated that taste of breast meat from broiler chickens which were fed a dietary medicinal herb extract

mix (at 0.3% level) consisting of mulberry leaf, Japanese honeysuckle, and goldthread at a ratio of 48.5:48.5:3.0 scored higher than control meat. Meanwhile, Lyon et al. (2004) examined the effect of diets with wheat or maize as the major carbohydrate source on commercial broilers with processing ages between 42 and 52 d. It was found that breast meat from wheat-fed broilers was harder, more cohesive, and chewier and had a larger particle size than meat from maize-fed broilers. Moreover, ‘‘brothy’’ scores were significantly higher in the meat from maize-fed broilers. The composition and amount of fat also determine the intensity of flavor in meat (Muchenje et al., 2010).

Except above mentioned factors, the “level” of meat flavor depends on other meat quality traits, e.g. pH. It is one of the important factors that influence the kind of volatile flavor compounds formed in the Maillard reaction, and then determine the final flavor characteristics of cooked food. It has been showed that as pH increases, color and polymeric compounds increase and nitrogen-containing compounds like pyrazines are favored, therefore it was assumed that higher ultimate pH in meat from grass-fed animals may favor the formation of thiazoles and thiophenones due to the availability of amino acid degradation products while decreasing other sulfur volatiles that favor lower pH (Ba et al., 2012). Also post-slaughter factors affect the meat flavor. The importance of ageing in the formation of flavor precursors has been emphasised, and have been suggested that microbial and enzymatic changes in the muscle alter the flavor profile of the meat (Elmore and Mottram, 2009). It has been noted that an increase in free amino acids during conditioning is responsible for meaty taste. These components serve either directly as flavor components or as a pool of reactive flavor intermediates that form many of the characteristic meat flavors after cooking (Jayasena et al., 2013).

2.3.2.3. Juiciness

Meat juiciness is the wetness during first bite and sustained juiciness likely due to fat in meat. Its sensation in chevon is closely related to the quantity and composition of the intramuscular fat and age of an animal (Ngambu et al., 2012). Until now, juiciness is still the least understood sensory trait of meat (Pearson, 1997). Sensory juiciness can be broken down into two parts: initial and sustained juiciness. Initial juiciness is the wetness during the first few chews produced by a rapid release of meat juices, while sustained juiciness is caused by fat in the sample that causes a slow release of saliva after continued mastication (reviewed by Woolley, 2014). It has been determined that initial wetness and overall juiciness could be combined into a single

attribute (Pearson, 1997). The initial juiciness of meat is related to water release during chewing, whereas final juiciness is determined by the lipid content of meat (Mikulski et al., 2011).

Juiciness increases flavor, helps soften meat - making it easier to chew, and stimulates saliva production in the mouth (FAO, 2014b). Value of this traits is influenced not only by meat-related factors (water, lipid content), but also by physiological and psychological factors inherent to individual tasters. Thus, earlier research separated initial and sustained juiciness (Juárez et al., 2012). Moreover, meat juiciness plays a key role in meat texture contributing between 10% and 40% to its variability. Unlike other key aspects of texture, juiciness remains a uniquely subjective property (Winger and Hagyard, 1994). Important is also the fact, that profound effect on the meat juiciness has cooking temperature (NIIR Board, 2005). End-point temperature is an important determinant of juiciness, and has been proposed as a method for obtaining meat of different juiciness levels for taste-panel training (Winger and Hagyard, 1994).

Sensory attributes, including juiciness, are difficult to measure and often require the use of taste panels to assess the complex parameters involved in the eating experience. Sensory analysis is unequivocally assigning in the scientific methods. It is one of the oldest means of quality control, but in principle is an essential part of the mandatory assessment of food quality, while also examining the deeper study of the interdependence between physiological and psychological phenomena in the very process of perception of sensory qualities. Many authors note that the sensory analysis, allowing manufacturers to identify, understand and respond to consumer preferences more effectively and in addition the identification of sensory characteristics and consumer preferences, helping manufacturers to increase competition in the market for other producers (reviewed by Adeyemo and Sani, 2013).

2.4. Microbiological aspect of meat

Muscles of healthy animals are regarded as sterile, but the slaughtering process of animals, including poultry, provides bacteria with an opportunity to colonize meat surfaces. It has been demonstrated that that microbiological contamination is more common than both microphysical and chemical contaminations. The relative number of illness due to foodborne microorganisms makes microbiological quality the most important food safety factor (Alum et al. 2016). People become infected by consuming

inadequately cooked poultry or other foods that become cross-contaminated via contact with poultry. Even infants riding in shopping carts containing raw poultry are at increased risk (Patrick et al., 2010). Food contaminated by pathogens or chemical substances is a serious issue because it can lead to a wide range of health problems. This is responsible for more than 200 diseases, including typhoid fever, diarrhea and cancers, and can lead to the death of unsuspecting. Besides diseases and death, the consumption of pathogen contaminated foods also creates economic impact that can be quite devastating on the food dealers, food companies, and ultimately on the on the consumers (Alum et al., 2016).

Contamination of meat is a continuing possibility from the moment of bleeding until consumption. In the abattoir itself there are many potential sources of contamination of meat by microorganisms, e.g. the contents of the gastrointestinal tract (if inadvertently released during dressing operations), airborne contamination, aqueous sources, various vessels and receptacles, and the personnel (via knives, equipment, the hands of workers) and also by cross-contamination from carcass to carcass (Holzapfel, 1998; Mohamed-Noor et al., 2012). Studies carried out on poultry species over the last few years show that the sites most heavily contaminated are the neck skin and less frequently on the back and the area around the vent. Fewer organisms are found around the breast, legs and under the wings. The presumable reason for the neck skin being the most heavily contaminated is that the washings from the rest of the carcass run down the neck while the carcass hangs on the conveyor (Mohamed-Noor et al., 2012). Among agents that are responsible for the microbiological meat contamination is pH of meat. It is well-known that microbial growth depends strongly on the pH of meat. In particular, breast meat characterized by high pH (> 6.0) is more likely to become contaminated with microbial growth and can trigger different types of spoilage microorganisms that impair taste, flavor and appearance (Petracci et al., 2015).

Healthy broilers entering slaughter processing might be highly contaminated by microorganisms, including food borne pathogens such as *Salmonella* species, *Campylobacter* species (Mohamed-Noor et al., 2012), *Clostridium perfringens*, *Escherichia coli* 0157 and *Listeria monocytogenes* (Mead, 2004b). Aforementioned *Salmonella* is one of the major foodborne causes of gastroenteritis and is frequently associated with contaminated poultry meat (Heyndrickx et al., 2002). Also *Campylobacter spp.* is recognized worldwide as important food pathogen (Granić et al., 2009). Thermotolerant *Campylobacter jejuni* is responsible for extraintestinal forms

(meningitis, peritonitis, pancreatitis, unrinary infections, neonatal sepsis and abortion) and some chronic immunomediated diseases (endocarditis nodal fever, reactive arthritis) (Salihu et al., 2009).

Meat and processed meat of broiler chickens can be contaminated also by *C. Perfringens* (Guran and Oksuztepe, 2013). In broiler chicken, it is responsible for necrotic enteritis, an acute enteric disease, and for a subclinical disease with focal necrosis in the intestine or *C. perfringens*-associated hepatitis, with cholangio hepatitis or fibrinoid necrosis in the liver (Silva, 2009). Meats are also common source of *Escherichia coli* contamination. *E. coli* is a normal inhabitant of the gastrointestinal tract of humans and animals; however, some strains are known to be pathogenic. These strains induce colibacillosis in chicken, which is an important cause of economic losses for the poultry industry (Furtula et al., 2010).

The most important factor in chicken meat quality is prevention from microbial contamination. Many broiler flocks can become infected with mentioned bacteria at many stages of the poultry production chain. Therefore, the only intervention strategy to reduce the exposure of humans to these microorganisms seems to be an integrated approach, with multiple control measures along the poultry production chain, for instance at farm level, during transport, at the slaughterhouse and/or at the product transformation step. Measures that are important to protect the flock include the washing of hands, the wearing of protective clothing and dedicated footwear, the respect of house cleaning and disinfection protocoles, feed and the removal of spent litter between two flocks (Vandeplas et al., 2008). Considering, the pre-slaughter period, the feed withdrawal deserve on special attention. It is a known procedure to lower the risk of contamination with faeces prior to slaughter, and the emptied intestinal tract also decreases the incidence of contamination of meat during dissection (Haslinger et al., 2007). Moreover, among post-slaughter methods used againt microorganisms in the chicken meat, are cooling, vapour-vacuum system, vapour pasteurization. Also, chemicals like chlorine and chlorine compounds, ozone, organic acids, trisodium phosphate are being widely used for decontamination purposes (Canan et al., 2007).

CHAPTER III

FREE-RANGE POULTRY PRODUCTION

3.1. Definition and basic assumptions

Currently, taking into account the animal production, have been observed growing interest in animal welfare. It is a multi-faceted issue which implies important scientific, ethical, economic and political dimensions. The term ‘welfare’ is not uniformly defined and used in the literature. This may be due to the different attitudes towards animals, but implies also the different methodologies used to evaluate welfare. The definitions of animal welfare proposed by various researchers reflect their different backgrounds. Among the main issues involved in the concept of welfare are the concepts of ‘suffering’ and ‘need,’ as well as the ‘five freedoms’ which are more related to animal husbandry and management by man (Carenzi and Verga, 2009). Recent research findings have indicated that an animal’s welfare is dependent on its genetic characteristics, environmental factors, and genetic-environmental interactions (Muir et al., 2014). Additionally, animal welfare is increasingly viewed as a factor affecting the quality of animal products while being an important tool of marketing strategy (Połtowicz and Doktor, 2011). The management system used in highly productive farms is often subjected to harsh criticism, one of the reasons being its failure to provide adequate welfare. In many countries, this fact has led to the development of poultry meat production under less intensive rearing conditions. Moreover, a survey of consumers, showed that they prefer to purchase food products obtained from livestock animals raised in production systems that are considered more animal welfare friendly, such as free-range system (Połtowicz and Doktor, 2011).

The free-range production systems focuses on low-input strategies and support of rural communities by maintaining the family farm (Olaniyi et al., 2012). Historically, free-range in poultry meant that the chickens were either totally unfenced or were kept in a field so large that the fences had little effect on their movement. More recently, the term “free-range” has been stretched and overused so much that its meaning is almost lost (Plamondon, 2003). Primarily, free-range chickens are known for no containing an artificial ingredients or preservatives and being minimally processed (Naufeld, 2002).

The free-range poultry production is in principle all forms of production in which the birds has access to outdoors fields, and that includes: the organic managed poultry production which is popular in some parts of the industrialized world, Label Rouge production system known from France, and the smallholder poultry production known from the developing countries.

Free-range production of chicken meat, taking place under the French programme termed Label Rouge is known since 1965, mainly for its association with the best quality poultry meat. This program involves all aspects of production from genetic breeders and farmers to processing plants where every part of production is controlled and must follow the Label Rouge requirements (Ferrer and Ames, 2012). The program meets consumer expectations of organic poultry production, and is regarded as providing branded low-fat meat products with exceptional flavor characteristics, highly appreciated by connoisseurs (Mikulski et al., 2011). Furthermore, Label Rouge has been transferred also to USA under the name: pasture-based poultry production (Sørensen and Berg, 2010). A similar concept is the organic system that promotes the use of organic and biodegradable inputs from the ecosystem in terms of poultry nutrition, birds health, their housing and breeding (Chander et al., 2013). Organic production system is defined within the European Union as the system of agriculture that aims to promote sustainable agricultural development. Its objectives are to produce products without chemical residues i.e. avoiding the use of artificial chemical pesticides, fertilizers and antibiotics and applying techniques prevent soil degradation. In the last decades increase of organic farming was observed around the world especially in Europe and United States. In Europe, organic area increased by about 500000 hectares per year in the last decade, and according to Eurostat data, in the EU 27 about 9.6 million hectares were managed organically in 2011 comparing to 5.7 million hectares in 2002 (Abbas and Ahmed, 2015). Aforementioned production systems have some common requirements, i.e.: average daily gain that not exceeds 30-35 g, access to outdoor facilities after 6 weeks of age, limited use of ingredients for feed, lower limit of age at slaughter that is 81 days for the Label Rouge and 56-63 days for organic produced chickens (Sørensen and Berg, 2010).

3.2. Breeds for free-range production

In alternative poultry rearing systems with access to outdoor fields, the pivotal role play the choice of bird strains suitable for free-range conditions. Breed suitability is

important in free-range systems for both broilers and turkeys, because the birds must be able to cope with a wide range of environmental conditions (especially temperature fluctuations and wet weather) and maintain health over a longer growth period (Jones and Berk, 2012). In systems with outdoor access, although a wide range of strains are used, only the slow-growing strains can fully benefit of free-range rearing systems (Castellini and Mourvaki, 2007). Mikulski et al. (2011) reported that compared with fast-growing birds, slower-growing chickens had a lower breast and thigh muscle yield, but they were characterized by higher survival rates at 65 days, a higher protein content and a lower fat content of breast meat. Researchers have suggested that perhaps the growth performance of slow-growing birds is less efficient than that of fast-growing birds, but slow-growing birds are more adapted to natural systems (Fanatico et al., 2005b). Slower growing breeds are often used and generally have less problems with foot pad lesions and hock burn than fast-growing breeds. Moreover, slow-growing breeds also have better walking ability and are more active, thus they are able to conduct more natural behaviors than fast-growing breeds (Jones and Berk, 2012). Simultaneously, most natural and organic poultry production in the United States utilizes the same fast-growing broiler genotype used in conventional production systems (Fanatico et al., 2006).

3.3. Advantages of free-range system

Among main advantages of free-range system are these related with bird health, physiological normality and also their behavior and affective state. Because the intensive system may cause stress and behavioral and physiological abnormalities, which adversely affects productivity and health, this type of technology is often abandoned or at least replaced by free-range system (Sosnówka-Czajka et al., 2010). Compared with the conventional confined system, outdoor systems without any confinement can reduce stress. This observation is very important considering the fact that animal stress is an important cause of reduced performance and increased susceptibility to disease. It has been observed that exposure to stressors for extended durations results in the secretion of the stress hormone corticosterone. If this hormone remains in circulation for long periods, impaired live performance can be expected because of corticosterone-induced gluconeogenesis. Moreover, corticosterone causes impaired immune system function and regression of the lymphoid tissues, often accompanied by an increased heterophil-to-lymphocyte ratio (Virden and Kidd, 2009).

Considering the secretion corticosterone, Pohle and Cheng (2009) demonstrated that level of this hormone increased in the hens housed in the conventional cages, but not in these housed in the furnished cages, suggesting that social stressor induced by housing conditions have a significant impact on the stress responses. Furthermore, stress causes broilers to preferentially metabolize glucose. This results in alterations in carbohydrate, protein, lipid, and mineral metabolism, which in turn causes the depletion of structural protein and the deposition of abdominal fat (Virden and Kidd, 2009).

Outdoor system can also improve skeletal health of birds. It has been noted that the major skeletal health issue of conventionally caged hens, as compared with loose housing systems, is the increased susceptibility to osteoporosis mainly due to lack of exercise. Levels of activity may also explain the improved bone strength of birds that had more floor space during production (Lay et al., 2011). Research conducted by Leyendecker et al. (2001) has shown the effect of poultry system production on bone strength of birds. In their study tibia and humerus breaking strength of a white layer line (Lohmann Selected Leghorn, LSL) and of a brown layer line (Lohmann Tradition, LT) was examined between three hen housing systems, battery cages, aviary and intensive free-range system. Bone strength was investigated at the end of the laying period of the hens aged about 13 months. The highest differences between the LT and the LSL-layer lines were obvious in bone breaking strength for hens kept in battery cages. For both layer lines the bone breaking strength was consistently higher for hens kept in the aviary or in the intensive free-range system as compared to battery cages. Also in the study of Fanatico et al. (2005b) conducted to assess the impact of genotype and outdoor access on growth rate and carcass yield was observed the effect of outdoor system on bone strength. In this experiment, housing system had an impact on bone-breaking strength as indicated by the stronger tibias in the outdoor birds. Authors suggested that perhaps the lower density and exercise in outdoor treatments led to stronger bones.

The favorable effects of the free-range system on the health of birds have also sunlight. Exposure to ultraviolet ray lead to an intensification of metabolism and breathing, increase the number of red blood corpuscles and stimulate the inner glands (Hussein Mahboub, 2004). Light has a stimulating effect on the pituitary, resulting of secretion of FSH and LH, which activates the ovary (Miao et al., 2005). Moreover, sunlight has a positive effect of animal health through its role in making vitamin D, that can influence a wide range of physiological processes. Considering this fact, it's important to emphasize that vitamin D plays an important role in the calcium and

phosphorus metabolism and helps ensure adequate levels of these minerals for metabolic functions and bone mineralization. Moreover, vitamin D sufficiency is pivotal for normal skeletal development. Vitamin D exerts also multiple effects on muscle health. Available literature showed that Vitamin D deficiency is associated with muscle weakness, predominantly in the proximal muscle groups and a reduction in performance speed (Wacker and Holick, 2013).

Furthermore, one of the major perceived benefits of free-range systems is the ability for birds to express the full range of natural behaviours (Lister and Nijhuis, 2012). This advantage is important especially for veterinarians. Behaviour is used widely in the clinical assessment of animal health and in particular in the assessment of pain. One example is the use of gait scores to assess the leg health of broiler. Chickens are observed during walking and is assigned them a score from 0 (healthy leg and normal walking) to 5 (unable to walk) (Dawkins, 2004). Among natural behaviours that can be expressed by birds in housing systems with outdoor access are foraging (e.g. pecking), locomotive (e.g. wing flapping) and resting (e.g. lying, dozing) behaviours, maintenance-comfort behaviour (e.g. preenting, starching, dustbathing, body shaking), social behaviour (e.g. threatening, chasing, crouching, vocalizing) and nest-and-laying behaviour sequence. Moreover, it has been found that access to free-range can allow birds to show complete sunbathing behaviour in direct sunlight while it is not shown in artificial light. Thus, it appears that the access to a free-range area is very important for poultry. The effect of the housing system on birds behaviour was investigated among others by Zhao et al. (2014). This research aimed to evaluate the effects of different two housing systems (indoor housing *versus* indoor with outdoor access) on behavioral activities, welfare and meat quality of two hundred fast-growing broilers. Their general behavior (feeding, drinking, fighting, standing, lying, walking, investigating, dust-bathing and preening) was observed, and tonic immobility, fluctuating asymmetry of legs and wings were measured. The results showed that the indoor-housed broilers with outdoor access had significant higher standing, walking, investigating, dust-bathing and preening than these indoor only. However, death rate in the outdoor run groups was significantly higher than that of the indoor ones.

It is also believed that “natural”, less intensive management systems provide chickens with higher welfare levels, resulting in much better meat quality. Consumers believe that the meat of free-range chickens is healthier than that of birds kept in a poultry house only (Połtowicz and Doktor, 2011). Numerous research findings

confirmed the hypothesis about the positive effects of alternative rearing systems on poultry meat quality (e.g. Chen et al., 2013). Have been observed that high kinetic activities (walking, running, foraging, exploring, crouching at pasture) and environment influence the muscle growth and fibre type composition of birds and consequently affect the overall quality of the meat (Fanatico et al., 2006; Dal Bosco et al., 2012). Among main differences in meat quality attributes between free-range and conventionally-reared chickens are related to flavor, pH, color and texture (Souza et. al., 2011). The potential to effect on flavor may have pasture, particularly if it is designed for poultry. Have been suggested, that flavor of the meat may be modified by the consumption of herbage or live protein. Different forages may result in different flavors, and diet manipulation could offer potential to enhance poultry flavor (Fanatico et al., 2006). Moreover, have been noted that meat from chickens reared with outdoor access characterized by higher value of other sensory traits. It has been confirmed in the study described by Lin et al. (2014). Experiment was conducted to evaluate the effect of an outdoor-grazed raising model on meat composition, physical properties and sensory attributes of Taiwan game hens. Authors of this research observed that birds form the free-range group produced significantly higher taste panel scores than the other groups. Thigh meat of the free-range group had high scores in flavor, juiciness, chewiness and overall acceptability, while their breast meat also had high scores in flavor, chewiness and overall acceptability. These results are in line with these presented by Castellini et al. (2002); they recorded markedly higher taste panel scores for juiciness and overall acceptability of breast meat for the organic rearing chickens than for the chickens that were reared conventional. The effect of housing system was observed in other livestock species. Several studies have shown that meat from lambs grazed on white clover pastures has a more intense flavor than that from lambs grazed on ryegrass (Fraser et al., 1996).

The rearing system can influences pH of meat as well. Broiler strains reared in alternative systems are more adapted to movement, and therefore, present higher capacity of reacting to pre-slaughter management. This may have lead to higher glycogen muscle consumption during the pre-slaughter period, reducing meat acidifying capacity (Souza et al., 2011). The effect of rearing system had been also observed in case of the most significant criteria of carcass selection at purchase- meat color. Mikulski et al. (2011) found that color of the breast and thigh muscles of chickens bred with outdoor access was significantly darker, compared with birds raised in

confinement. Changes in the color of meat in their study were accompanied by a better water-holding capacity of breast muscles and lower juiciness of breast from free-range chickens. Meanwhile, Ponte et al. (2008b) demonstrated that chickens using free-ranges were characterized by a brighter color of meat and a higher contribution of yellow color in muscles, which according to these authors is due to the content of natural carotenoids in green forage ingested by the birds using free-ranges. Moreover, the muscles of free-range birds have a higher level of physical activity, which can result in tougher meat due to increased intramuscular collagen content (Joubert, 2013).

Recent research findings have indicated also that a basic chemical composition of meat is dependent on rearing system. Broilers reared in alternative production systems are encouraged to forage and benefit from the vegetation, thereby increasing their choice of environment and food source (Castellini et al., 2002; Grashorn, 2005; Husak et al., 2008). Hence, one of the principal expectations from outdoor access and feeding on organic diets is improvement in the functional quality of chicken meat. Tougan et al. (2013b) reported that the chickens reared under free-range breeding system had total protein content higher than the chickens bred in confinement breeding system (20.8 % *versus* 20.2 % of raw meat). These results are in line with these presented in research by Bogosavljevic-Boskovic et al. (2010), in which the protein content of breast muscle was significantly higher in both male and female broilers from free-range system than in extensive indoor females. Meanwhile, Castellini et al. (2002) have observed a reduced content of fat (abdominal fat in particular) in free-range broilers as attributable to more intensive locomotor activity. They also reported higher levels of omega-3 and omega-6 fatty acids, and increased levels of total polyunsaturated fatty acids in free-range birds, which enhance meat quality and have consumer health benefits of reducing the risk of different types of cardiovascular disease (Betti et al., 2009). In other farm animals, such as lambs and beef (Enser et al., 1998, Realini et al., 2004) a large effect of grass-based diets on the levels of α-linolenic acid and others n-3 PUFA was observed as well. This positive trend could be partly due to the different compositions of the ingested foods, caused by grass intake.

3.4. Disadvantages of free-range poultry production

The alternative systems of poultry production have also some disadvantages. In general, management of free-range and organic birds is more complicated and requires more skills than management of birds kept inside. In free-range system feed

consumption is higher as a consequence of extra locomotion and of thermoregulation at lower temperatures due to outside access, and lower density of birds in the house in organic systems (Leenstra et al., 2014). It is also connected with slaughter age of birds in free-range system, because higher final age resulting in higher average final weight, and further, higher final weight resulting in higher feed intake (Leinonen et al., 2012). There are also the differences in environmental impact of different poultry housing systems. Research done by Williams et al. (2006) has shown that free-range and organic poultry production are more environmentally harmful than intensive production systems (Table 3.1). While, other field crops and animal products consume less primary energy and have less environmental burdens when grown organically, poultry meat and eggs are exceptions, because of the much lower bird performance and low efficiency of feed conversion in alternative housing systems (Rodić et al., 2011).

Table 3.1. Comparison environmental burdens of different production systems (adopted from Williams et al., 2006).

Impact and land used	Poultry meat systems (per tonne)		Egg production system (per 20,000 eggs)	
	Traditional	Free-range	Cage	Free-range
Primary Energy used, GJ	12	14.5	13.6	15.4
Global worming potential (GWP)	4.6	5.5	5.3	6.2
Eutrophication potential (EP)	49	63	75	80
Acidification potential (AP)	173	230	300	312
Land use, ha	0.64	0.73	0.63	0.78

Moreover, birds managed on free-range had a higher mortality. According to Sogunle et al. (2012) this might be due to the age at which birds were introduced to the field and cold weather. Furthermore, production systems that are considered more animal welfare friendly may create new or reintroduce old risks to public health. Adverse public health aspects related to outdoor housing can affect food safety, but a more open farming structure also may result in more risk of transfer of zoonotic pathogens to livestock, as happens with avian influenza outbreaks. Compared with conventional (indoor) systems, outdoor husbandry systems are inherently less controllable from a hygiene point of view and can be affected by pollutants (e.g., heavy

metals) that have not been an issue for most modern farmers. An example of a health issue that can be directly connected to outdoor animal husbandry is the protozoan parasite *Toxoplasma gondii*. When animals infected with *T. gondii* defecate outdoors, a substantial load of infectious oocysts can be found in the environment, resulting in a substantial risk of toxoplasmosis (reviewed by Kijlstra et al., 2009).

A significant problem in case of free-range systems, is also *Salomnella*, an intestinal bacterium that can be transmitted by animals. Especially, poultry remains an important vehicle of *Salmonella* transmission to humans, occurring mainly via contaminated meat (Alali et al., 2010). Many consumers assume that the organic chickens shed less *Salmonella* than the broilers because of the particular system management (low density, access to outside and special diet) (Bailey and Cosby, 2005). In fact, a higher number of chickens in the farm can increase the chance of infection with *Salmonella*. However, in free-range, organic farms the access to outside may amplify the risk of the infection with *Salmonella* through the contact with feaces of wild birds (Pieskus et al., 2008), insects, rodents droppings, and other potential carriers of *Salmonella* (Bailey and Cosby, 2005). Cui et al. (2005) found that organically raised broilers had higher prevalence of *Salmonella* than broilers raised conventionally.

After salmonellosis, campylobacteriosis is the next serious disease that is mainly caused by consumption of contaminated poultry products. The higher incidence of *Campylobacter* infections found in free-range chicken flock could be associated with few facts. Firstly, broiler from alternative systems live longer than conventional chickens and the increased life span also increases the chance of becoming infected (Kijlstra et al., 2009). Moreover, characteristics of extensive broiler productions, including the access to an open-air range, could be associated with a higher prevalence of *Campylobacter* than conventional standard production (Vandeplas et al., 2010). Among reasons responsible for the higher incidence of *Campylobacter* infections also could be less vigilant farmer attitude toward general farm hygiene (Kijlstra et al., 2009). However, there exist a variety of ways which allow to reduce the problem with occurring both salmonellosis and campylobacteriosis in free-range poultry flocks. For alternative systems, disease risk can be limited by utilizing pasture rotation to regenerate soil, regularly mowing or grazing to keep short vegetation on pasture, using only land with good drainage, removing heavily contaminated soil around the house before introducing a new flock, and installing fencing and bird mesh to exclude wild birds and other animals (Shields and Duncan, 2006).

Except zoonotic pathogens, in free-range systems many cases have been observed in which birds have started to perform an abnormal behavior, such as feather pecking (Hermansen et al., 2004). It is a kind of deviant behavior defined as the pecking at and pulling out of feathers of another chicken (Bestman and Wagenaar, 2003). Knowledge of individual variation in feather pecking is important in understanding the development and spread of this behaviour within a group (Wechsler et al., 1998). Bestman and Wagenaar (2003) demonstrated that feather pecking in organic laying hens is associated significantly with several housing and management practices. Their study showed that no severe feather pecking was seen as soon as 66% of the birds used the outdoor run.

The alternative bird's rearing systems affects the meat characteristics not only in positive but also in negative ways; this systems allows to obtain a leaner and tastier meat, but it contains a greater amount of free radicals and has a shorter shelf-life. Latter mentioned meat quality trait is related with lipid peroxidation usually evaluated by TBARS (thiobarbituric reactive substances) concentration. It was observed that the meat obtained from chickens reared in an alternative systems, had a higher TBARS level, which could be ascribed to a higher content of metallic ions that catalyze peroxidation (Castellini and Mourvaki, 2007). Available literature has shown that shown that metals such as iron, copper, cadmium, chromium, lead, mercury, nickel, and vanadium exhibit the ability to produce reactive oxygen species, resulting in lipid peroxidation, DNA damage, depletion of sulfhydryls, and altered calcium homeostasis. However, comparative evaluation of these ions revealed that Fe (II) is more effective catalyst of lipid oxidation than other mentioned ions. Iron play key role of in the initiation of lipid peroxidation; have been presented that lipid peroxidation requires both Fe(III) and Fe(II), probably as a dioxygen-iron complex. Most iron is complexed, and little free iron actually exists in nature (Stohs and Bagchi, 1995). It is known that physical activity, which is more intensive in free-range systems, increases the amount of heme-iron particularly in the more oxidative muscles (Castellini et al., 2002). Taking into account all fact about the role of iron ions in lipid oxidation, it is not surprising that modifications in dietary iron level altered the development of lipid oxidation in turkey dark muscles and pork muscles (Erickson, 2008).

Higher TBARS level of meat obtained from animals raised in alternative systems could be related also with a greater degree of unsaturation of intramuscular lipids (Song and Miyazawa, 2001). Have been reported that the main targets for

oxidation in the lipids are the polyunsaturated fatty acids (PUFA) and phospholipids that are vulnerable to the action of $HO^{\cdot}$. The presence of carbon double bonds in PUFA weakens the C-H bonds and makes the H^{+} vulnerable for oxidation reactions triggered by $HO^{\cdot}$, $HO^{2\cdot}$, $RO^{\cdot}$, and $ROO^{\cdot}$ that are generated in mitochondrial and microsomal membranes. These radicals ($HO^{\cdot}$, $HO^{2\cdot}$, $RO^{\cdot}$, and $ROO^{\cdot}$) are able to abstract hydrogen atoms from methylene groups of fatty acids that can initiate lipid oxidation (Bekhit et al., 2013).

From limited research carried out in the area of muscle foods, Nilzén et al. (2001) and Castellini et al. (2002) compared meat from conventionally reared animals with that of meat from animals reared on either free-range or organic production systems. They suggested that meat produced from free-range or organic systems may be more prone to lipid oxidation. Also findings presented by Lawlor et al. (2003) have indicated that organic broiler meat is characterized by shorter shelf-life. They studied changes in chicken burger meat manufactured, cooked and stored in a modified atmosphere at 4^{o}C under fluorescent light for 1-7 days. Malondialdehyde thiobarbituric acid (MDA-TBA) values used to monitor oxidative changes were in the following order throughout each storage period: organic > free-range > conventional. It was concluded that cooked breast burgers from broilers fed organic diets had a lower shelf-life compared with cooked breast burger from broilers raised on free-range on conventional systems.

Castellini et al. (2006) suggested that nto account the peculiar oxidative status of birds reared under alternative conditions different strategies should be considered: (1) avoid excessive carcass processing and reduce storage time; (2) use strains more adapted to kinetic activity and poor environment conditions; (3) provide more natural antioxidants. Considering the last listed strategy, it is necessary to mention that available grass provides a range of bioactive compounds which are a source of primary lipid-soluble antioxidants in biological systems and thus may lead to improvements in meat quality (Ponte et al., 2008a). One of the most important lipid-soluble antioxidant is vitamin E, a potent peroxyl radical scavenger, that prevents the propagation of free radical damage in biological membranes (Traber and Packer, 1995).

CHAPTER IV

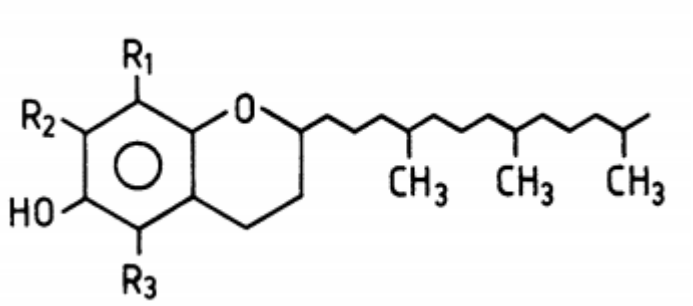
VITAMIN E

4.1. Chemical structure

Vitamin E is a fat-soluble vitamin, essential for health. The main sources of vitamin E are nuts and vegetable oils including wheat germ, and sunflower. Vitamin E occur ubiquitously in cereal grains such as wheat, rice and barley, and certain vegetable oils like palm oil or bran oil (Wojcik et al., 2010). Vitamin E was discovered in 1922 by Evans and Bishop as a necessary dietary factor for reproduction in rats. Subsequent studies showed that the presence of rancid fat in the experimental diets fed to rats and chickens was the causative agent of various pathologies in these animals and that these abnormalities could be "cured" by wheat germ oil concentrates that later were demonstrated to contain tocopherols (Traber and Atkinson, 2007). In 1936 vitamin E (α-tocopherol) was isolated from wheat germ oil by a research team led by Evans. One year later, β- and γ-tocopherol were isolated from vegetable oil (β-, γ-T). The following year the structure of α-tocopherol was determined, followed by defining the process of its synthesis and it was confirmed that it is the most effective of the known tocopherols in the prevention of vitamin E deficiency. In 1947 the scientists identified four naturally occurring tocotrienols (Zielińska et al., 2014).

Vitamin E is the term for a group of organic chemical compounds soluble in lipids. The basic unit for vitamin E family is tocol, the chemical name of which is 2-methyl-2-4,8,12-trimethyltridecylchroman-6-ol (Figure 4.1.) (Zielińska et al., 2014).

Figure 4.1. Tocol structure (Bjørneboe et al., 1990).



Eight different forms of vitamin E can be found in nature: 4 tocopherols (α, β, γ, δ; Table 4.1.) and 4 tocotrienols (α, β, γ, δ; Table 4.2.). The difference between α, β, γ, δ forms finds its origin in the incorporation of methyl group at different places (Bouts and Gasthuys, 2003).

Table 4.1. Tocopherols (adopted from Zielińska et al., 2014).

Trivial name	Chemical name	Abbreviation	Ring position		
			R_1	R_2	R_3
α-tocopherol	5,7,8-trimethyltocol	α-T	CH_3	CH_3	CH_3
β-tocopherol	5,8-dimethyltocol	β-T	CH_3	H	CH_3
γ-tocopherol	7,8-dimethyltocol	γ-T	H	CH_3	CH_3
δ-tocopherol	8-methyltocol	δ-T	H	H	CH_3

Tocopherols and tocotrienols have the same basic chemical structure characterized by a long chain attached at the 2-position of a chromane ring. Tocotrienols differ from tocopherols because they possess a farnesyl rather than a saturated isoprenoid C16 side chain (Colombo, 2010). Moreover, the tocotrienols are more rapidly metabolised than the corresponding tocopherol forms (Raederstorff et al., 2015). Tocopherols are present in oil seeds, leaves, and other green parts of higher plants. α-Tocopherol is found mainly in the chloroplasts of plant cells, while other tocopherols (β, γ and δ) are usually located outside these organelles. While, tocotrienols are not found in the green parts of the plants, but are widely distributed in the bran and germ fractions of certain seeds and cereals (Surai, 1999).

Table 4.2. Tocotrienols (adopted from Zielińska et al., 2014).

Trivial name	Chemical name	Abbreviation	Ring position		
			R_1	R_2	R_3
α-tocotrienol	5,7,8-trimethyltocotrienol, the name: tocochromanol-3 has also been used (formerly known as ζ or ζ2 -tocopherol)	α-T3	CH_3	CH_3	CH_3
β-tocotrienol	5,8-dimethyltocotrienol (formerly known as ε-tocopherol)	β-T3	CH_3	H	CH_3
γ-tocotrienol	7,8-dimethyltocotrienol, the name: plastochromanol-3 has also been used (formerly known as η-tocopherol)	γ-T3	H	CH_3	CH_3
δ-tocotrienol	8-methyltocotrienol	δ-T3	H	H	CH_3

Vitamin E derivatives have three main distinct domains, described as: (a) the functional domain, responsible for the antioxidant activity and, therefore, vitamin E properties, epitomized by the hydroxyl group in α-tocopherol; (b) the signalling domain, comprised of the aromatic rings (phenol-, chromanol-) and activated by the monoesterifi cation of dicarboxylic acids with the phenol oxygen; and (c) the hydrophobic domain, responsible for docking the agents in circulating lipoproteins and biological membranes (Augustyniak et al., 2010). All forms of vitamin E resemble colorless or light yellow, viscous oils and they are soluble in lipids or organic lipid solvents (Zielińska et al., 2014). With respect to tocopherols, α-tocopherol is the most important form of vitamin E present in plasma and tissues. The synthetic α-tocopherol (all racemic α-tocopherol) consists of an equal racemic mixture of the eight stereoisomers (RRR, RSR, RRS, RSS, SRR, SSR, SRS, and SSS), whereas the natural form of α-tocopherol is found only in the RRR configuration (Gagné et al., 2009). Have been noted that one IU of vitamin E is the activity of 1 mg of synthetic dl-α-tocopheryl acetate, 0.135 mg d-α-tocopheryl acetate,0.671 mg d-α-tocopherol or 0.909 mg dl-α-tocopherol (Surai, 1999).

4.2. Metabolism

Poultry cannot synthesize vitamin E, therefore, vitamin E requirements must be given from dietary sources (Ziaei et al., 2013). The dietary recommendations of vitamin E for poultry species vary among species. Poultry feed can be supplemented with 10 IU of vitamin E per kg feed (1 IU = 0.67 mg *dl*-α-tocopheryl acetate) for chickens aged up to six weeks, 5 IU/kg feed for chickens aged over six weeks, 12 IU/kg feed for turkeys aged up to eight weeks, and 10 IU/kg feed for turkeys aged over eight weeks. For ducks and Japanese quail, feed can be supplemented with 10 IU/kg feed and 12 IU/kg feed, respectively, for starting and growing birds (Rengaraj and Hong, 2015).

Absorption of vitamin E is closely related to fat absorption. Mentioned process is facilitated by the lipase enzymes of the bile and pancreas. Moreover, vitamin E is absorbed as an alcohol whereby esters are firstly hydrolyzed before absorption (Bouts and Gasthuys, 2003). Necessary is also highlight that in chickens, absorption of vitamin E is impaired by severe selenium deficiency and selenium alleviates vitamin E deficiencies by permitting higher levels of vitamin E to be absorbed (Ziaei et al., 2013). Regarding transport, vitamin E is transported in plasma lipoproteins. After its intestinal absorption vitamin E is packaged into chylomicrons, which along the lymphatic pathway are secreted into the systemic circulation. By the action of lipoprotein lipase (LPL), part of the tocopherols transported in chylomicrons are taken up by extrahepatic tissues, and the remnant chylomicrons transport the remaining tocopherols to the liver. Here, by the action of the "alpha-tocopherol transfer protein", a major proportion of alpha-tocopherol is incorporated into nascent very low density lipoproteins (VLDL). Besides the LPL action, the delivery of alpha-tocopherol to tissues takes place by the uptake of lipoproteins by different tissues throughout their corresponding receptors (Herrera and Barbas, 2001).

In case of animals important is also the fact that the distribution of vitamin E isoforms varies from tissue to tissue. For example in the study carried out on mice was observed that, in individuals that were fed a diet not specifically enriched with tocotrienols, up to 15% of total vitamin E was composed of tocotrienols; the brain contained no detectable α-tocotrienol levels; in other tissues, 99% of the vitamin E was present as α or γ-tocopherol. These observation indicates that tissues may possess the ability to regulate the vitamin E composition individually (Packer et al., 2001). Small amount of vitamin E will persist for a longer time in the body but the major stores will be exhausted rapidly due to polyunsaturated fatty acids in tissues (Bouts and Gasthuys,

2003). Before excretion vitamin E is extensively metabolized. In the middle of previous century two major urinary metabolites of α-tocopherol, tocopheronic acid and the tocopheronolactone derived therefrom, were described. Both metabolites are excreted in the urine as glucuronides or sulfates (so-called Simon metabolites) (Brigelius-Flohé et al., 2002). Compared to α-T, these compounds have an open hydroxychroman ring and a shortened side chain. Measurement of Simon metabolites in urine may therefore represent a useful, non-invasive marker that reflects the extent to which α-T has reacted as a radical scavenger *in vivo*. However, the existence of these metabolites has been questioned, with some evidence suggesting that they are formed only as artefacts during sample preparation. As urinary α-T metabolites are thought to be excreted as conjugates of glucuronides or sulfates, urine samples have to be hydrolysed chemically or enzymatically prior to their analysis (Wu and Croft, 2007).

4.3. Biological functions

Vitamin E is an interesting group of compounds, able to exert many and different biological activities in plant, animal and human cells. α-tocopherol has the greatest biological activity when compared to other forms of vitamin E, because it has higher absorption, higher deposition in tissues and low fecal excretion (Cortinas et al., 2005).

4.3.1. Vitamin E as an antioxidant

Vitamin E is an efficient scavenger of lipid peroxyl radicals and, hence, it is able to break peroxyl chain propagation reactions (Wang and Quinn, 1999). The rate of scavenging of lipid peroxyl radicals by cr-tocopherol is considered to be much lower in the membrane than in homogenous solution. Nevertheless, vitamin E plays a major role as a chain-breaking antioxidant of the membranes (Surai, 1999). For better understanding this property of vitamin E is necessary to explain the general role of antioxidants, that are capable of neutralizing the harmful effects of free radicals in the human and animal body (Jeeva et al., 2015). Halliwell (2007) defined antioxidants as "any substance that delays, prevents or removes oxidative damage to a target molecule". In the same year, Khlebnikov et al. (2007) defined antioxidants as "any substance that directly scavenges ROS or indirectly acts to up-regulate antioxidant defences or inhibit ROS production".

There are many intrinsic free radical scavenger systems, which involve enzymatic and no enzymatic reactions. Regarding enzymatic antioxidants they are divided into primary and secondary enzymatic defences. With regard to the primary defence, it is composed of three important enzymes that prevent the formation or neutralize free radicals: (1) glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenoles and also eliminates peroxides as potential substrate for the Fenton reaction; (2) catalase, that converts hydrogen peroxide into water and molecular oxygen and has one of the biggest turnover rates known to man, allowing just one molecule of catalase to convert 6 billion molecules of hydrogen peroxide; (3) superoxide dismutase converts superoxide anions into hydrogen peroxide as a subtract for catalase (Rahman, 2007). The secondary enzymatic defense includes (1) glutathione reductase, that plays an important role in protecting hemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathone in the process of aerobic glycolysis, and (2) glucose-6-phosphate dehydrogenase, that activity is important for cell growth (Chang et al., 1978; Tian et al., 1998).

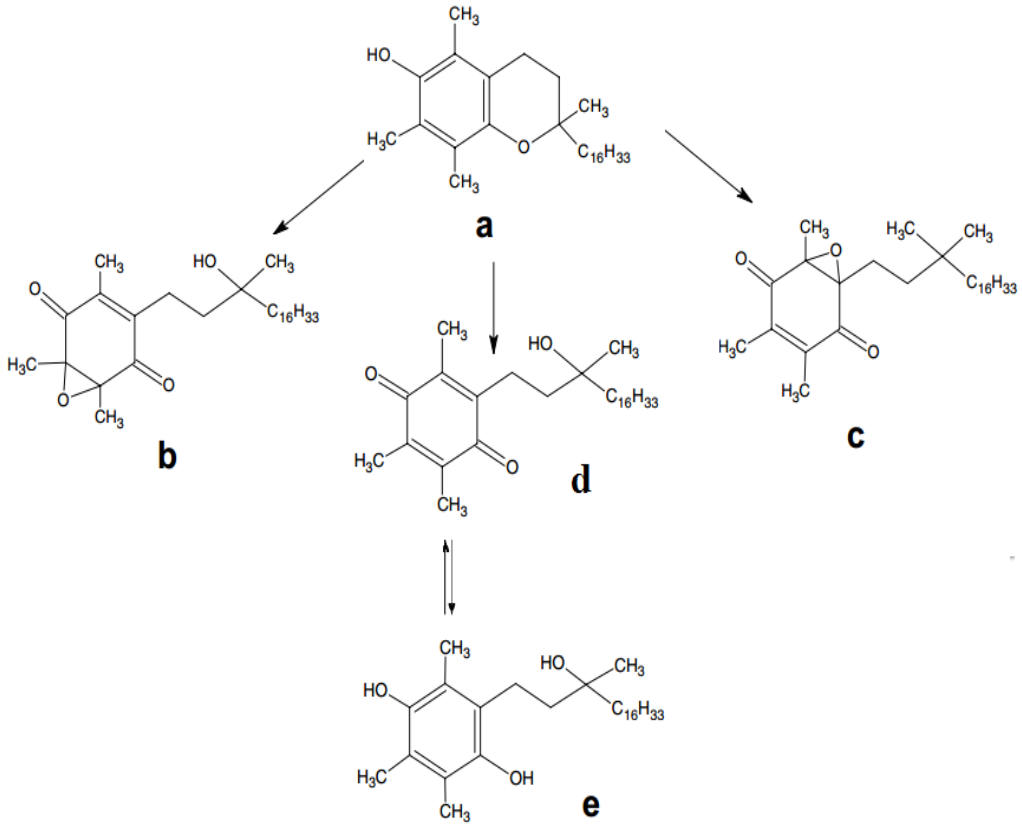
Since efficient antioxidant protective mechanisms are crucial to inhibit the oxidative stress, searching for non-enzymatic antioxidants in has received much attention in recent years (Wojcik et al., 2010). Non-enzymatic antioxidants include natural fat-soluble antioxidants (vitamins A, E, carotenoids, ubiquinones, etc.) and water-soluble antioxidants (ascorbic acid, uric acid, taurine, etc.) (Surai et al., 2016)

Non-enzymatic antioxidative systems are not as specific as enzymatic ones, but, nevertheless, they are in the first line of antioxidative defense and are therefore of high importance in cellular response to oxidative stress (Augustyniak et al., 2010). Additionally, non-enzymatic antioxidant can be divided into two types: natural, and synthetic. Natural antioxidants are present in plants (berries, fruits, vegetables, medicinal, aromatic plants, spices and other botanicals), and this is why the basis source of these compounds for humans and animals are plant-derived products. Moreover, important group of natural antioxidants in animal-derived food products are aminocompounds: aminoacids, peptides and proteins. Antioxidant activity of these compounds is connected mainly with amino acids which possess thiol groups (metionine, cysteine). Proteins, acting as antioxidants, scavenging of free radicals formed in biochemical processes of cells. Antioxidant activity of proteins from animal-derived products can be also connected with addition (in food technology) of

concentrates and isolates gained from high-protein plants origin (legumes seeds) and animal origin (milk, eggs) raw materials (Sikora et al., 2008). Some natural antioxidant, especially plant-derived compounds (carnosol, rosmanol, rosmariquinone, and rosmaridiphenol) are better antioxidants than synthetic antioxidants (Brewer et al., 2011). Among these synthetic antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG). With regard to their comparative stability against thermal oxidation the order found was BHT > PG > BHA > TBHQ (Marmesat et al., 2010). However, important is to highlight that synthetic antioxidants have recently been reported to be dangerous for human health. Thus, natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; they are more readily acceptable to the modern consumer (Tavasalkar et al., 2012).

Antioxidant properties of vitamin E are a result of the presence of hydroxy group in the chromanol ring (6' position). The antioxidative activity of tocopherols *in vivo* is created in the following order: α-T > β-T > γ-T > δ-T. Their activity *in vitro* is exactly opposite: δ-T > γ-T ~ β-T > α-T (Zielińska et al., 2014). It is generally considered that the stability of different vitamin E forms in the feed is inversely proportional to their antioxidant activity. This means that α-tocopherol with the highest antioxidant potential is oxidised first (Surai, 1999). It has been shown that the principle oxidation metabolites of α-T include α-tocopherylquinone, 5,6-epoxy-α-tocopherylquinone and 2,3-epoxy-α-tocopherylquinone (Figure 4.2.) (Wu and Croft, 2007).

Figure 4.2. α-tocopherol and its principle oxidation products: (a) α-tocopherol; (b) 5,6-epoxy-α-tocopherylquinone; (c) 2,3-epoxy-α-tocopherylquinone and (d) α-tocopherylquinone (adopted from Wu and Croft, 2007).



Vitamin E does not work in isolation from other antioxidants; rather it is part of an interlinking set of redox antioxidant cycles, which has been termed the "antioxidant network". It is hypothesized that vitamin E acts catalytically, i.e., it is efficiently reduced from its free radical (chromanoxyl) form, which arises after quenching lipid radicals, to return back to its reduced native state. This catalysis occurs through the interactions between water- and lipid-soluble substances by both non-enzymatic and enzymatic mechanisms that regenerate vitamin E from its tocotrienoxyl or tocopheroxyl radical back to tocotrienol and tocopherol, respectively (Packer et al., 2001). Moreover, vitamin E (α-tocopherol) is a good antioxidant but can act also a pro-oxidant to facilitate lipid peroxidation in LDL particles. However, this pro-oxidant activity of α-tocopherol is prevented by ascorbate acting as a co-antioxidant (Gagné et al., 2009).

Dietary supplementation of α-tocopherol increases incorporation of the antioxidant into the phospholipid membrane region where the polyunsaturated fatty acids are located. Including α-tocopherol in livestock diets has been shown to have significant effects on antioxidative activities of their tissues and the stability of meat

derived from them (Brewer et al., 2011). As an antioxidant molecule, vitamin E supplementation can alleviate negative effects of heat stress, that occurs when the amount of heat produced by an animal surpasses the animal's capacity to dissipate the heat to its surrounding environment. This property of vitamin E plays pivotal role because animals experiencing heat stress tend to reduce their heat production by limiting feed intake, with subsequent negative effects on growth performance. Therefore, heat stress has been a great concern among scientists and poultry producers for many decades, particularly in arid (dry, hot all year) and in tropical (wet, hot all year) regions of the world, as well as in other climates due to surges in temperature during the spring and summer months (Akbarian et al., 2016). Maini et al. (2007) supplemented vitamin E in feed during the summer months; they observed that the broilers from experimental group showed reduced lipid peroxidation and high activity of the glutathione peroxidase, catalase, superoxide dismutase and glutathione reductase in erythrocytes, compared to the control diet. Therefore, vitamin E supplementation improves enzymatic and non-enzymatic antioxidative systems of red blood cells in animals subjected to heat. The interesting results were obtained also in the study conducted earlier and described by Sahin et al. (2002). The goal of this research was to determine if the negative effects of high ambient temperature (34^{o}C) on egg production, egg quality, digestibility of nutrients, and mineral content of egg yolk could be alleviated by dietary vitamin E (dl-α-tocopheryl acetate) supplementation in laying Japanese quails. Results of the this study conclude that supplementation with 250 mg α-tocopherol acetate/kg of diet can be considered to be protective management practice in a quail diet, reducing the negative effects of heat stress. These results are in line with these presented by Ajakaiye et al., (2011) in the research in which the effects of heat stress on egg quality profile in layer hens supplemented with vitamins C and E were investigated. They concluded that dietary supplementation of laying hens with vitamins C and E singly or in its combined form, can at least in part alleviate heat stress induced oxidative damage. Furthermore, it has been suggested that vitamin E should be added not only before heat stress but also during and after the stress (Lin et al., 2006). However, is important to mention that stress in poultry production is not only restricted to heat but also physiological stress, e. g. as a result of increasing stocking density. It has been noted that chickens performance and health can be influenced by very high stocking density thereby it is important to ensure that adequate floor space is available for each bird. If the stocking density is too high, the temperature may rise dangerously

since there will be more metabolic heat being added to the house air than was planned for. In the experiment described by Adebiyi (2011) in which the main aim was to assess the influence of optimal dose of vitamin E supplementation on broilers reared under increased stocking density, have been observed that broiler chicks could be stocked up to 20 birds/m^2 only if the diet is supplemented with 100 mg/kg vitamin E. In this same study was observed that supplementation with vitamin E in case of increased stocking density improved the plasma constituents of broiler chicks as judged by plasma AST and ALT during the experimental period.

4.3.2. Other properties of vitamin E

Vitamin E has a wide range of functions in the body; except anioxidative properties it is primarily crucial for fertility in humans and livestock species. As was already mentioned, vitamin E was discovered as a "vitamin of reproduction" in 1922 (Surai et al., 2006). Until now several reports have been published on the beneficial effects of vitamin E on improving reproductive traits in male poultry (e.g. Lin et al., 2005, Cerolini et al., 2006). For cockerels, vitamin E has traditionally been known as anti-sterility vitamin. The importance of this vitamin can be seen from the fact that it constitutes 88% of chicken semen antioxidant capacity. It was observed that high level of vitamine E in the diet improve the semen physical characteristics (Alm El-Dein et al., 2013).

From the point of view of laying hen vitamin E is essential for normal hatchability. According to Amiri Andi et al. (2006) vitamin E level of 40 IU/kg in Arian broiler breeder diet is the best level for egg production and persisting of hatchability. These results are compatible with the findings described in the work of Hossain et al. (1998). Additionally, have been observed that increased dietary vitamin E supplementation of the maternal diet is associated with increased vitamin E concentrations in the egg yolk, embryonic tissues and their increased resistance to oxidative stress (Surai et al., 2016). Meanwhile, in the study of An et al. (2010) have been observed that addition of vitamin E at 100 mg/kg of diet significantly reduced lipid peroxidation in the egg yolk, and then promoted the development of heart and liver of the progeny.

Vitamin E also has been shown to be a requirement for normal development and function of the immune system. The immunomodulatory effects of vitamin E have been demonstrated in humans and a variety of animal species, and were most evident in very

young, very old, and immunocompromised individuals. Gore and Qureshi (1997) reported enhanced cell-mediated and humoral immunity in broiler chicks receiving 10 IU VE *in ovo* on day 18 of incubation. Moreover, vitamin E has been shown to improve immunity to *Escherichia coli* infection, coccidiosis, infectious bursal disease and Newcastle disease. It has been noted that dietary vitamin E supplementation in the form of α-tocopherol acetate, significantly affect T cell differentiation in the thymus and alter the proportions among T cell subsets in the thymus and spleen of broiler chickens (Erf et al., 1998). Simultaneously, Lin and Chang (2006) have reported that excessive dose of vitamin E may depress specific immune response.

It has been demonstrated *in vitro* and in excised mouse muscle that α-tocopherol improves muscle membrane repair and rescues myocytes from necrosis. A recent study in rats showed that the plasma membrane repair capacity is impaired in skeletal muscle fibres when the animals are deprived of vitamin E. Thus, in myocyte plasma membranes, the presence of vitamin E promoted membrane repair. This is evidence that suggests a function for α-tocopherol in membrane repair (Raederstorff et al., 2015). Vitamin E plays also a role in the blood clothing system by inhibiting platelet integration, and provides protection against the toxicity of heavy metals (cadmium, mercury, arsenic, selenium, lead) (Bouts and Gasthuys, 2003). Moreover, α-tocopherol is able to alter gene expression, modulating cell signaling. It also has marked effects on platelet adhesion, redox-regulated transcription, cytokine signaling. In addition, α-tocopherol inhibits protein kinase C, 5-lipoxygenase and phospholipase A2, and activates diacylglycerol kinase (Gagné et al., 2009). Except α-tocopherol, on attention deserve also γ-tocopherol, which has a number of beneficial effects, including reducing in platelet aggregation, increasing endogenous SOD activity. γ-tocopherol and its metabolite can also reduce synthesis of PGE2, which plays a key role in inflammation. In addition, γ-tocopherol is superior to α-tocopherol in controlling damage caused by reactive nitrogen oxide species and in reducing oxidative DNA damage (Gagné et al., 2009). Meanwhile, tocotrienols in animal cells inhibit cholesterol biosynthesis by suppressing 3-hydroxy-3-methyl glutarylCoA reductase enzyme (HMGR): the key-enzyme in the sterologenic pathway, resulting in less cholesterol being manufactured by liver cells (Colombo, 2010).

4.4. Effects of deficiency and supplementation of vitamin E

There is numerous of symptoms connected with vitamin E deficiency that vary, depending on the species affected. This deficiency is an effect not only of a severe malnutrition, an inadequate level of vitamin in the diet but also might occur when there is an impaired pancreatic or liver function and/or when the release of bile (obstruction) is abnormally delayed or stopped (Bouts and Gasthuys, 2003). Symptoms connected with vitamin E deficiency include disorders of the nervous system, skeletal system, circulatory system, muscular system, cardiovascular system, immune system, and reproductive system (Rengaraj and Hong, 2015). Regarding the muscular system, muscular dystrophy associated with vitamin E deficiency in chicks has proved to be a complex deficiency involving selenium and sulfur amino acids as well as excess dietary linoleic acidl. It has occurred on diets containing levels of antioxidants which have prevented encephalomalacia (Cheville, 1966). Moreover, vitamin E deficiency may manifest itself in a number of disorders of liver, kidney, and lungs; and it causes gizzard myopathy in turkeys and ducks. Vitamin E deficiency may increase the risk of ischemic heart disease, the incidence of infections, and it promotes susceptibility to dietary and environmental stress in humans and animals. Vitamin E deficiency can lead also to membrane lipid peroxidation, affecting hepatic mitochondria and microsomes, as well as to an accumulation of ceroid in adipose tissues, and to cerebellar encephalomalacia in chickens. Encephalomalacia is a vitamin E deficiency disease, which readily occurs in chickens fed on a diet containing high levels of polyunsaturated fatty acids of the linoleic acid series and low levels of vitamin E. Available literature suggest that vitamin E acts as an antioxidant that protects chickens against aforementioned encephalomalacia by preventing the breakdown of linoleic acid to 12-oxo-cis-9-octadecenoic acid (keto acid). In addition, vitamin E deficiency impairs feather development in chickens. Taking into account high dose administrations, data from animal studies show that vitamin E toxicity is low and that the vitamin is not mutagenic, carcinogenic, or teratogenic (Rengaraj and Hong, 2015). However, it has been declared that the supplementation of vitamin E decreases plasma cholesterol and triglyceride levels and increases the ALP level in hens (Arslan et al., 2001). Rabbits fed the vitamin E-free diet showed higher concentrations of total cholesterol than rabbits fed the 60 mg/kg all-rac-a-tocopheryl acetate diet; on the contrary, HDL cholesterol was constantly lower in rabbits fed the vitamin E-free diet,with a minimum of 4.4% of total cholesterol at the 20^{th} wk (Oriani et al., 1997). Furthermore, other works provides evidence that

intramuscular injections of vitamin E, given to growing lambs in adequate form and dose, reduces collagen maturity (Maiorano et al., 2007) and lipid oxidation (Maiorano et al., 2016) in the *Longissimus* muscle of growing lambs.

PART II: RESEARCH WORKS

CHAPTER V

Research n^{o} 1: Effects of rearing system and vitamin E on the performance and meat quality of Kabir

5.1. Aim

Animal's welfare is increasingly viewed as a factor affecting the quality of animal products while being an important tool of marketing strategy (Połtowicz and Doktor, 2011). Recent research findings have indicated that an animal's welfare largely dependent on environmental factors (Muir et al., 2014). Despite this fact, the management system used in highly productive farms is characterized by failure to provide adequate welfare, thus is often subjected to harsh criticism. In many countries, this situation has led to the development of poultry meat production under less intensive rearing conditions. Moreover, a survey of consumers, showed that they prefer to purchase food products obtained from livestock animals raised in production systems that are considered more animal welfare friendly, such as free-range systems (Połtowicz and Doktor, 2011).

Free-range production presents an alternative to factory farm poultry and eggs that conveys a positive image of animals living outdoors as "nature intended". The term "Free-range" refers to these birds that are not confined to large indoor commercially intensive livestock buildings (Tserveni-Gousi et al., 2005). The free-range production system focuses on low-input strategies and support of rural communities by maintaining the family farm. This low input/output system has been a traditional component of small farms all over the developing world (Olaniyi et al., 2012).

Free-range system allow to perform natural patterns of behaviour such as moving, flying, wing flapping, scratching, pecking, foraging and feeding (Sosnówka-Czajka et al., 2010). Moreover, the environment' elements to which free-range poultry birds are exposed (the outdoor access, the feed they consume, climatic factors and management systems) affect the performance of the birds and quality of their meat

(Chen et al., 2013). First off all, meat from chickens reared outdoor has significantly better results of sensory evaluation and is well received by the sensory panel or panelists compared with chicken meat conventional (Lin et al., 2014). Some poultry producers are raising their birds by giving them outdoor (pasture) access because it is considered that the bird reared in free-range rearing system giving meat with better flavor. Moreover, the available literature showed that the systems with outdoor access can positively influence chewiness, hardness, fracturability, meat shear force values, and physicochemical properties, such as pH and color of meat (Cheng et al., 2008; Souza et al., 2011; Mikulski et al. 2011).

Rearing system affects the chemical composition of poultry meat as well. The chickens reared under free-range breeding systems have total protein content higher than the chickens bred in confinement breeding system. Birds reared outdoor can have also significantly higher microelements content, such as zinc and iron, than these reared intensively. Moreover, the reduced content of fat (abdominal fat in particular) and the decreased calorie content in muscles of free-range broilers were observed (Lin et al., 2014). The findings of numerous studies indicated the increased level of unsaturated fatty acids in flesh of free-range birds (Castellini et al., 2002; Pavlovski et al., 2013). However, high degree of unsaturation of intramuscular lipids results in shorter shelf-life of meat and meat products. Therefore, it is necessary to highlight the role of antioxidants that may lead to improvements in meat quality (Ponte et al., 2008d).

The principal antioxidant widely used in animal diet is vitamin E. It is the most effective chain-breaking lipid-soluble vitamin. Due to its lipophilic property, the vitamin E absorption is dependent on animals' fat digestion and absorption (Zouari et al., 2010). Vitamin E has been recognized as an essential nutrient for health and growth of all species of animals. The diverse roles of vitamin E are due to its involvement in nutritional myopathy, immune responsiveness and prostaglandin biosynthesis (Adebiyi et al., 2011). Dietary vitamin E supplementation results in elevated concentrations of α-tocopherol within cell membranes, increasing the days of retail display life without compromising microbiological quality by preventing the oxidation of membrane phospholipids during storage which inhibits the passage of sarcoplasmic fluid through the muscle cell membrane (Castillo et al., 2013). In the studies conducted on lambs, intramuscular injection of DL-α-tocopheryl acetate increased metacarpal growth plate width, influenced intramuscular collagen (IMC) characteristics, increased lipid oxidation, maintained the redness of meat, and improved the nutritional value and

consumer acceptability of lamb meat (Maiorano et al., 1999, Maiorano et al., 2007, Maiorano et al., 2016). However, according to the available information, no research has yet been conducted to study the effect of vitamin E administrated as the intramuscular injection on the slaughter performances and meat quality features of broiler chickens.

The aim of the present study was to evaluate the effects of rearing system (outdoor versus indoor) and of DL-α-tocopheryl acetate single intramuscular injection on carcass traits and meat quality of slow-growing broilers (Kabir).

5.2. Material and methods

This study was carried out during from August to November 2015 in Molise (Italy) on the farm located in Bonefro (at 628 m above sea level, 41°42′18″ N , 14°56′03″ E). For the experiment were used sixty Kabir male chicks. Animal handling followed the recommendations of European Union directive 86/609/EEC and Italian law 116/92 regarding animal care.

5.2.1. Kabir breed

The Kabir (from Arabic “large”) breed was established in Israel by the Katz family and has been owned for the past years by the Italian Avizoo company that is the leader in the Italian colored broiler market.

The Kabir gene pool includes a number of pure lines used to provide a range of products with specific colored and naked neck characteristics (Moye and Van Den Berg, 2009). Kabir chicken has been distributed throughout the world (Italy, other countries of southern Europe, China, Malaysia, Thailand, USA) (Moye and Van Den Berg, 2009), gaining popularity because of its exceptional characteristics. The advantages of raising Kabir chicken are as follows: (1) is superior for meat conversion because of its medium growth, good body conformation and efficient feed conversion; (2) is resistant to disease and heat stress; (3) is large in size and produce meat that has a “native” taste and texture; (4) when cross-breed with native chickens, the Kabir qualities are retained in the new breed; (5) feeding cost is low since Kabir chicken can also survive by themselves; (6) Kabir hen’ eggs are low in cholesterol. Since its introduction into the production, Kabir has become known as the chicken most-in-demand for backyard and small enterprise flocks (Lelis, 2013).

5.2.2. Experimental material

The trial was carried out on sixty Kabir male chickens (Figure 5.1). The animals were vaccinated against Marek disease and Coccidiosis. Birds were kept together after hatching until 14 d of age in an environmentally controlled poultry house, with temperature ranging between 30 and 32°C and relative humidity between 65 and 70%. At 15 d of age, healthy male chicks of similar body weight were transferred to the experimental farm, and randomly selected and assigned to one of two raising systems (indoor and free-range) with thirty birds in each system.

Figure 5.1. Kabir male chickens (Bonefro, Italy).

The birds of indoor system were raised in pens (stocking density 0.12 m^2/bird) fitted with the solid floor, in a poultry research house that contained side curtains. The indoor and outdoor areas were separated by the house wall. In the free-range system chickens were raised with free access to the grassy paddock (4 m^2/bird; 8 hours/day). Birds were confined to indoor pens at night. Chickens from both rearing systems were fed *ad libitum* the same starter and grower-finisher diets. Access to feed and water was freely available, and all diets were formulated to contain adequate nutrient levels (Table 5.1).

At 84 d of age, thirty chickens from both examined rearing systems (i.e. 15 birds per system) received, into the right-side breast fillet, single intramuscular injection with 1 ml of DL-α-tocopheryl acetate (50 IU of Vitalene® E, Fatro, Bologna). Kabir chickens assigned to the control group received injection of physiological saline.

Table 5.1. Ingredients and chemical analysis of diets

	Diet	
Item (% unless noted)	starter	finisher
Ingredients		
Corn	22	31.6
Wheat	19.5	15
Soybean meal	31.5	25
Wheat middlings	13	15
Corn gluten	10	10
Soybean oil	1.32	1.1
Calcium carbonate	1.2	1
Dicalcium phosphate	0.5	0.5
NaCl	0.2	0.2
Sodium bicarbonate	0.1	0.1
Vitamin-mineral premix 1[1]	0.3	-
Vitamin-mineral premix 2[2]	-	0.3
Phytase	0.1	0.1
Coccidiostat	0.1	0
Color additives	0.1	0.1
Methionine	0.08	-
Chemical composition, %		
CP	24.00	21.00
Lipid	4.50	4.50
Crude Fiber	4.50	4.00
Ash	7.00	6.00
Lysine, %	1.10	1.00
Methionine, %	0.35	0.30
Calcium, %	1.30	1.10
Available P, %	0.70	0.60
Sodium, %	0.15	0.20

[1]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,600 IU; vitamin E, 50.1 mg; vitamin B1, 3 mg; vitamin B12, 0.04 mg; vitamin B2, 6 mg; vitamin B6, 3.99 mg; $CuSO_4$ $5H_2O$ (Cu, 10mg), 38.26mg; $Ca(IO_3)_2$ (I, 1.50mg), 2.31mg; $FeCO_3$ (Fe, 45mg), 93.15mg; MnO (Mn, 36mg), 46.44mg; $MnSO_4$ (Mn, 35mg),110.88mg; Na_2SeO_3 (Se, 0mg), 0.43mg; ZnO (Zn, 51mg), 63.24mg.

[2]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 3,000 IU; vitamin E, 41.68 mg; vitamin B1, 2.90 mg; vitamin B12, 0.03 mg; vitamin B2, 5 mg; vitamin B6, 3.33 mg; $CuSO_4$ $5H_2O$ (Cu, 8mg), 32.72mg; $Ca(IO_3)_2$ (I, 1.25mg), 1.93mg; Fe_2O_3 (Fe, 560mg), 800.8mg; $FeCO_3$ (Fe, 38mg), 77.63mg; MnO (Mn, 30mg), 38.70mg; $MnSO_4$ (Mn, 30mg), 92.40mg; Na_2SeO_3 (Se, 0mg), 0.36mg; ZnO (Zn, 43mg), 52.7mg.

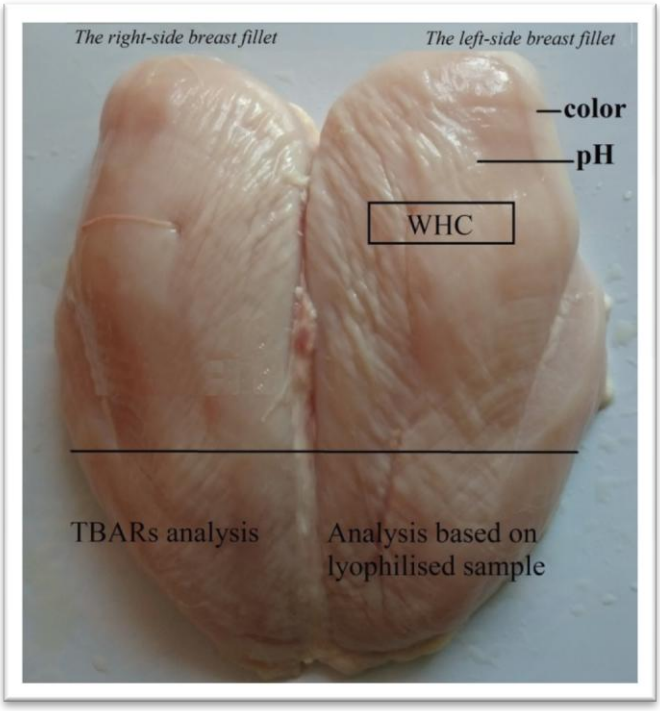
5.2.3. Slaughter surveys

Chickens were slaughtered, after 12 hours of fasting, at 94th day of age. Animals were individually weighted just before slaughter for the final body weight determination. The carcass weight was measured after removing the blood, feathers, skin, head, feet, and all internal organs (evisceration). Carcass yield was calculated as the ratio between the carcass weight and final body weight after fasting. The breast muscle (including pectoralis major and pectoralis minor), leg muscle (including thigh and drumstick), wings and back+neck were removed from the carcass and then weighted. The weight percentages of breast and leg muscles, wings and back+neck were calculated as a percentage of eviscerated carcass weight.

5.2.4. Meat quality traits

After slaughter and carcass dissection, breast muscles (both right- and left-side fillets) from the examined Kabir chickens were collected for the future analysis aimed at evaluation of meat quality features. Aforementioned analysis were performed with using schedule presented on Figure 5.2. Laboratory analysis of chemical composition of chickens meat were carried out in the Department of Agricultural, Environmental and Food Sciences, University of Molise, Italy.

Figure 5.2. Determination of chicken meat quality traits.



5.2.4.1. Physicochemical characteristics

The following analyses were carried out on Kabir chickens breast muscle: (i) ultimate pH was measured 24 hours *post-mortem* on the upper part of the left-side breast fillet using a Mettler Toledo FG2/EL2 portable pH Meter Compact instrument for pH measurement in agriculture. pH meter was calibrated prior to experiment and adjusted for the temperature of meat samples. (ii) color (Commission Internationale de l'eclairage; L^* = lightness, a* = redness, and b* = yellowness) was measured 24 hours *post mortem* in triplicate on the bone-side surface of left-side breast fillet using a Chroma Meter CR-300 (Minolta Corporation, Italia s.r.l., Milano). The instrument was standardized with the white and black tiles provided by the manufacturer before sample measurements; (iii) water-holding capacity (WHC) was measured 24 hours after slaughter by applying filter paper compression method (Grau and Hamm, 1952). Meat samples weighing 300 mg were placed on Whatmann filter paper (diameter 55 mm, area 23,6 cm^2), and pressured using the laboratory hydraulic press (50 kg / cm^2) for 5 minutes. A planimeter (317E, Haff, Germany) was used to determine the area (in cm^2) of the two patches formed by the pressed meat juices and of the meat. The value of water-holding capacity was expressed in percentage as the area of the emitted water divided by the area of the filter, multiplied 100.

5.2.4.2. Intramuscular collagen analysis

Breast muscle samples (from down part of left-side breast fillet*)* collected from Kabir male chickens were thawed, at room temperature, trimmed of fat and epimysium, lyophilized for 24 h (Genesis Pilot Lyophilizer, SP Scientific), and stored frozen (at temperature -20°C) until collagen analyses. The lyophilized muscle tissue (100 mg) was hydrolyzed in Duran tubes in 5 ml 6N HCl at 110°C for 18 to 20 hours (Etherington and Sims, 1981) for the determination of hydroxyproline and crosslinking. The hydrolyzate was filtered (Whatman filter paper, Grade 1; diameter 90 mm) and diluted with distillated water. An aliquot of the hydrolyzate was removed for hydroxyproline determination.

The intramuscular collagen concentration (4-hydroxyproline) was quantified using the colorimetric procedure described by Woessner et al. (1961). The hydroxyproline was oxidated with sodium p-toluenesulfonchloramide (chloramines T; $C_7H_7ClNO_2S{\cdot}Na$ ($3H_2O$)) which then was destroyed by the addition of perchloric acid

($HClO_4$). A solution of p-dimethylaminobenzaldehyde (Ehrlich solution) was added and the tube was placed for 20 minutes in water bath at 60°C. The solution absorbance was then determined by spectrophotometer Jasco V-730 (Germany) at 557nm. The hydroxyproline concentration was determined from the standard curve of L-hydroxyproline ($C_5H_9O_3N$). Intramuscular collagen concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as μg hydroxyproline per milligram of lyophilized tissue.

5.2.4.3. Total lipid and fatty acid composition

Lipid extraction from breast muscle was performed by Folch et al. (1957) method. The extracted lipids were esterificated and then analyzed by gas chromatography (GC). Analysis was performed using a GC Trace 2000 (ThermoQuest EC Instruments) equipped with a flame ionization detector (260°C) and a fused silica capillary Column (Omegawax 320, Phenomenex, Torrance, CA, USA) 30 m x 0.32 mm x 0.25 μm film thickness. The carrier gas was helium (25cm/sec). The oven temperature was maintained constant at 200°C. The individual fatty acid peaks were identified by comparison of retention times with these of known mixtures of standard fatty acids (PUFA-2, Supelco, Bellofonte, PA, USA) run under the same operating conditions. Results were expressed as percentage of the total fatty acids identified. To assess the nutritional implications, the n-6 fatty acids/n-3 fatty acids, n-3 FA/n-6 FA and the PUFA/SFA ratios were calculated. Moreover, in order to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, respectively the atherogenic index (AI) and the thrombogenic index (TI) were calculated, according to the formulas suggested by Ulbricht and Southgate (1991).

AI = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3);

TI = (C14:0 + C16:0 + C18:0)/(0.5 * MUFA + 0.5 * PUFA n-6 +3 * PUFA n-3 + n-3/n-6).

5.2.4.4. Measurement of oxidative stability

For oxidative stability evaluation, breast muscle samples were were analyzed as raw meat storage at 4°C for 24 hours and after stored 1 month in the freezer at temperature -18^{o}C).

Lipid oxidation was determined by the TBA reactive substances (TBARS) method as described by Vyncke (1970, 1975) and with modifications according to Sørensen and Jørgensen (1996). Briefly, 5.0 g of minced meat was homogenized in 15 ml 7.5% trichloroacetic acid with 0.10% propyl gallate and 0.10% EDTA using an Ultra-Turrax T 25 BASIC (Ika-Werke, Staufen, Germany) and filtered; 1.0 ml of the filtrate was mixed with 1.0 ml of TBA 0.020 M and incubated at 100 °C in a water bath for 40 min. Absorbance was measured at 532 nm and 600 nm at room temperature. The TBARS value was expressed as mg of malondialdehyde (MDA) per kilogram of sample using a standard curve prepared from 1,1,3,3-tetraethoxy-propane.

5.2.5. Statistical analyses

Data were analysed by ANOVA using GLM procedure of the SPSS package (SPSS, 2010). The multi-comparison Scheffe's test was used to separate the differences among the mean for statistical significance ($P < 0.05$)

5.3. RESULTS AND DISCUSSION

5.3.1. Slaughter traits

Table 5.2. shows the effect of rearing system and vitamin E treatment on final body weight and carcass traits of Kabir chickens.

Chickens reared without outdoor access were heavier (+6.40%) that these reared outdoor ($P < 0.05$). This finding corroborate the results described by Lima and Nääs (2005) and Wang et al. (2009). They reported that slaughter weight of the chickens in the free-range treatment were much lower than that of the chickens in the indoor floor treatments ($P < 0.05$). However, literature data suggest that the effect of free-range production system on the body weight of broilers is inconclusive. Ponte et al. (2008b) showed that the final body weight of birds consuming pasture were significantly greater than that of the birds kept under the same environmental conditions but not allowed to forage. In the current study, the lower body weight of free-range birds may be related to increased physical activity of outdoor chickens that favored myogenesis over lipogenesis (Castellini et al., 2002). In the presented study, the carcass weight and carcass yield have been not affected negatively by the free-range system. The same tendency regarding the effect of free-range system on carcass weight was observed in chickens (Cheng et al., 2008), partridges (Yamak et al., 2016), pigs (Hoffman et al.,

2003; Parunović et al., 2012) and kids (Herrera et al., 2011). Although, the carcass yield was similar, chickens kept indoors showed a better carcass muscling. In fact, birds reared indoor had higher breast weight ($P < 0.05$) and sliglty higher breast yield ($P = 0.074$). Leg weight and leg yield also were lower for free-range birds (+5.27% and +0.08%, respectively), however the differences were not significant. These results are in contrast to the findings of Castellini et al. (2002), who found that percentages of breast and thigh meat increased when birds had outdoor access. Our results are not in accordance also with the results reported by Tong et al. (2014). They observed that the breast yield increased linearly with increasing free-range days ($P < 0.05$), whereas the leg yields decreased linearly ($P < 0.05$). Differently, Wang et al. (2009), Mikulski et al. (2011), Połtowicz and Doktor (2011) observed no significant differences in the dressing percentage and percentage of breast muscles, leg muscles between chickens reared in free-range and conventional systems. Moreover, in the current study the birds in the free-range treatment showed markedly lower ($P < 0.001$) wings weight and yield than birds in the indoor treatment. In agreement with literature (Santos et al., 2005; Wang et al., 2009; Tong et al., 2014), the housing system had no significant effect on either back+neck weight and back+neck content in the carcass.

Table 5.2. Effect of rearing system and vitamin E treatment on slaughter traits of Kabir chickens.

Traits	Rearing system (RS)		Treatment (T)		SEM	Significance		
	Indoor	Outdoor	Control	Vit E		RS	T	RS x T
Final body weight (g)	3288.73	3090.93	3205.00	3182.25	32.99	*	ns	ns
Carcass weight (g)	2101.67	1989.97	2021.50	2057.98	23.12	ns	ns	*
Carcass yield (%)	64.02	64.38	63.13	64.73	0.51	ns	ns	ns
Breast weight (g)	405.17	366.43	383.00	387.20	5.56	*	ns	**
Breast yield (%)	19.26	18.41	18.92	18.80	0.16	ns	ns	ns
Leg weight (g)	745.43	706.13	728.20	724.58	11.28	ns	ns	*
Leg yield (%)	35.51	35.43	36.04	35.19	0.341	ns	ns	ns
Wings weight (g)	217.50	188.67	207.25	201.00	2.77	***	ns	***
Wings yield (%)	10.37	9.46	10.26	9.75	0.08	***	***	ns
Back+neck weight (g)	689.00	655.80	682.00	667.60	9.30	ns	ns	*
Back+neck yield (%)	32.87	32.96	33.74	32.51	0.33	ns	ns	ns

$P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

The data of research clearly show that the single intramuscular injection of vitamin E is inefficient in improving the chicken body weight (Table 5.2). No differences ($P > 0.05$) were found between control group and experimental group also in case of carcass weight and carcass yield, it ranged from 2021.50 to 2057.98g, and from 63.13 to 64.73%, respectively. No effect of supplementation with vitamin E on aforementioned slaughter characteristics of broilers was also observed by Leonel et al. (2007) and Adebiyi et al. (2011). Furthermore, our finding corroborate the results of studies carried out on other species, such as pigs (Niculita et al., 2007), lambs (Maiorano et al., 2007; Atay et al., 2009) and bulls (Neto et al., 2012). The weights of the main cuts of the carcass (breast and leg) and mean yields of these parts did not differ ($P > 0.05$) between the evaluated groups. The lack of significance influence of vitamin E supplementation on carcass muscling of chickens was observed by Nobakht et al. (2012) as well. Chickens from control group characterized by higher ($P < 0.01$) value of wing yield (+ 0.51%) than these from Vit E group. Presented study revealed no vitamin E effect ($P > 0.05$) for values of wings weight, back+neck weight and back+neck yield. Despite above observations regarding the lack of influence of vitamin E on chickens final weight and carcass traits, marked effect ($0.05 > P < 0.01$) of interactions between rearing system x vitamin E treatment was recorded for most of slaughter performances. These interactions indicated that significance differences exist between chickens injected with vitamin E in different rearing systems (with/without outdoor access).

5.3.2. Physicochemical properties

Effects of rearing system and treatment with vitamin E injected intramuscularly on pH, color of breast muscle after 24 hours *post-mortem* and water-holding capacity are presented in Table 5.3.

Breast meat ultimate pH value (ranging from 5.77 to 5.82) was not significantly affected by both rearing system and vitamin E. In the chicken, normal ultimate pH value is around 5.8 (Fletcher, 1999; van Laack et al., 2000). Thus, can be assumed that the recorded pH values are within the acceptable range for commercial meats, with no evidence of pre-slaughter stress. A slight increase in muscle pH was reported by Alvarado et al. (2005) in chicken reared with free-range system. The tendency of having high pH could be attributed to lower energy intake due to undernutrition and/or preslaughter stress (Apple et al., 1995; Sañudo et al., 1998). Differently, Culioli et al. (1990), Castellini et al. (2002), Fanatico et al. (2007) and Dou et al. (2009) found that

outdoor access decreases muscle pH in comparison to pH of muscle obtained from chickens reared in intensive systems. Results of these research indicated that active birds are more prone to stress, which leads to rapid breast muscle acidification. This fact is worth to noticing, because acidifying poultry meat induces a destructuration of the myofibrillar network, which also induces a marked decrease in water-holding capacity (Barbut, 1997).

Regarding the effect of vitamine E on pH of meat, our findings corroborate the results described by Leonel et al. (2007) and Li et al. (2009). They reported that supplemental vitamin E in diet of chickens did not influence pH value markedly. Moreover, the addition of vitamin E to diet did not appear to affect pH values of meat from other species: lambs (Atay et al., 2009), cattle (O'Grady et al., 2001) and pigs (Guo et al., 2006). In contrast, Lauridsen et al. (1999) observed increased ultimate pH in pigs fed 200 mg of dl-α-tocopheryl acetate/kg compared to pigs supplemented with 0 or 100 mg of dl-α-tocopheryl acetate/kg feed. Also Maiorano et al. (2007) have reported that 1200 IU of DL-α-tocopheryl acetate (150 IU/week) intramuscular injected in lambs influence the pH of longissimus muscle which was higher in treated animals when compared to control animals (5.72 *versus* 5.62 for vitamin group and control, respectively; $P < 0.05$). However, a difference in pH would not be expected because vitamin E has not been reported to have a direct effect on glycolytic potential or *post-mortem* glycolysis (Hasty et al., 2002).

Table 5.3. Effect of rearing system and vitamin E treatment on pH, color parameters (L*, a*, b*) and water-holding capacity in breast muscle of Kabir chickens.

Traits	Rearing system (RS)		Treatment (T)		SEM	Significance		
	Indoor	Outdoor	Control	Vit E		RS	T	RS x T
pH_{24}	5.77	5.82	5.77	5.80	0.01	ns	ns	ns
Color 24 h								
L*	52.51	46.32	47.34	50.45	0.43	***	***	**
a*	2.58	1.97	3.24	1.80	0.16	ns	***	ns
b*	9.10	11.33	9.03	10.82	0.44	*	*	ns
WHC	12.32	12.16	11.96	12.39	0.18	ns	ns	ns

P < 0.05. ** P < 0.01. *** P < 0.001.

pH is also associated with meat color, that is an important quality attribute which influences consumer acceptance of many food products, including poultry meat (Qiao et al., 2001; Kralik et al., 2014). In our study, color parameters measured in breast muscle after 24 hours *post-mortem*, were affected by rearing system. Access to outdoor reduced the values of ($P < 0.001$) L* (lightness) and a* (redness) ($P > 0.05$). Likewise, Fanatico et al. (2007) demonstrated that the meat of chickens kept indoors characterized by lighter color than that of free-range reared birds. While, Castellini et al. (2002) reported that the organic production system with free-range access increased the lightness of meat, which was paler than the meat of birds kept indoors. Lightness is one of the most important meat quality parameter that is correlated with fiber composition of type I and IIB (Ryu and Kim, 2006). The change in L* can be attributed to the alterations for sarcoplasmatic and myofibrillar proteins and subsequent alternations of the meat surface properties (Tao et al., 2014). Soares et al. (2009) have proposed the following criteria for classification of breast meat into quality categories: $L^* \geq 53$ for like-PSE, $L^* \leq 44$ for like-DFD and $44 < L^* < 53$ for normal meat. Taking this under consideration, analyzed indoor and free-range chicken breast samples should be classified as a normal meat. Regarding redness (a*), our finding is consistent with study conducted by Fanatico et al. (2005a). These authors have found that meat of slow-growing chicken genotypes with outdoor access was characterized by lower a* value compared with the meat of birds reared indoor. Available literature explained that observed in presented research the decrease in redness of meat from outdoor chickens is probably due to the increased oxidation of myoglobin and/or heme displacement and release (Tao et al., 2014). Regarding yellowness (b*) index, in the studies described by Alvarado et al. (2005), Fanatico et al. (2005a, 2007) and Puchała et al. (2015), outdoor access resulted in the same increasing impact on the yellowness of meat as was recorded in our research. Meanwhile, findings of studies conducted by Mikulski et al. (2011), Połtowicz and Doktor (2011) demonstrated that the free-range rearing system did not affect the b* value of chickens' meat. In current study, the relatively high values of yellowness of breast meat, may be due to the access of outdoor and the natural pigments present in the plant material (Fanatico et al., 2005a). Among main pigment compounds of plants that could alter the meat color are carotene (golden), pheophytin (olive green), chlorophyll a (blue-green), chlorophyll b (yellow-green), lutein (yellow), xanthophylls (yellow), betalains (yellow, orange, red, purple), and anthocyanins (red) (Boonsong et al., 2011).

Together with other technological parameters, water-holding capacity (WHC) is generally used to predict the degree of meat quality (Joo et al., 1999; Fischer, 2007). In presented study, WHC was not affected (P = 0.308) by the rearing system. On the contrary, Castellini et al. (2002), Fanatico et al. (2007) and Cheng et al. (2008) have observed that an outdoor production system lowered WHC value. The decrease in water-holding capacity could be explained in terms of protein denaturation resulting in a loss of gel matrix integrity and the concomitant loss in gel strength and water-holding capacity which determines the volume of fluid formed on cooking (Joseph and Olanrewaju, 1999). Additionally, lower WHC indicates losses in the nutritional value through exudates that are released and this results in drier and tougher meat (Dabes, 2001).

Meat color was also influenced by vitamin E treatment (Table 5.3). Suman (2012) reported that lipid oxidation could promote myoglobin oxidation. Consequently, the factors affecting lipid oxidation in meat, such as vitamin E, can also influence meat color. The α-tocopherol has been reported to stabilize the oxymyoglobin complex (Lanari et al. 1994) and there is considerable evidence that this results in an improvement in the color life of fresh pork (Asghar et al., 1991), beef (Arnold et al., 1993), and lamb (Wulf et al., 1995). Compared to the control group, the intramuscular injection of vitamin E increased (P < 0.001) the lightness (L*: 50.45 *versus* 47.34 for vitamin E and control, respectively) of breast muscle. Similar trend has been observed for yellowness (b*: 10.82 *versus* 9.03 for vitamin E and control, respectively). Our findings are in agreement with these recorded by Xiao et al. (2011). They found that dietary supplementation of vitamin E significantly enhanced the lightness (L*) and slightly elevated the yellowness (b*) of chicken thigh meat compared with the color of chicken thigh meat from the control group during 7 days of refrigerated storage. Furthermore, results of current research are partially comparable to these of Hasty et al. (2002), who noted no effects of α-tocopheryl acetate supplementation to the pigs diet on L* or a* values. Simultaneously, they reported a tendency for vitamin E to increase b* values linearly indicating increased yellowness. Significant differences were also observed for redness (a*); value of this parameter was higher (P < 0.001) in control group in comparison with Vit E group (+80%). Taking under consideration redness of meat, our findings are partially consistent with result of study conducted by Xiao et al. (2011). These authors have found that chickens of control group had lower a* value compared with these fed the diet supplemented with antioxidants (500 IU of vitamin E

+ 200 mg/kg of butylated hydroxyanisole); but in their study the differences were not statistically significant. While, the study of Zouari et al. (2010) showed that the red coordinate a* values decreased over time and were not significantly different between thigh meat from vitamin E fed chickens or control ones upon refrigeration. At this point worth to mention that the effect of endogenous vitamin E on color quality is more evident in species having higher levels of myoglobin (e.g. beef and lamb meat) (Zouari et al., 2010). Moreover, significant interaction ($P < 0.01$) between rearing system and vitamin E treatment was observed for the value of L* parameter.

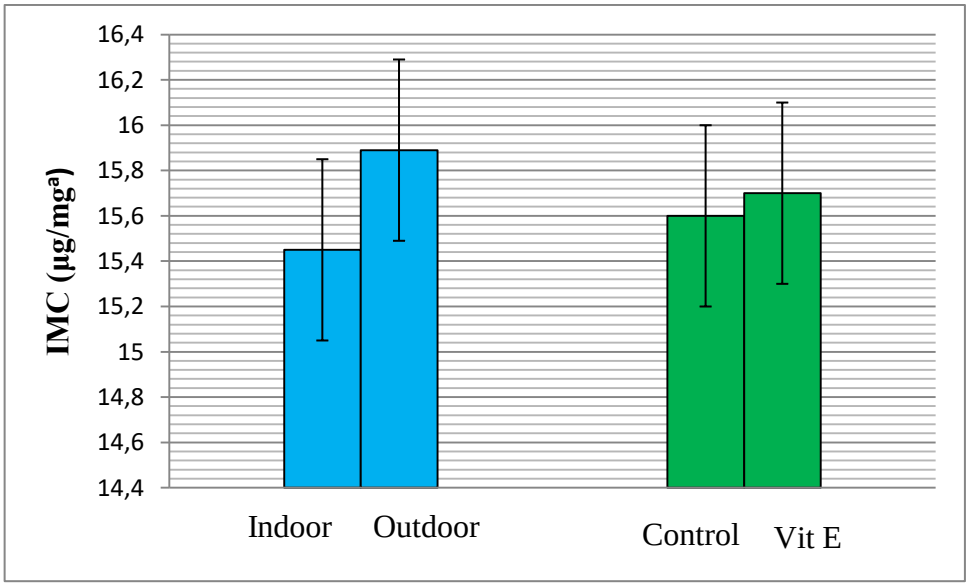
Water-holding capacity was not affected by Vit E treatment. Our result regarding this trait is in line with the finding of study conducted by Adebiyi et al. (2011). They recorded no significant differences in the WHC of both raw and the cooked meat for all the treatments (50 mg/kg, 100 mg/kg and 150 mg/kg vitamin E supplementation). Despite the lack of marked differences, important is to highlight that higher water-holding capacity usually equates to juicier and more palatable sensory perceptions and an overall improvement in meat quality. From the processor's perspective, increased WHC equals increased yield (Smith and Acton, 2010).

5.3.3. Intramuscular collagen content

Effects of rearing system and treatment with vitamin E injected intramuscularly on intramuscular collagen (IMC) level in chickens breast muscle are presented in Figure 5.3. Mentioned trait have great impact on eating quality of meat. Collagen characteristics, mainly content and solubility, determined the contribution of connective tissue to meat toughness (Konga et al., 2008). In the current study, based on the obtained data it could be concluded that collagen concentration in meat of Kabir chickens was slightly higher for group reared with outdoor access (+2.77%). Although, observed difference was not significant ($P > 0.05$). Regarding collagen content in meat of free-range and conventional chickens, our results are in consistent with Puttaraksa et al. (2012). They found that insoluble and total collagen content of breast meat was not significantly different among treatments ($P > 0.05$), whereas the outdoor chickens had lower soluble collagen content in breast meat than indoor chickens. No difference between IMC content in meat of pigs from group that was housed in a pen, and the outdoor-reared animals that had access to a paddock was observed by Maiorano et al. (2013). Meanwhile, Maiorano et al. (2003) did not find any effect of rearing system on *longissimus* muscle collagen content, however they noted an increase of HLP content

and HLP/IMC ratio in *m. semimembranosus* of outdoor pigs than their confinement-reared counterparts. The effect of rearing system on collagen content was observed also in study described by Hanekom (2010). In this research, the *Bicpes femoris* of the lambs from the extensive production system contained more ($P < 0.05$) insoluble collagen than mentioned muscle from intensively raised lambs. There is also an investigation on the adaptation of collagen properties to outdoor rearing systems in case of rabbits. Combes et al. (2003) documented that collagen content is similar or slightly higher in organic outdoor rabbits than in confined animals, however, the heat-solubility of collagen is not modified by the rearing system.

Figure 5.3. Effect of rearing system (indoor *versus* outdoor) and vitamin E (control group *versus* Vit E group) on intramuscular collagen (IMC) content in breast muscle of Kabir chickens.



[a] of lyophilized muscular tissue.

Results regarding the effect of vitamin E on intramuscular collagen (IMC) content are reported in Figure 5.3. It has been observed that vitamins E can affects collagen renewal. Supplementation with vitamins E may increase meat tenderness by increasing collagen renewal and turnover (Archile-Contreras et al., 2012). The data of this research clearly shows that the single intramuscular injection of vitamin E doesn't have marked effect on IMC concentration in breast muscle of Kabir chicken. Meat samples of birds from control group characterized by only slightly lower IMC content than those from experimental group injected intramuscularly with DL-α-tocopheryl

acetate (+0.64%). The results of the present study are in line with the findings provided by Maiorano et al. (2007), Maiorano et al. (2015b) who reported that dl-α-tocopheryl acetate did not influence IMC amount in lambs meat. While, in the study of Maiorano et al. (1999) collagen concentration increased in meat of lambs injected with 625 IU of dl-α-tocopheryl acetate.

5.3.4. Fatty acids profile and total lipid content

Table 5.4 shows the effect of rearing system and vitamin E treatment on total lipid content, fatty acids composition (% of total fatty acids) and nutritional ratios in breast muscle of Kabir chickens.

Total lipid content in breast muscle, ranging from 1.30 to 1.39 g/100 g, was not affect ($P > 0.05$) by both rearing system and vitamin E. Lipids are important components in foods which directly impact on sensory properties and diet consumer, mainly due to unsaturated fatty acids present on lipid fraction. Therefore, total lipid determination is essential for known food composition (Vasconcellos de Alcântara et al., 2015). There are few investigation about the effect of rearing system on total lipids content in chicken meat. Our finding is in agreement with other works conducted on chickens by Mikulski et al., (2011), Küçükyılmaz et al. (2012) and Michalczuk et al. (2014). These studies did not demonstrate any significant effect of the rearing system on fat level in muscles. Differently, Castellini et al. (2002) observed minimum differences in protein content and substantial differences in fat content in m. *pectoralis major* and m. *peroneus longus* of conventional broilers and organic broilers. Namely, at 56 days of age, organic broilers had a fat content of 0.72% in the *pectoralis major* and conventional broilers 1.46%. At 81 days of age, the respective values were within a wider range of 0.74 and 2.37%. A similar trend was observed for the m. *peroneus longus*. The same tendency regarding the effect of vitamin E on total lipid content was observed in the other studies on chickens (Skřivan et al., 2010), ducks (Schiavone et al., 2010), goose (Łukaszewicz et al., 2016); no effect of supplementation with vitamin E on lipid content also was observed in case of other livestock species, e.g. pigs (Morel et al., 2006; Bahelka et al., 2011).

Generally, poultry fat is characterized by more desirable composition than other animal fats, which facilitates its absorption in the body. Poultry may synthesize saturated and monounsaturated fatty acids from non-fat feed mixtures. In contrast, polyunaturated fatty acids are not synthesized by poultry and ought to be provided to

birds with feed mixtures (Michalczuk et al., 2014). It is widely known that the composition of dietary fat will affect the composition of fat deposited as carcass fat. The difference in fatty acid composition in meat is likely due to dietary fatty acid intake (Molee et al., 2012). Thus, it could be concluded that fatty acid profile of poultry meat may depends on rearing system, and connected with it feeding system.

Overall, the PUFA were the most abundant fatty acids with 39.44±0.30%, followed by SFA with 36.55±0.348% and MUFA with 24.04±0.45%. In the current research rearing system did not significantly affect the total SFA content and the proportion of single SFA. Quantitatively, the palmitic acid (C 16:0) was the most concentrated saturated fatty acid (25.96-26.16%). Our findings partially corroborate the results described by Kralik et al. (2005). In their research content of total SFA did not differ statistically ($P > 0.05$) between investigated groups; when compared to the outdoor group, chickens of the indoor group exhibited considerably higher portion of myristic (C 14:0), and palmitic acid (C 16:0). However in this study reported difference between examined chickens group were significant. Lack of marked differences between birds reared indoor and outdoor regarding content of SFA, myristic acid (C 14:0), palmitic acid (C 16:0) and stearic acid (C 18:0) was observed by Skomorucha and Sosnówka-Czajka (2015). Also in their study, among SFA the most abundant was the palmitic acid (C 16:0) that concentration ranged from 26.74 to 27.10%. Palmitic acid, whose name comes from the tree, is the main fatty acid occurring naturally in animals and vegetables as well as is the main component of human milk fats. Palmitic acid has been positively related to high serum cholesterol levels (both LDL- and HDL-cholesterol) and, as a consequence, to an increase in the risk of cardiovascular diseases (Fattore and Finelli, 2013).

Table 5.4. Effect of rearing system and vitamin E on total lipid content (g/100g), fatty acids composition (% of total fatty acids), and nutritional ratios in breast muscle of Kabir chickens.

Item	Rearing system (RS)		Treatment (T)		SEM	Significance		
	Indoor	Outdoor	Control	Vit E		RS	T	RS x T
Total lipid	1.30	1.37	1.24	1.39	0.10	ns	ns	ns
C 14:0	0.19	0.18	0.15	0.20	0.01	ns	*	ns
C 16:0	26.16	25.96	25.61	26.29	0.30	ns	ns	ns
C 16:1	1.18	1.30	1.05	1.33	0.08	ns	ns	ns
C 18:0	10.29	10.37	10.59	10.20	0.19	ns	ns	ns
C 18:1 n-9	22.52	23.16	21.91	23.31	0.38	ns	ns	ns
C 18:2 n-6	26.03	25.87	25.96	25.95	0.32	ns	ns	ns
C 18:3 n-3	0.62	0.59	0.55	0.63	0.02	ns	ns	ns
C 18:3 n-6	0.09	0.09	0.09	0.09	0.01	ns	ns	ns
C 20:1	0.11	0.10	0.10	0.11	0.01	ns	ns	ns
C 20:3 n-3	0.51	0.38	0.54	0.40	0.03	*	*	ns
C 20:4 n-6	9.36	9.27	10.35	8.80	0.32	ns	*	ns
C 20:5 n-3	0.09	0.06	0.13	0.06	0.02	ns	*	ns
C 22:4 n-6	1.66	1.57	1.67	1.59	0.06	ns	ns	ns
C 22:5 n-3	0.28	0.24	0.29	0.25	0.02	ns	ns	ns
C 22:6 n-3	0.96	0.85	1.00	0.86	0.05	ns	ns	ns
Partial sum								
ΣSFA	36.64	36.51	36.35	36.69	0.34	ns	ns	ns
ΣMUFA	23.81	24.56	23.06	24.75	0.45	ns	ns	ns
ΣPUFA	39.62	38.93	40.59	38.62	0.30	ns	**	ns
Σn-6	37.15	36.80	38.08	36.43	0.28	ns	**	ns
Σn-3	2.47	2.12	2.51	2.19	0.07	*	*	ns
Nutritional ratios								
n-6/n-3	15.54	17.47	15.58	16.97	0.41	*	ns	ns
n-3/n-6	0.07	0.06	0.07	0.06	0.01	*	ns	ns
PUFA/SFA	1.08	1.07	1.12	1.06	0.01	ns	*	ns
Atherogenic index	0.42	0.42	0.41	0.43	0.01	ns	ns	ns
Thrombogenic index	0.97	0.99	0.96	0.99	0.02	ns	ns	ns

* $P < 0.05$. ** $P < 0.01$.

Rearing system did not affect ($P > 0.05$) total MUFA content and the individual MUFA values. Quantitatively, the oleic acid (C 18:1 n-9) was the most concentrated MUFA (ranging from 21.91 to 23.31%) followed by palmitoleic acid (C 16:1; ranging from 1.05 to 1.33%) and gadoleic acid (C 20:1; ranging from 0.10 to 0.11%). From a nutritional point of view, oleic acid has a relevant importance in the human diet because it acts on lipaemia reducing both LDL cholesterol and the triglycerides (Mattson and Grundy, 1985; Stachowska et al., 2010) and providing other health benefit, such as a reduced risk of stroke and a significant decrease in both systolic and diastolic blood pressure in susceptible populations (Kris-Etherton, 1999). In general, some

epidemiological studies have suggested an inverse relationship between MUFA intake and mortality rates to cardiovascular disease (Hu et al., 1997; Kris-Etherton, 1999).

Also the total PUFA content was not influenced ($P > 0.05$) by rearing system. Similar result was obtained by Michalczuk et al. (2014) and Skomorucha and Sosnówka-Czajka (2015), who reported that the difference in polyunsaturated FA level between indoor and outdoor chickens was not significant. Quantitatively, the linoleic (C 18:2 n-6; ranging from 25.87 to 26.03%) was the most abundant PUFA followed by arachidonic (C 20:4 n-6; ranging from 9.72 to 9.36%), docosatetraenoic (C 22:4 n-6; ranging from 1.57 to 1.66%), docosahexaenoic acid (DHA, C 22:6 n-3; ranging from 0.85 to 0.96%), α-linolenic acid (ALA, C 18:3 n-3; ranging from 0.55- to 0.63%), and eicosatrienoic (C 20:3 n-3; 0.51% *versus* 0.38% in indoor and outdoor, respectively; $P < 0.05$). Similar result was obtained by Kralik et al. (2005). In their research have been reported that dominant FA among polyunsaturated are linoleic and arachidonic acid. Linoleic acid is a major fatty acid in plant lipids. In animals it is derived mainly from dietary plant oils. Arachidonic acid is a major component of membrane phospholipids throughout the animal kingdom, but very little is found in the diet (Rustan and Drevon, 2005). These acids, in particular arachidonic acid, are the precursors of important hormonal substances (prostaglandins and leukotrienes), and play important roles in the mechanism of action of second messengers, but their structural role is also important since they modulate the structure, enzymatic activities, and function of the membranes (Bourre et al., 1996). Eicosatrienoic acid is an n-3 PUFA that is widely distributed in the phospholipids of most animal tissues, it rarely account for more than 1% of the fatty acid total (Chen et al., 2015). Therefore, the presence of eicosatrienoic acid isn't considered in numerous scientific studies.

No significant difference ($P > 0.05$) was found for the total n-6 FA amount. This kind of PUFA is involved in the synthesis of eicosanoids, biologically active in very small quantities and with properties much more inflammatory than eicosanoids from the n-3 PUFA. Therefore, nutritional guidelines recommend minimizing the intake of n-6 fatty acids relative to n-3 fatty acids (Parunović et al., 2012). The statistically significant n-6 FA content-reducing effect of outdoor access was observed by Michalczuk et al. (2016). In our experiment, the n-3 was affected ($P < 0.05$) by the rearing system; value of this trait was higher in breast muscle of birds reared indoor in comparison to outdoor chickens. These n-3 FA are associated with a number of physiological and health beneficial effects. The n-3 fatty acids are known to have potential in the prevention and

treatment of cardiovascular disease, diabetes, and some types of cancer (Molee et al., 2012). Moreover, the n-3 PUFA influence macrophages to be less inflammatory, enhance antibody responses, and suppress cell-mediated response compared with n-6 PUFA (Maroufyan et al., 2012). Our observation, regarding n-3 FA, is in contrast with these described by Ponte et al. (2008d), Molee et al. (2012), and Michalczuk et al. (2016). In their study the level of n-3 fatty acids was greater in breast meat of broiler in free-range-pastured system. In presented experiment, also n-6/n-3 and n-3/n-6 ratios were influenced by outdoor access significantly. The ratio n-6/n-3 was lower ($P < 0.05$) in breast muscle of birds reared indoor in comparison to outdoor chickens. Mentioned ratio is an important determinant of health. A lower ratio of n-6 to n-3 fatty acids is more desirable in reducing the risk of many of the diseases. Michalczuk et al. (2014) observed a opposite tendency as that reported in our study. Their research showed a slightly lower n-6/n-3 ratio in the group of birds allowed to use free-range. Our finding isn't consistent with result of study conducted by Molee et al. (2012). In this study, the ratio of n-6 to n-3 fatty acids was lower in breast and thigh meat of free-range group than that of control group. Regarding, the n-3/n-6 ratio we have observed the siginificant impact of examined factor; value of this trait was higher in breast muscle of birds reared indoor in comparison to outdoor chickens. It has been suggested that this ratio is a good standard to compare the nutritional value of lipids presented in meat poultry.The n-3/n-6 ratio of 1:1, or 1:1.5 can contribute to a healthy diet in humans (Chávez-Mendoza et al., 2014). Moreover, in presented research the PUFA/SFA (polyunsaturated fatty acids/saturated fatty acids) ratio was evaluated. The PUFA/SFA is used for assessment of lipids on the basis of the proportions of the different fatty acids groups. It plays a significant role for cell membrane properties such as fluidity, contributing to the normal cell metabolism (Merdzhanova, 2014). In our study, the PUFA/SFA ratio was not affected ($P > 0.05$) by rearing system. Similar results (1.11 and 1.13% for broiler chickens reared outdoor and indoor, respectively) were obtained by Skomorucha and Sosnówka-Czajka (2015).

In the present study, two distinct indexes were investigated: 1) atherogenic index (IA); and 2) thrombogenic index (IT). These indexes take into account the different effects that single fatty acid might have on human health and in particular on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Garaffo et al., 2011). In the present research, both the

atherogenic index and thrombogenic index were not affected (P > 0.05) by the free-range treatment.

Based on the data from Table 5.4 it could be concluded that total SFA content and palmitic acid (C 16:0) and stearic acid (C 18:0) level in chickens meat were not affected (P > 0.05) by intramuscular injection of dl-α-tocopheryl acetate. Differently, vitamin E increased (P < 0.05) the content of myristic acid (C 14:0). Our results partially corroborate with these carried out on chickens by Cortinas et al. (2004) and Leonel et al. (2007). In their studies, total SFA amount, and myristic acid content were only slightly (P > 0.05) influenced by vitamin E supplementation. While in the research conducted on pigs by Martino et al. (2014) rearing system showed significant effect on myristic acid. Myristic acid, also called tetradecanoic acid, has a widespread occurrence, occasionally as a major component (Rustan and Drevon, 2005). Myristic acid has the greatest effect and are abundant in diets rich in dairy products and meat. Have been concluded that intake of foods rich in myristic acid should be replaced by fats with a lower content of these particular fatty acids (Hu et al., 1997). Respect to myristic acid, Bellizzi et al. (1994) found a positive correlation with coronary heart disease mortality. Moreover, the same authors noted that myristic acid was the major cause of hypercholesterolemia induction. This effect was related both to repression of hepatic low density lipoprotein (LDL) receptor synthesis and to direct stimulation hepatic LDL synthesis.

Presented study revealed no significant difference (P > 0.05) for total MUFA content and the sigle MUFA acids, as palmitoleic acid (C 16:1), gadoleic acid (C 20:1) and oleic acid (C 18:1n-9) between control and vitamin E group. In the experiment described by Schiavone et al. (2010) this same tendency regarding the effect of vitamin E on C 18:1 n-9 and total MUFA content was reported. Moreover, our results are in accord with these of Bölükbaşi et al. (2006), who observed that breast muscle of birds from control and vitamin E group had similar level of oleic acid (C 18:1) and total MUFA level. The statistically significant total MUFA content-reducing effect of vitamin E was observed in sheep by Zhao et al. (2013). While, in the study carried out on pigs by Guo et al. (2006) dietary vitamin E supplementation effectively changed the tissue fatty acid profiles by increasing the total MUFA concentration.

Intramuscular injection of dl-α-tocopheryl acetate did not significantly affect the proportion of single PUFA, except for the eicosatrienoic acid (C 20:3 n-3), arachidonic acid (C 20:4 n-6) and eicosapentaenoic acid (C 20:5 n-3) content. Levels of

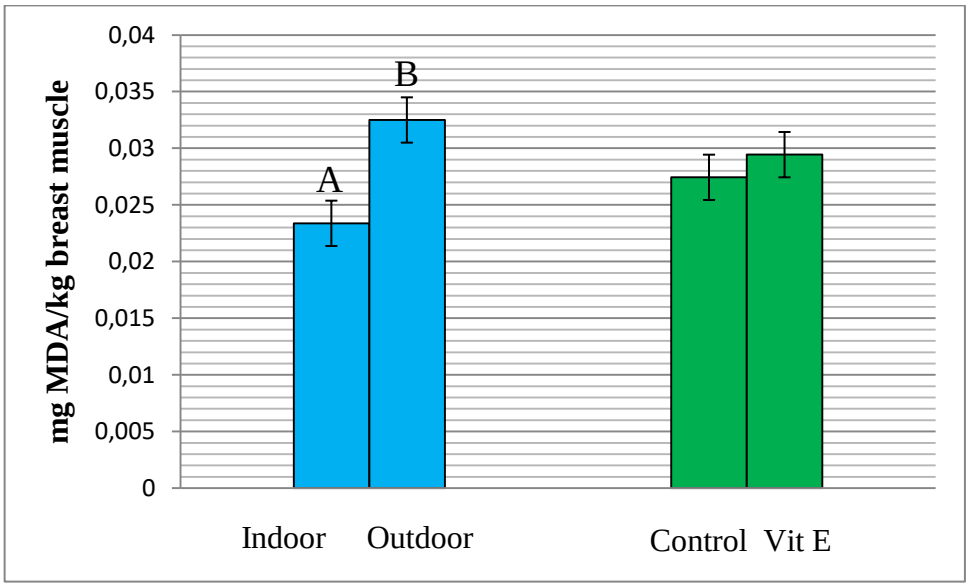
these PUFA were markedly higher ($P < 0.05$) in breast muscles of birds from control group than that in muscles of birds injected with vitamin E. Eicosatrienoic acid (C 20:3 n-3), arachidonic acid (C 20:4 n-6) and eicosapentaenoic acid (C 20:5 n-3) were detected also in study described by Leonel et al. (2007). However, in their research dietary supplementation of vitamin E didn't changed significantly content of these FA. Moreover, vitamin E reduced ($P < 0.01$) the total PUFA content compared to control. Considering this trait, our findings aren't in agreement with these recorded by Schiavone et al. (2010). They did not found any difference for the total PUFA concentration between control and experimental group, that received dietary supplementation of vitamin E.

In the current study, the breast muscles of chickens from Vit E group were distinguished by significantly lower content of Σn-6 ($P < 0.01$) and Σn-3 ($P < 0.05$) in comparison to control group. Regarding both mentioned ratios, our results aren't in line with data described by Zhao et al. (2013). In their study Σn-6 PUFA concentration was significantly lower in meat of control group animals in comparison to individuals from vitamin E groups; while n-3 PUFA level weren't affected by vitamin E supplementation. In contrary to our observations are also these described by Schiavone et al. (2010). They reported that supplemental vitamin E in diet of ducks did not influence Σn-6 and Σn-3 markedly. In presented study, the PUFA/SFA ratio was affected by vitamin E; value of this trait was higher ($P < 0.05$) in breast muscles of birds from control group than that of vitamin E group (1.12 *versus* 1.06, respectively). According to FAO and WHO, the PUFA/SFA ratio in human diets should amount to 0.45. Therefore, from the point of view of human health, obtained in our research result concerning the PUFA/SFA ratio seems to be too high. The opposite trend regarding mentioned ratio was reported by Liu et al. (2013) and Zhao et al. (2013). In their study an increasing tendency for the ratio of PUFA/SFA in muscles of animals treated with vitamin E was detected. In our study, no significant differences ($P > 0.05$) were found between control and vitamin E group for n-6/n-3, n-3/n-6 ratios, the atherogenic index (AI) and thrombogenic index (TI). Moreover, lack of marked effect of interactions between rearing system x vitamin E treatment was recorded for all detected fatty acids, calculated ratios, and nutritional indexes.

5.3.5. TBARS: Oxidative stability

Thiobarbituric acid reactive substances (TBARS) values during storage are provided in Figure 5.4. The level of TBARS was significantly ($P < 0.01$) affected by the rearing system. Value of this traits was markedly higher for outdoor chickens in comparison to indoor birds. TBARS level reflects oxidation processes in meat lipids. Lipid oxidation is one of the primary causes of quality deterioration in meat and generates compounds potentially dangerous. The conversion of muscle to edible meat after slaughter can unbalance the equilibrium between pro-oxidative and anti-oxidative factors, resulting in initiation and propagation of lipid oxidation (Min and Ahn, 2005). The rearing system is one of the factors that potentially can influence the lipid oxidation.

Figure 5.4. Effect of the rearing system (indoor *versus* outdoor) and vitamin E (control group versus Vit E group) on the evolution of TBARS (mg MDA/kg, means ± SE) in breast muscles after 1 month in the freezer at temperature -18°C.



$^{A, B}$ values within the same group differ significantly ($P < 0.01$)

Our results are in line with these reported by Castellini et al., (2008). They observed a higher TBARS in meat from free-reared poultry, which could be due to the higher content of metallic ions (total and heme Fe) that catalyze peroxidation, and to the greater degree of unsaturation of intramuscular lipids. Moreover, lipid oxidation can be affected by physical activity of birds. Castellini et al. (2002) found that organically reared chickens had a lower lipid stability (as shown by higher TBARS values) than in

conventionally house-reared chickens, which he attributed to greater physical activity by organic birds. Similarly, Petersen et al. (1997) speculated that a greater degree of physical fitness increases the muscle oxidative capacity. This same tendency, as in presented study, was reported by Nudda et al. (2013) in lamb. In their research the fatty acids oxidation, expressed as mg MDA/kg muscle, was higher for outdoor than indoor lambs. On the other hand, our findings aren't in agreement with the results of several other studies (Gatellier et al., 2005; Santé-Lhoutellier et al., 2008; Warren et al., 2008). Authors of these experiments observed the protective effect of grass-based rearing system against oxidative processes in meat.

Oxidation of muscle components *post-mortem* can be inhibited by antioxidants in the diet, of which α-tocopherol is the most important. Vitamin E might control the level of oxygenated radical scavenging systems such as GSH-Px and depress antioxidant enzyme activities by homeostatic compensation (Bayraktar et al., 2011). It is well-established that α-tocopherol confers greater lipid stability and is therefore widely used as an additive in dietary supplements (Sun et al., 2012). In the current study, the level of TBARS wasn't significantly ($P > 0.05$) affected by vitamin E. Value of this traits was only slightly higher for birds from Vit E group in comparison to chickens from control group. Our observations aren't in line with these noted by Sun et al. (2012). According them the vitamin E can be found at higher concentrations in muscles from free-range birds than in muscles from animals raised on concentrates. The rich dietary source of antioxidants eaten by broilers reared at pasture provides a more effective inhibition of lipid oxidation, and as a consequence, the lower values of TBARS observed in meat.

5.4. CONCLUSIONS

The main goal of this study was to verify the hypotheses about the effect of the rearing system and intramuscular injection of vitamin E on performance and meat quality traits of Kabir chickens.

Birds reared indoor were heavier that those reared outdoor ($P < 0.05$). The carcass weight has been insignificantly reduced by the free-range system. While, the carcass yield of chickens reared indoors was only by 0.36% lower than that of outdoor chickens. Breast weight and breast yield were significantly higher for birds reared indoor in comparison to the group with outdoor access; while leg weight and leg yield were not significantly affected by different rearing system. The birds in the free-range

treatment showed lower ($P < 0.01$) wings weight and wings yield than birds in the indoor treatment. The housing system had no significant effect on either back+neck weight and back+neck content in the carcass.

The data of research clearly show that the single intramuscular injection of vitamin E did not affect the bird growth and carcass traits; however, the wings yield was higher ($P < 0.01$) in control group than in vitamin E ones.

A marked effect ($0.05 > P < 0.01$) of interactions between rearing system x vitamin E treatment was recorded for most of slaughter performances.

Breast meat ultimate pH value was not significantly affected by both rearing system and vitamin E; however, the recorded pH values are within the acceptable range for commercial meats. Color parameters were significantly affected by rearing system. Access to outdoor reduced ($P < 0.01$) the values of L* (lightness). The free-range system oppositely influenced the value of yellowness (b*) index, that was higher ($P < 0.05$) in breast muscle collected from chickens with outdoor access. Water-holding capacity was not affected by rearyng system.The intramuscular injection of vitamin E increased the lightness (L* value) ($P < 0.001$) and yellowness (b*) ($P < 0.05$) of breast muscle. On the contrary, value of a* parameter was higher ($P < 0.01$) in control group in comparison with Vit E group. The water-holding capacity wasn't affected by vitamin E.

The data of this research clearly shows that the rearing system and the single intramuscular injection of vitamin E don't have marked effect on intramuscular collagen concentration in breast muscle of Kabir chicken.

Total lipid content in breast muscle was not affect ($P > 0.05$) by both rearing system and vitamin E. Overall, the PUFA were the most abundant fatty acids, followed by SFA, and MUFA. Rearing system did not significantly affects the total SFA and MUFA content and the proportion of single SFA and MUFA. Quantitatively, the palmitic acid (C 16:0) was the most concentrated SFA, while among MUFA the most abundant was oleic acid (C 18:1 n-9). Also the total PUFA content was not influenced ($P > 0.05$) by rearing system. Quantitatively, the linoleic (C 18:2 n-6) was the most abundant PUFA followed by arachidonic (C 20:4 n-6), docosatetraenoic (C 22:4 n-6), docosahexaenoic acid (DHA, C 22:6 n-3), α-linolenic acid (ALA, C 18:3 n-3; ranging from 0.55- to 0.63%), and eicosatrienoic (C 20:3 n-3; 0.51% *versus* 0.38% in indoor and outdoor, respectively; $P < 0.05$). No significant difference ($P > 0.05$) was found for the total n-6 FA amount. While, the total n-3 was affected ($P < 0.05$) by the rearing system; value of this trait was higher in breast muscle of birds reared indoor in comparison to

outdoor chickens. The ratio n-6/n-3 was lower ($P < 0.05$) in breast muscle of birds reared indoor in comparison to outdoor chickens, while value of the n-3/n-6 ratio was higher ($P < 0.05$) in breast muscle of birds reared indoor in comparison to outdoor chickens. The PUFA/SFA ratio, and both the atherogenic index and thrombogenic index were not affected ($P > 0.05$) by the free-range treatment.

Total SFA content, palmitic acid (C 16:0) and stearic acid (C 18:0) level in chickens meat were not affected by intramuscular injection of dl-α-tocopheryl acetate. Differently, vitamin E increased ($P < 0.05$) the content of myristic acid (C 14:0). No significant difference for total MUFA content and the sigle MUFA acids, as palmitoleic acid (C 16:1), gadoleic oleic acid (C 20:1) and oleic acid (C 18:1 n-9) between control and vitamin E group was observed. Intramuscular injection of dl-α-tocopheryl acetate did not significantly affect the proportion of single PUFA, except for the eicosatrienoic acid (C 20:3 n-3), arachidonic acid (C 20:4 n-6) and eicosapentaenoic acid (C 20:5 n-3) content. Levels of these PUFA were markedly higher ($P < 0.05$) in breast muscles of birds from control group than that in muscles of birds injected with vitamin E. Moreover, vitamin E reduced ($P < 0.01$) the total PUFA content compared to the control group. The breast muscles of chickens from Vit E group were distinguished by significantly lower content of Σn-6 ($P < 0.01$) and Σn-3 ($P < 0.05$) in comparison to control group. The value of PUFA/SFA ratio was higher ($P < 0.05$) in breast muscles of birds from control group than that of vitamin E group. No significant differences ($P > 0.05$) were found between control and vitamin E group for n-6/n-3, n-3/n-6 ratios, the atherogenic index and thrombogenic index. Moreover, lack of marked effect of interactions between rearing system x vitamin E treatment was recorded for all detected fatty acids, calculated ratios, and nutritional indexes.

At the light of the above mentioned, this research contributes to extend existing knowledge on free-range broiler chickens by providing new data regarding their performance and meat quality trait in comparison to birds reared indoor. Presented study have confirmed the effect of the increased physical activity of birds, and the natural pigments present in the plant material on slaughter and poultry meat quality traits. Moreover, the current research showed the effect of intramuscular injection of vitamin E on the aforementioned traits of Kabir broiler chickens. The main aim of studies concerning the potential effect of vitamins in livestock animals is establishing the optimal dietary dose of those nutrients that may positively influence the health, level of production and quality of animals' products. Until now the optimal dose of vitamin E

administered by intramuscular injection in case of poultry species has not been determined. Insignificant effect of examined vitamin on almost all slaughter traits of Kabir chickens may be connected with the inadequate amount and/or mode of administration of dl-α-tocopheryl acetate. Although the treatment effects noted in this study were subtle, further research is warranted to elucidate the effect of vitamin E on chicken growth

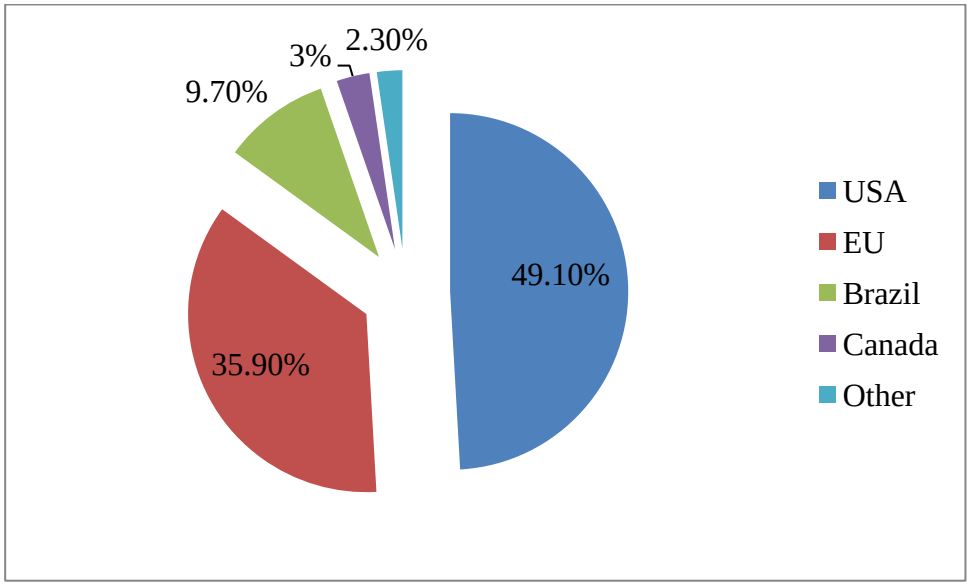
CHAPTER VI

Research n° 2: Biochemical parameters in the blood and meat quality of white hybrid XL turkeys

6.1. Aim

Throughout the world, consumption of poultry meat continues to rise in both developing and developed countries (Mead, 2004a). Despite the fact that during last decades the poultry industry has faced a number challenges (e.g. avian influenza), poultry meat and eggs continue to be hugely important source of animal protein. The category of poultry covers many species, among which (Stenhouse, 2008) chicken is the most common type all over the world. After chicken broiler, the important position in poultry meat production occupy turkey. The leadership in turkey production belongs to United State of America (Figure 6.1), however the next place on the podium belongs to European Union. Among the top European producing turkey meat countries are France, Germany, Italy and Poland (avec, 2014).

Figure 6.1. World turkey meat production in 2014 (avec, 2015).



Although, turkeys production, consumption and trade are much lower than for chickens. It's necessary to highlight that turkeys can be raised successfully almost

anywhere in the world if they are well fed and protected against diseases, predators and adverse weather conditions. Moreover, turkeys grow faster than broiler chickens and have a slaughter weight that is about double that of the broiler chicken at the age of twelve weeks. However, turkeys are more delicate than chickens (Emmah, 2006). Thus, the fundamental role in the maximization of animals productivity plays constant monitoring of their health state (Starkey et al., 1995; Pearson et al., 1997), which is also one of the criteria for welfare assessment.

Essential to controlling the health status of animals is testing of physiological indicators (Radkowska and Herbut, 2014). Serum biochemical references constitute important panels in the diagnosis, prognosis and treatment of livestock diseases via the investigations of myriads of parameters influencing serum biochemical indices (Onasanya et al., 2015). These indices are often helpful in revealing health disorders already in the preclinical stage (Piotrowska et al., 2011). Serum biochemical values have been established in most domestic mammalian species. However, limited information is available for domestic avian species, and even less has been established for turkey species (Ogundu Uduak et al., 2013).

Biochemical analyses are necessary to monitor the health status of poultry that directly influences animals production, including quantity and quality traits of obtained products. The quality of meat in general and hence poultry meat is an extremely complex notion that can be evaluated from different points of view. From the standpoint of consumer interests and the slaughter industry, broilers should have high slaughter yields and desirable carcass conformation scores and also good technological, sensory and nutritional characteristics. From the point of view of nutritionists, poultry meat is a valuable source of proteins, unsaturated fatty-acids, vitamins and minerals, and has a relatively low fat content (2.8 g/100 g of breast and 13 g/100 g of thigh) (Barroeta, 2007; Bogosavljević-Bošković et al., 2010). The mentioned quality traits are dependent upon a number of factors. These factors can be either extrinsic (e.g. conditions of breeding, and slaughtering, feed, technological treatments and post mortem biochemical changes) and intrinsic (such as species, race, sex, genetic origin and slaughtering age) (Tougan et al., 2013b). Among internal factors that substantially affect certain meat quality traits, poultry muscle type plays an pivotal role. The chemical composition of muscle tissue of major primal cuts is an important element of broiler meat quality (Bogosavljević-Bošković et al., 2010).

As well as being sold as a whole, turkey meat is also marketed in pieces to provide various cooking and taste alternatives. Whole bird has basically three large parts i.e. breast, legs and wings. With growing interest in healthy, low-calorie diet have been noticed that costumer who preferred drumsticks recently shifted to breast parts (Chae et al., 2007; Kleczek et al., 2009). Except low calorific value, the flight (breast) muscle of turkey meat is characterized by wide range of another advantages. Reasons for the increasing demand of chicken and turkey breast meat are attributed also to its healthy nutritional profile, sensory properties that make breast meat very flexible for any type of home-cooking style as well as for manufacturing processed products. Moreover, mild flavor and the high tenderness of breast meat allow imparting a wide range of desired favor profiles and textures of processed meat products that meet market needs by targeting different groups of consumers. Additionally, breast meat is very suitable for easy and quick home-cooking, which is important in modern societies where people tend to spend increasingly less time on preparation of meals at home (Petracci et al., 2015).

Second kind of the main poultry muscles is a group of lower limb muscles. In comparison to pectoralis muscle that is homogeneous in type IIB white fibers, thigh and drumstick muscles are comprised of numerous individual muscles, and have a preponderance in type I and type IIA red fibers (Xiong and Blanchard, 1994). The red color of birds muscle fiber is attributed to the high myoglobin content, which absorbs oxygen carried by the small blood vessels and serves as an oxygen reserve for contraction of the living muscle (Heinz and Hautzinger, 2007; Jacob and Pescatore, 2013). Moreover, flavor tends to be more specific in poultry thighs than breasts, what is connected with higher fatness of leg muscle. This property results from the fact that fat acts as one of precursors of taste by combining with amino acids from proteins and other components when heated (Thu, 2006). Stronger flavor of poultry drum and thighs in comparison to the breast muscle is connected with the iron content of the muscle as well (McKee, 2003). It has been also noted that leg meat contains almost twice as much total tocopherols as breast meat. This favorable distribution of tocopherols may be significant in retarding oxidative changes of cooked leg tissues during post-cooking storage (Ang et al., 1990).

The main objective of our study was to examine the effects of muscle type on chemical composition and nutritional properties of turkey meat. A secondary objective was to evaluate some blood serum biochemical parameters of turkeys.

6.2. Material and methods

6.2.1. Animals

The study was carried out at the Morky Petránek, s.r.o., in Čremošné (Slovak Republic). Ten broad-breasted female white turkeys hybrid XL were used in trial. Experiment lasted 18 weeks. The birds were reared under the same husbandry conditions. Experiment was conducted in environmentally controlled farm building with light, temperature, and humidity controls. Turkeys were housed on deep litter. Conditions of animals care, manipulation and use corresponded with the ethical instructions (Figure 6.2.).

Figure 6.2. Broad-breasted female white turkey hybrid XL, Slovak Republic.

Birds in the experiment were fed standard fattening complete feed mixture according to the age. Nutrient composition of feed mixture is presented in Table 6.1. Birds were provided free access to feed and water throughout the trial. All birds were fed the same feed. Turkeys were slaughtered after fasting at 18 weeks of age.

Table 6.1. Nutrient composition of complete feed mixture.

	DM	CP	F	CF	NFE	Starch	T.s.	ME_N
	$g*kg^{-1}$	$g*kg^{-1}$ of dry matter						$MJ*kg^{-1}$
KR1	906.3	297.7	56.9	37.4	541.8	343.5	54.1	11.79
KR2	903.9	271.9	51.8	40.5	557.7	362.8	46.7	11.40
KR3	895.9	252.3	70.1	40.7	564.8	357.7	46.1	11.55
KR4	915.0	211.4	82.5	42.1	601.9	417.5	42.0	12.47
KR5	905.4	193.8	70.5	41.7	630.9	471.6	39.2	12.50
KR6	908.3	183.4	93.6	42.3	631.2	464.3	37.0	12.98
Wheat	896.3	146.5	15.2	26.9	792.9	667.5	33.2	12.88

DM: dry matter, CP: crude protein, F: crude fat, CF: crude fibre, NFE: nitrogen free extract, T.s.: total sugars, ME_N: metabolisable energy, KR1-KR6: complete feed mixtures for turkeys fattening

6.2.2. Evaluation of serum biochemical parameters

During slaughter the blood of 10 turkeys was collected from jugular vein and after all serum was separated. Laboratory analysis of biochemical parameters of turkeys blood serum was carried out at the Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovakia.

The blood serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of following serum parameters: glucose (Glu), triglycerides (Tg), total cholesterol (Chol), total protein (TP), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were analysed. The aforementioned parameters were determined using automatic analyser RX Monza (Randox, United Kingdom; Figure 6.3) and microprocessor-controlled analyser EasyLite (Medica, Bedford, USA) according to the manufacturer's instructions.

Figure 6.3. Automatic analyser Randox RX Monza.

6.2.3. Evaluation of meat quality traits

After slaughter of turkeys, breast and leg muscles of eight individuals chosen randomly, were collected for future analysis. Each meat sample was separately comminuted into fine fibre fragments in a meat homogeniser after which they were packaged in bags, labelled and stored in a frostless freezer. Laboratory analysis of nutritional composition of turkeys meat was carried out in the laboratory of quality and nutritional value of feeds at the Department of Animal Nutrition in Slovak University of Agriculture in Nitra, Slovakia.

6.2.3.1. Chemical composition analysis of turkeys muscles

Chemical composition of turkeys meat was determined by standard laboratory methods and procedures described by Association of Official Analytical Chemists (AOAC, 2000). The dry matter content was determined by drying of the sample by gravimetric method, crude protein by Kjeldahl method (mineralization, distillation, titration), and ash by complete combustion of the sample in a muffle furnace at 550°C (4-6 hours). Crude fat was analyzed after acid hydrolysis and extraction in Soxtec

System. The metabolizable energy was calculated, according to the formula suggested by Zelenka and Zeman (2006):

ME (MJ/kg) = 34.31 x crude fat (g/g) + 15.51 x crude protein (g/g) + 16.69 x starch (g/g) + 13.01 x sugar (g/g)

6.2.3.2.Collagen analysis

100 mg of lyophilized muscle tissue was hydrolyzed in Duran tubes in 5 ml 6N HCl at 110°C for 18 to 20 h (Etherington and Sims, 1981) for the determination of hydroxyproline and crosslinking. The hydrolyzate was filtered (Whatman filter, Grade 1) and diluted with destilated water. An aliquot of the hydrolyzate was removed for hydroxyproline determination and the remaining part was subjected to crosslink analysis. The intramuscular collagen concentration (4-hydroxyproline) was quantified using the colorimetric procedure of Woessner et al. (1961). The hydroxyproline was oxidated with sodium p-toluenesulfonchloramide (chloramines T) that in the next step was destroyed by perchloric acid. A solution of p-dimethylaminobenzaldehyde (Ehrlich solution) was added and the tube was placed in water bath at 60°C for 20 minutes. The solution absorbance was then determined by spectrophotometer Jasco V-730 (Germany) at 557nm. Intramuscular collagen concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as μg hydroxyproline per milligram of lyophilized tissue.

6.2.3.3. Analysis of muscles mineral composition

The contents of mineral nutrients were determined by High Resolution Continuum Source Atomic Absorption Spectrometer ANALYTIK JENA contrAA 700 (Ca, Mg, Na, K, Zn, Cu, Fe, Mn) and 6400 Spectrophotometer (P). The determination of individual elements' content was based on the absorptions measured at the following wavelengths: Ca content was detected at 422.7 nm, P at 666 nm, Mg at 285.2 nm, Na at 589.0 nm, K at 766.5 nm, Cu at 324.7 nm, Zn at 213.9 nm, Fe at 248.3 nm.

6.2.3.4. Fatty acids analysis

For the characterization of the lipid fraction, the triglycerides were hydrolyzed (saponified) into glycerol and free fatty acids. Fatty acids were derivatized to the methylesters (FAMEs). After the FAMEs preparation, they were separated according to the carbon number (number of carbon atoms in the fatty acid chain, excluding the

methyl ester carbon) and the degree of unsaturation by gas chromatography (GC) with flame ionisation detector (FID). For column check-out, a 37-component mixture (Supelco 47885-U) was used. The standard was diluted with 10 ml hexane (final concentration was 0.2–0.4 mg ml-1 per FAME) before the use. The total of 200 mg of sample in a 20 ml test tube was used. Dissolution of the sample in 5 ml hexane and addition of 1 ml 2 N potassium hydroxide in methanol was used. The tube was closed and shaken for 30 sec. The tube was heated for 30 sec at 60 °C in a water bath. After 1 minute, 2 ml of 1 N HCl was added and the tube was shaken. The upper (organic) layer was transferred into a 2 ml autosampler vial after passing it through a bed of anhydrous Na2SO4. The analyses were performed on an Agilent 6890A GC (Agilent technologies, U.S.A.) analyzer with a flame ionization detector (FID). Automated split injection was performed using an Agilent autosampler (Agilent technologies, U.S.A.). FAMEs were separated on DB-23 analytical column and identified by FID.

6.2.3.5. Amino acids analysis

The amino acid content was determined by using of automatic amino acid analyzer. Chromatographic estimation of hydrolysates of each sample was assessed by sodium-citrate buffer and post colon ninhydrin derivation. Samples were hydrolyzed by hydrogen chloride acid (6 $mol.dm^{-3}$) during 23 hours at 110 °C under an N_2 atmosphere. After filtering of the hydrolysed samples, the hydrolysates were neutralized with sodium hydroxide solution and completed to the volume of 100 cm^3 using sodium citrate buffer with a pH of 2.2. The hydrolysates were held at 4°C to silage for 24 hours in fridge. Methionine content was estimated as methionine sulphon, cysteine was determined as cysteic acid after oxidation by performic acid and hydrolysis. The samples were hydrolysed for 16 hours at 110 °C. After the vaporization at vacuum rotary device and supplementation with sodium citrate buffer (pH = 2.2) to 50 cm^3 volume and following the dilution the analysis of amino acids was performed. The following amino acids were measured: Asp- asparagines, Tyr- tyrosine, Ala- alanine, Ser- serine, Glu- glutamine, Pro- proline, Gly- glycine, Cys- cysteine, Val- valine, Ile- isoleucine, Leu- leucine, Thr- threonine, Phe- phenylalanine, His- histidine, Arg- arginine, Lys- lysine, Met- methionine.

6.2.4. Statistical analyses

For biochemical parameters and enzymes activities in turkeys blood, mean, the sample maximum, the sample minimum and the standard deviation were calculated. Data on chemical and nutritional properties of breast and leg muscles were analyzed by one-way analysis of variance. All data were performed using SPSS package (SPSS, 2010).

6.3. RESULTS AND DISCUSSION

6.3.1. Blood serum biochemical parameters

The results for 11 blood serum biochemical parameters of turkeys are shown in Table 6.2. The blood biochemical profile was investigated because it may be a useful indicator about the physical condition of animals, making them a useful tool in differentiating healthy animals from diseased states (Jatoi et al., 2013). The glucose (Glu) concentration in turkeys was 13.06±0.45 mM/L (235.32±8.11 mg/dL) and ranged from 12.33-13.48 mM/L. While, the mean serum triglycerides (Tg) content in birds of presented research was 0.55±0.07 mM/L (48.68±6.19 mg/dL). Our results are partially in line with these obtained by Krauze et al. (2012), who examined the effect of water extract of raw garlic and garlic preparation administered to drinking water for turkey hens and their impact on selected biochemical indicators of blood serum. Compare to results of current study they recorded similar Glu levels (12.94-13.64 mmol/L) and higher Tg concentrations in turkeys blood serum (0.59-0,81 mmol/l). Both aforementioned parameters play undeniably important role in the organism. Glucose and triglycerides are the major metabolites that are closely related to the sustainability of energy supply for the implementation of the physiological and biochemical functions in the body (Hernawan et al., 2012). In the present research, also the serum cholesterol (Chol) concentration was assessed. Available literature shows that the cholesterol metabolism in avian species is similar to that of mammals, but plasma cholesterol level can significantly increase during vitellogenesis and egg formation in birds (Keçeci and Çöl, 2011). Moreover, the level of plasma cholesterol depends on a gamut of factors especially the dietary intake of saturated fatty acids with polyunsaturated and monounsaturated fatty acids (Nwaoguikpe, 2010). The serum Chol level in examined turkeys was 3.29±0.38 mM/L (127.03±14.67 mg/dL) and ranged from 2.60-3.88 mM/L. Our result contrasted with these of Huff et al. (2008), who found higher values of this

trait (158.4-170.4 mg/dL). The research conducted on turkey poults by Etuk et al. (2012) also showed higher levels of serum cholesterol (150.00-169.00 mg/dL) then this recorded in the present experiment. The total protein (TP) concentration in turkeys was 39.99±4.1 g/L and ranged from 34.77-47.45 g/L. Compared to our results, in the research conducted by Silva et al. (2007) lower levels of total protein were recorded (2.96-3.23 g/dL). Also Filipović et al. (2007) noted slightly lower values of this trait (27.00-35.60 g/L). Blood plasma proteins have an exceptional significance in homeostasis maintenance. Total concentration of blood serum proteins of birds is about the same as half its value in mammals. In mammals it is 50-70 g/L, while in birds it is approximately 40 g/L.

Table 6.2. Some biochemical parameters in the blood plasma of turkey females.

Parameter	Mean	Minimum	Maximum	Standard Deviation
Glucose (Glu, mM/L)	13.06	12.33	13.48	0.45
Triglycerides (Tg, mM/L)	0.55	0.47	0.69	0.07
Cholesterol (Chol, mM/L)	3.29	2.60	3.88	0.38
Total protein (TP, g/L)	39.99	34.77	47.45	4.1
Calcium (Ca, mM/L)	2.86	2.66	3.00	0.11
Phosphorus (P, mM/L)	2.00	1.78	2.14	0.12
Magnesium (Mg, mM/L)	0.76	0.62	0.97	0.09
Sodium (Na, mM/L)	150.93	149.30	155.10	1.71
Potassium (K, mM/L)	5.24	4.64	5.67	0.30

Among evaluated in our study biochemical indices were also some serum mineral parameters, including calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). These minerals are necessary for birds growth and they are involved in many digestive, physiological and biosynthetic processes within the body (Piotrowska et al., 2011). The mineral content in the blood serum of birds is considerably dependent on its mineral concentration in feeds as well as factors influencing the degree of their absorption in the digestive tract (Bogusławska-Tryk et al., 2012). As in case of previously described biochemical serum indices, blood minerals levels may be different as a function of the methodology applied, but also breed and genetic line may also influence these parameters (Silva et al., 2007). In our

study, serum Ca concentration in turkeys was 2.86±0.11 mM/L (11.44±0.44 mg/dL) and ranged from 2.66-3.00 mM/L. While, the mean serum P level was 2.00±0.12 mM/L (6.19±0.37 mg/dL) and ranged from 1.78-2.14 mM/L. Similar Ca content in blood serum of turkeys is shown by Szabó et al. (2005) and Majewska et al. (2009). Regarding serum Ca and P concentrations, our results are higher than these reported by Huff et al. (2008). They observed lower levels of both mentioned minerals compared to these from the present study. Ca and P are an important parameters that indicate animal health status. Calcium is mainly essential for the ossification of bones, regulation of muscle activity and catalization of enzyme and hormone systems, while phosphorus is an important constituent of nucleic acids and phospholipids (Piotrowska et al., 2011). Furthermore, it was observed that in mammals Ca changes very little throughout the life cycle but in fowls the calcium increases over 100% at the time of egg production. Regarding phosphorus, it has been shown that P content in the blood of the fowl is peculiar in that it is much greater than that of mammals and the inorganic P content of the serum, as usually determined, composes only a small part of the whole (Heller et al., 1934). Have been stated that exist the numerous interactions or interrelationships between the major elements such as Ca, P and Mg (Al-Ankari, 2006). The latter from mentioned minerals is vitally involved in the metabolism, mostly as a catalyst of a wide array of enzymes (Piotrowska et al., 2011). In the present research, serum Mg concentration in turkeys was 0.76±0.09 mM/L (1.52±0.18 mEq/L) and ranged from 0.62-0.97 mM/L. Our result is in line with result presented by Huff et al. (2008). They observed Mg content at level 1.6 mEq/L.

For a special attention deserve serum Na and K contents as well. Sodium is primarily responsible for determining the volume of the extracellular fluid and its osmotic pressure. The normal ranges of serum sodium in mature birds is 130-150 mmol/l (Simaraks et al., 2004). While, potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na^+/K^+ -ATPase (Soetan et al., 2010). The level of Na in turkey blood serum was 150.93±1.71 mM/L, and serum K content was 5.24±0.30 mM/L. Our results are in contrast to these presented by Szabó et al. (2005), who reported higher serum Na level (165 mM/L) and lower K concentration (2.57 mM/L) in blood serum of 16 weeks old turkeys. While, Ognik and Merska (2012) noted lower serum Na (119.5 mmol/L) and K (3.83 mmol/L) content if compared with these reported in our study.

6.3.2. Enzymes in the blood plasma

Data regarding the activity of selected enzymes in the blood plasma of slaughter turkey females are presented in Table 6.3. Among the most sensitive and widely used liver enzymes are the aminotransferases (Celebi et al., 2009). In fowls, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are synthesized not only in liver, but also in muscles, skeletal and cardiac (Fernandez et al., 1994). In the present study, the activity of AST in turkeys was 7.32±2.59 μkat/L (440.96±156.02 U/L) and ranged from 0.77-9.28 μkat/L. While, the mean ALT activity was 0.25±0.03 μkat/L (14.71±1.76 U/L) and ranged from 0.21-0.31 μkat/L. Regarding these enzymes, our results are in contrast to these described by Huff et al. (2008). They reported higher level of AST enzyme (515.0 U/L) and lower of ALT enzyme (6.23 U/L) if compared with these reported in our study. In study presented by Mikulski et al. (2008) the levels of the above blood serum biochemical indicators were higher (489.0 and 39.0 U/L, respectively) than that in the present experiment. Currently, AST activity is considered as a very sensitive but nonspecific indicator of hepatocellular disease in avian species, and is frequently used with the muscle-specific enzyme creatine kinase to differentiate between liver and muscle damage (Keçeci and Çöl, 2011). The next mentioned enzyme-ALT is a cytoplasmic enzyme that catalyzes the transamination of α-ketoglutarate and l-alanine, forming glutamate and pyruvate (Scholtz et al., 2009). The results of study conducted by Diaz et al. (1999) showed that ALT is one of the most valuable tool in the diagnosis of fatty liver-hemorrhagic syndrome in a flock of layers. Among evaluated in our study enzymes is also alkaline phosphatase (ALP), that activity in turkey serum was 33.12±4.33 μkat/L (1948.24±254.71 U/L). Obtained in the present research results are not in accordance with the findings described by Krauze et al. (2012). They noted lower serum activity of ALP enzyme (1169.2-1292.4 U/L), which is one of the most frequently used biochemical markers of osteoblast activity. The higher ALP activity is essential for initiating mineralization, because ALP can decompose phosphoric acid of organic matter, increase inorganic phosphoric acid concentration and thereby enhance mineralization (Guo et al., 2011b).

Table 6.3. Activity of selected enzymes in the blood plasma of turkey females.

Parameter	Mean	Minimum	Maximum	Standard Deviation
Aspartate Aminotransferase (AST, μkat/L)	7.32	0.77	9.28	2.59
Alanine Aminotransferase (ALT, μkat/L)	0.25	0.21	0.31	0.03
Alkaline Phosphatase (ALP, μkat/L)	33.12	26.32	41.65	4.33

6.3.3. Chemical composition of turkey muscles

The data on comparison of breast and leg muscles in terms of their chemical composition are presented in Table 6.4. No difference ($P > 0.05$) was found between examined muscles for the dry matter. This finding corroborate the results described by Ognik and Merska (2012). They reported that the dry matter content in pectoralis muscle of female turkeys was slightly higher than that in leg muscles (26.4 and 24.9%, respectively). This same tendency was observed in other poultry species, i.e. broiler chicken (Sogunle et al., 2010), pheasant (Večerek et al., 2005) and duck (Marzoni et al., 2014). The muscle type affected significantly ($P < 0.01$) crude protein and crude fat level in dry matter of assessed muscles. Crude protein content was higher in turkeys breast muscles than in leg muscle (+8.95%), while in case of latter mentioned trait the opposite tendency was reported. Turkey leg muscles were characterized by higher concentration of crude fat (+8.63%) in comparison to breast muscles. Described trends regarding crude protein and fat content in turkeys muscles, have been observed also in studies presented by Apetroaei et al. (2012b), and Herkel et al. (2016). The fat and protein content of muscles is a complex trait simultaneously affected by a large number of genetic and non-genetic factors (Bogosavljević-Bošković et al., 2010).

Table 6.4. The effect of muscle type on chemical composition of turkey meat.

Component	Breast muscle	Leg muscle	SEM	*P*-value
Dry matter (DM, %)	26.22	25.60	0.18	0.087
Crude protein (% of DM)	86.18	77.23	1.49	<0.001
Crude fat (% of DM)	5.21	13.84	1.48	<0.001
Ash (% of DM)	4.43	4.29	0.04	0.102
Energy (kJ)	436.48	472.56	9.31	0.046
Intramuscular collagen (IMC, μg/mg[a])	17.53	29.13	1.82	<0.001

[a] of lyophilized muscular tissue.

No statistically significant difference was found to exist between breast muscles and leg muscles for ash content (4.43 and 4.29% of dry matter, respectively). Our results are in line with these obtained by Batkowska et al. (2011), who noted slight difference in crude ash content between breast and thigh muscles of turkey females. On the other hand, energy level was markedly influenced by the muscle type; value of this trait was higher (+ 8.27%; $P < 0.05$) for leg muscles than that of breast muscles. The same trend was observed in studies conducted on broiler chickens by Haščík et al. (2013, 2015). The amount of energy provided is dependent on the protein (1 gram of protein = 17 kJ/4 kcal of energy) and on the fat (1 gram of fat = 37 kJ/9 kcal) content (Gerber, 2007). Thus, it can be said that turkey leg muscles were characterized by higher calorific value in comparison to breast muscles due to fat higher energy density.

An important role in shaping meat quality also is played by connective tissue, which has been shown to be a critical factor in meat tenderness. Studies conducted on livestock species, indicated that the morphology, composition and amount of intramuscular connective tissue depends on numerous factors, including muscle type (reviewed by Wojtysiak, 2013). This observation is confirmed in the current study, where intramuscular collagen (IMC) concentration was significantly ($P < 0.01$) affected by the muscle type. Leg muscles were distinguished by markedly higher content of IMC in comparison to breast muscles (+39.82%). Our results are in line with these presented by Jaturasitha et al. (2008) and Voutila et al. (2009), who reported lower amount of collagen in breast muscles than that of leg muscles. Above mentioned differences in IMC are considered to be closely related to the muscle type and physiological function

of muscle (Palokangas et al 1992; Kuypers and Kurth, 1995). Our results are consistent with the conclusions of Kuypers and Kurth (1995) and Harper (1999) that variation in IMC properties with muscle type and function leads to the well-known differences in background toughness among meat cuts.

6.3.4. Mineral composition of turkey muscles

The mean mineral composition of breast and leg muscles from turkeys is presented in Table 6.5. Mineral components play an important role in the metabolism of skeletal muscles. Some elements, including sodium, calcium, potassium, phosphorus and magnesium, are essential for enzymatic processes and are responsible for normal muscle function and for the course and extent of post-mortem changes in muscles (Połtowicz and Doktor, 2013). The data of this research clearly show that even if the breast muscle was slightly richer in Ca (+11.69%) than leg muscle, the differences were not significantly ($P = 0.489$). Our results corroborate with these of Połtowicz and Doktor (2013), who determined the effect of slaughter age of broiler chickens on macro minerals levels of breast and leg muscles and their association with meat quality. While, Zapata et al. (1998) noted significantly higher Ca amount in dark meat than that in light meat. Moreover, in our study similar ($P > 0.05$) values were found between examined muscles in case of sodium and potassium. Furthermore, similar to other studies conducted on poultry, K was quantitatively the most important mineral in turkey meat, followed by P and Na (Demirbas et al., 1999; Podgórski et al., 2001). Evaluation of content of above mentioned minerals plays pivotal role, according to available literature, chloride (Cl) together with Na and K are the most essential for acid-base balance. These are sometimes referred to as “strong ions” (Borges et al., 2004). The significant influence of the muscle on Na and K content has been observed in broiler chickens (Połtowicz and Doktor, 2013), beef (Barge et al., 2005) and fish (Mnari Bhouri et al., 2010) as well. In the present study, marked difference has been recorded for phosphorus content, that was higher ($P < 0.01$) in leg muscle. The opposite tendency was observed in case of magnesium concentration. Turkey breast muscle was characterized by higher content of Mg in comparison to leg muscle (+7.06%; $P < 0.01$). Regarding P and Mg levels in muscles, our results are partially in line with data described by Zapata et al. (1998). In their study phosphorus and magnesium concentrations were significantly higher in dark meat when compared to that in light meat of broilers.

Table 6.5. The effect of muscle type on mineral composition of turkey meat.

Minerals	Breast muscle	Leg muscle	SEM	*P*-value
Macroelements (mg/kg of DM)				
Calcium (Ca)	186.62	167.08	13.30	0.489
Phosphorus (P)	9,222.17	9,971.32	156.24	0.008
Magnesium (Mg)	957.04	893.95	12.75	0.005
Sodium (Na)	2,738.21	2,779.39	111.87	0.864
Potassium (K)	13,465.47	13,752.07	180.55	0.454
Microelements (mg/kg of DM)				
Copper (Cu)	3.32	5.03	0.28	<0.001
Iron (Fe)	19.14	46.62	4.22	<0.001
Zinc (Zn)	23.63	65.85	6.63	<0.001

Considering the effect of turkey muscle type on microelements concentrations in meat, in the presented study the leg muscle was distinguished from breast muscle by significantly higher ($P < 0.01$) content of copper (Cu), iron (Fe) and zinc (Zn) (+51.5%, +143.6% and +178.7%, respectively). These trace minerals present in tissues of animals serve a variety of functions in their bodies (Yang et al., 2011). Copper is an essential metal for the well-being of an organism (Szymczyk and Zalewski, 2003). In respect of human nutrition, a deficiency of this metal in many cases leads to an anemia and also to a deterioration of elastin creation process in blood vessels and collagen in bone system. Moreover, Cu is essential for an absorption of Fe (Trojanowski et al., 2009), that is a constituent of multiple enzymes and metalloproteins that participate in redox processes. In muscles Fe is a constituent of myoglobin - a red pigment of muscles that collects oxygen from red blood cells and utilizes it for the work of muscles (Wójcik et al., 2009). Zinc, like copper, is an important element in the animal organism. High concentrations of Zn occur in the liver, kidneys and bones (Szymczyk and Zalewski, 2003). Presented in this study results on microelements content in turkey females muscles partially corroborate with these of Makarski and Makarska (2010), who determined the concentration of Ca, Mg, Zn, Cu and Fe in the consumable tissues of turkey cocks. They observed that the levels of Cu and Zn in the breast muscle were lower when compared to that in leg muscle; the opposite trend for Fe level they recorded.

6.3.5. Fatty acids profile of turkey meat

Considering the nutritional value, except the mineral composition, on attention deserve also the fatty acids profile of meat. Fatty acids (FA) composition of breast and leg muscles of turkeys is shown in Table 6.6. From a biochemical point of view, the FA have attracted the greatest interest recently. It is connected with the fact that FA in the human and birds diets are essential nutrients needed for a series of metabolic interactions in addition to their caloric contribution to a balanced nutritional regime (Shin, 2010). Furthermore, the growing interest in FA is evidently related with the effect of fatty acid profile of food products, such as meats, oils and dairy products, on health state of consumers (Benatti et al., 2004). For instance, it has been reported that a high intake of SFA from the diet may be associated with elevated cardiovascular disease risk. Thus, nowadays, the association between diet and health is a decisive factor in consumer food selection (Martemucci and D'Alessandro, 2012). In our research, the fatty acid profile exhibits a dominance of two classes: monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA); the third position belongs to the polyunsaturated fatty acids (PUFA). Muscle type did not significantly affect the total SFA content and the proportion of single SFA, except for a lauric (C 12:0) and myristic (C 14:0) acids content. Levels of these SFA were markedly higher in leg muscle of examined birds than that in breast muscle (+0.21, $P < 0.01$ and +0.09%, $P < 0.05$, respectively). Our results partially corroborate with these of Cortinas et al. (2004). In their study, in comparison to breast muscles, chicken thighs were characterized by higher total SFA amount, lauric and myristic acids content. Simultaneously, in above mentioned research, as in this carried out on turkeys and described by Nobar et al. (2010), myristic acid content in breast muscle was evidently lower than this in our study. In the presented study, quantitatively, the palmitic acid (C 16:0) was the most concentrated saturated fatty acid (24.95-25.12%) followed by the stearic acid (C 18:0; 7.14-7.55%). Regarding domination of the palmitic acid among other SFA, our result is in line with findings of studies conducted on turkeys (Salamatdoustnobar, 2010), broiler chickens (Leonel et al., 2007), ducks (Wołoszyn et al., 2006), beef (de Almeida et al., 2006), lamb (Maiorano et al., 2015a) and goats (Szymanowska et al., 2009). Taking under consideration all above mentioned SFA, it's worth to highlight that lauric acid and myristic acid, have a greater total cholesterol raising effect than palmitic acid, whereas stearic acid has a neutral effect on the concentration of total serum cholesterol, including no apparent impact on either LDL or HDL (Daley et al., 2010).

Table 6.6. The effect of muscle type on fatty acid composition in turkeys meat (% of total fatty acids).

Fatty acids	Breast muscle	Leg muscle	SEM	P-value
C 12:0	0.66	0.87	0.04	0.003
C 14:0	1.32	1.41	0.02	0.038
C 14:1	0.30	0.26	0.01	0.089
C 15:0	0.16	0.14	0.01	0.179
C 16:0	25.12	24.95	0.12	0.510
C 16:1	6.82	5.91	0.21	0.021
C 17:0	0.24	0.24	0.01	0.937
C 18:0	7.14	7.55	0.16	0.221
C 18:1 n-9	34.29	33.14	0.32	0.063
C 18:2 n-6	18.13	19.90	0.38	0.011
C 18:3 n-3	1.15	1.21	0.02	0.174
C 20:1 n-9	0.28	0.28	0.003	0.874
C 20:2 n-6	0.22	0.22	0.01	0.973
C 20:3 n-6	0.05	0.04	0.02	0.749
C 20:4 n-6	0.62	0.78	0.05	0.102
C 24:1 n-9	0.04	0.02	0.02	0.532
Partial sum				
ΣSFA	34.64	35.16	0.19	0.166
ΣMUFA	41.73	39.61	0.46	0.012
ΣPUFA	20.20	22.14	0.43	0.016
Σn-3	1.15	1.21	0.19	0.170
Σn-6	19.03	20.94	0.42	0.013
Nutritional ratios				
n-6/n-3	16.51	17.36	0.19	0.013
PUFA/SFA	0.58	0.63	0.13	0.059

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Muscle type affected the total MUFA amount; value of this trait was higher for breast muscle compare to leg muscle (+2.12%; $P < 0.05$). Regarding single MUFA concentration, breast muscle was characterized by higher ($P < 0.05$) palmitoleic acid content (C 16:1) than the other investigated muscle. The most abundant MUFA was the

oleic acid (C 18:1 n-9), that was slightly higher (P = 0.063) in breast muscle compared with leg muscle. Oleic acid is present in considerable quantities in both animal and plant sources (FAO, 2008). Other MUFA detected in analyzed tissues were not affected by muscle type. In the experiment described by Azman et al. (2005) this same tendency on palmitoleic acid concentration was reported. On the other hand, in this same study have been observed higher total MUFA amount in thigh muscle than in breast muscle. Moreover, our results are not in accord with these of Shin et al. (2011), who observed that broiler chickens breast muscle had lower level of palmitoleic acid than thigh (4.96 and 6.18%, respectively).

The second class of unsaturated fatty acids consist of PUFA. There is increasing recognition of the health benefits of PUFA in general, and of n-3 fatty acids in particular, because these fatty acids are essential for humans (Starčević et al., 2014). Available literature shows that PUFA play an active role in the prevention and management of several pathologies, such as coronary heart disease, hypertension, type 2 diabetes, renal disease, ulcerative colitis, chronic obstructive pulmonary disease and Crohn's disease. Moreover, besides the health benefit of PUFA, it has been reported in chickens that inclusion of PUFA in the diet reduces abdominal fat and total body fat compared with SFA-rich diets (Schiavone et al., 2010). In the current research, total PUFA amount was higher ($P < 0.05$) in leg muscle (+1.94%) than in breast muscle. Muscle type did not markedly affect the proportion of single PUFA, except for a linoleic acid (C 18:2 n-6, LA) content that was higher ($P < 0.05$) in leg muscle when compared to that in breast muscle. Similar result was obtained by Cortinas et al. (2004). In their research have been reported that thighs of chickens have higher linoleic acid content than breast muscles. The opposite tendency was recorded in the study described by Haraf et al. (2014), who determined fatty acids profile of muscles and abdominal fat in geese of Polish native varieties. Additionally, the values of linoleic acid levels in muscles found in the present study are higher if compared with these reported in both above mentioned experiments. Lower LA content was observed in beef (Chail et al., 2005) and pigs (Hăbeanu et al., 2010) muscles as well. Linoleic acid deserves for the special attention for the second reason; quantitatively it is the most concentrated PUFA among these detected in our study (18.13-19.90%). The next most abundant PUFA is α-linolenic acid (C 18:3 n-3, ALA). Both LA and ALA can be further metabolized to long chain PUFA through a series of desaturation and elongation steps. LA is metabolized to arachidonic acid, while ALA can be metabolized to eicosapentaenoic acid (EPA) and

ultimately docosahexaenoic acid (DHA) (Anderson and Ma, 2009). EPA and DHA have a number of biologic effects, among them beneficial effects like reduction in cardiovascular mortality and morbidity (Heuvel, 2012).

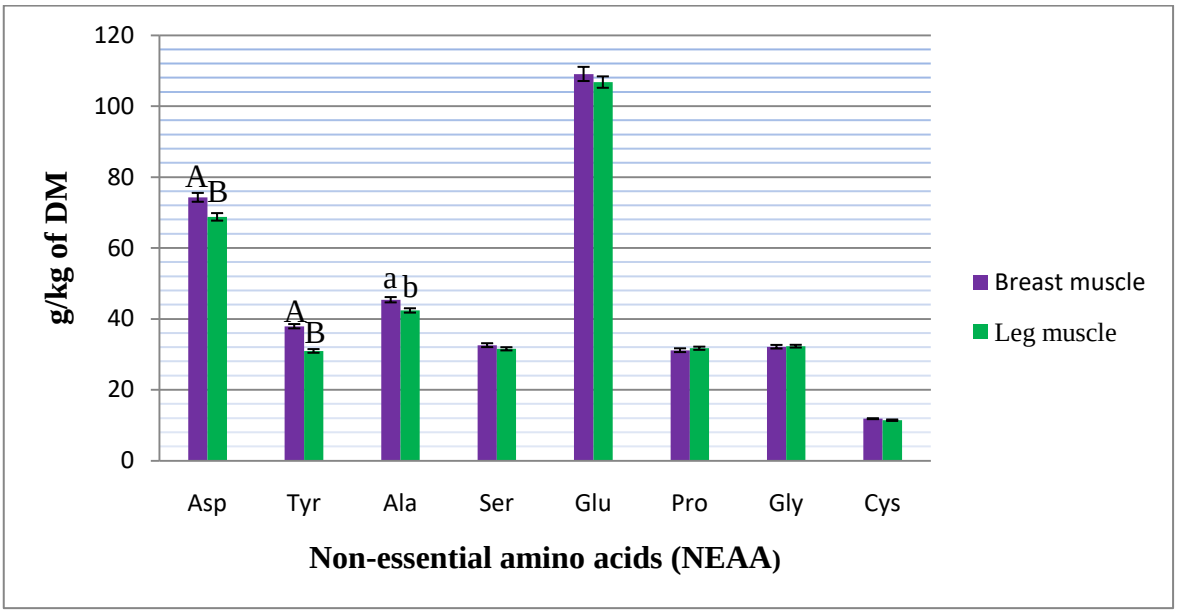
Essential fatty acids can be separated into two kinds of PUFA that including n-3 and n-6 PUFA, these two classes of essential fatty acids are not inter-convertible and often have important opposing physiological functions (Calder, 2003). In the current research, levels of n-3 and n-6 were higher in leg muscles in comparison to breast muscles. The significant difference was reported only in case of Σn-6 concentration ($P < 0.05$). n-6 fatty acids play essential roles in many biological functions, e.g. n-6 fatty acids have long been known to reduce serum total and low-density lipoprotein cholesterol (Willett, 2007). Moreover, because n-6 fatty acids are the precursors of proinflammatory eicosanoids, higher intakes have been suggested to be detrimental (Willett, 2007). Our results are not in line with these presented by Celebi et al. (2011). In their research breast muscle in laying hens had higher level of n-6 FA than thigh muscle (21.42 *versus* 21.00%). While, in the experiment described by López-Ferrer et al. (1999), content of n-6 in breast muscle of chickens was clearly similar to n-6 level in thigh muscle. The n-6/n-3 ratio, commonly used criterion to describe the dietetic value of fat, was also affected by the muscle type. It was lower in white meat than that in dark meat of thighs (16.51 *versus* 17.36, respectively; $P < 0.05$). The PUFA/SFA ratio have also a great nutritional implications and it is taken as a measure of the propensity of the diet to influence the incidence of coronary disease (Wood et al., 2003). In the current study, the PUFA/SFA ratio was slightly higher ($P = 0.059$) in leg muscle in comparison to breast muscle (0.63 *versus* 0.58, respectively). It was recommended that an ideal ratio of n-6/n-3 should be in range between 4.0 (maximum) and 0.45 (minimum). The higher values are harmful to health and may promote cardiovascular diseases. In this study aforementioned parameter in both turkeys muscles exceed the maximum value of 4.0 (HMSO, 1994). Generally, the chicken meat and poultry in general is characterized by high ratios n-6/n-3 (15 and 18, Rule et al., 2002), much higher values to the ideal value of 1 (Wood et al., 2003). The obtained values of n-6/n-3 ratio are higher than that reported in turkeys (Bedeković et al., 2014) and other poultry species, such as broiler chicken (López-Ferrer et al., 1999; Starčević et al., 2014), japanese quail (Aksu Elmali et al., 2014), and duck (Witak et al., 2008). The values of this trait found in the present study are lower if compared with this reported in turkeys by Lisitsyn et al. (2013). In poultry this relationship can be easily reduced by including dietary sources of n-3 fatty

acids, such as linolenic acid (C18: 3 n-3) (López-Ferrer et al., 2001; Kouba and Mourot, 2011), with obvious advantages from the nutritional point of view and the healthy.

6.3.6. Amino-acids profile of turkey meat

The results for non-essential and essential amino acids in breast and leg muscles of turkeys are shown in Figure 6.4 and 6.5. The proteins from animal sources are most desirable since they meet the human nutritional requirements. Poultry meat is rich in high quality proteins (Ramanea et al., 2011). Proteins consist of amino acid (AA) combined by peptide bonds and other linkages (Dozier et al., 2008). Dietary AA concentrations should match needs for both maintenance and skeletal muscle accretion to effectively allow for increased synthesis of white meat (Vieira and Angel, 2012). AA are also key precursors for syntheses of hormones, cell signalling molecules, being regulators of gene expression and the protein phosphorylation cascade (Takahashi et al., 2011). AA could be divided into two broad types. Some of amino acids can be formed within the body, assuming there are adequate substrate molecules, such as nitrogen. These amino acids are called dispensable, or non-essential amino acids – they are required by the body each day but because our body can make them, they are not essential to be supplied via our diet. Conversely, nine of the amino acids are classified as indispensable or essential. These amino acids cannot be formed within the body, so must be supplied via our diet, and are found within the proteins in foods we eat every day (Fanning, 2016).

Figure 6.4. Effect of muscle type on non-essential amino acids composition of turkey meat.



DM- dry matter; Asp- asparagines; Tyr- tyrosine; Ala- alanine; Ser- serine; Glu- glutamine; Pro- proline; Gly- glycine; Cys- cysteine; [a,b] values within different muscles differ significantly ($P < 0.05$); [A,B] values within different muscles differ significantly ($P < 0.01$).

Taking into account non-essential AA (Figure 6.4), in the present study, marked differences between examined muscles have been recorded for asparagine (Asp) and tyrosine (Tyr) contents. Levels of these AA were higher ($P < 0.01$) in breast muscle than that in leg muscle (+7.42, and +18.38%, respectively). Moreover, turkey breast muscle was characterized by higher content of alanine (Ala) in comparison to leg muscle (45.39 *versus* 42.37 g/kg of dry matter; $P < 0.05$). Our findings are in agreement with the conclusions drawn by Straková et al. (2002) who found that the levels of individual amino acids in breast muscles were significantly higher than these in thigh muscles. Moreover, our results are partially in line with these presented by Straková et al. (2006). They analyzed the differences in amino acids content between breast and thigh muscles within the species (broiler chickens and pheasants). In their study, the average levels of Asp and Tyr in breast muscles of broiler chickens and pheasants were significantly higher than these in thigh muscles. Additionally, in this research breast muscle of chicken broiler was characterized by higher level of Ala in comparison to leg muscle. However in case of pheasants were observed opposite tendency regarding Ala content. The data of presented research clearly show that the turkey muscle type did not change significantly ($P > 0.05$) other non-essential AA, such as serine (Ser), glutamine (Glu), proline (Pro), glycine (Gly), and cysteine (cys). In our study, the most abundant non-

essential AA in turkey breast and leg muscles was the glutamine (C 106.77-109.07 g/kg of dry matter). Glu is an amino acid of interest to human medical use. Hence, the potential use of glutamine in broilers diet has been discussed, and many benefits have been noted in different studies (Ebadiasl, 2011). Glu may have positive effects on central obesity and metabolic syndrome, can modulate mind-body interactions, has beneficial effects on the hypothalamus, hippocampus and amygdala. Moreover, Glu is also involved in the activation of ATP-sensitive K+ (KATP) channels by H_2O_2 and glutamate-dependent inhibition of striatal dopamine release (Takahashi et al., 2011).

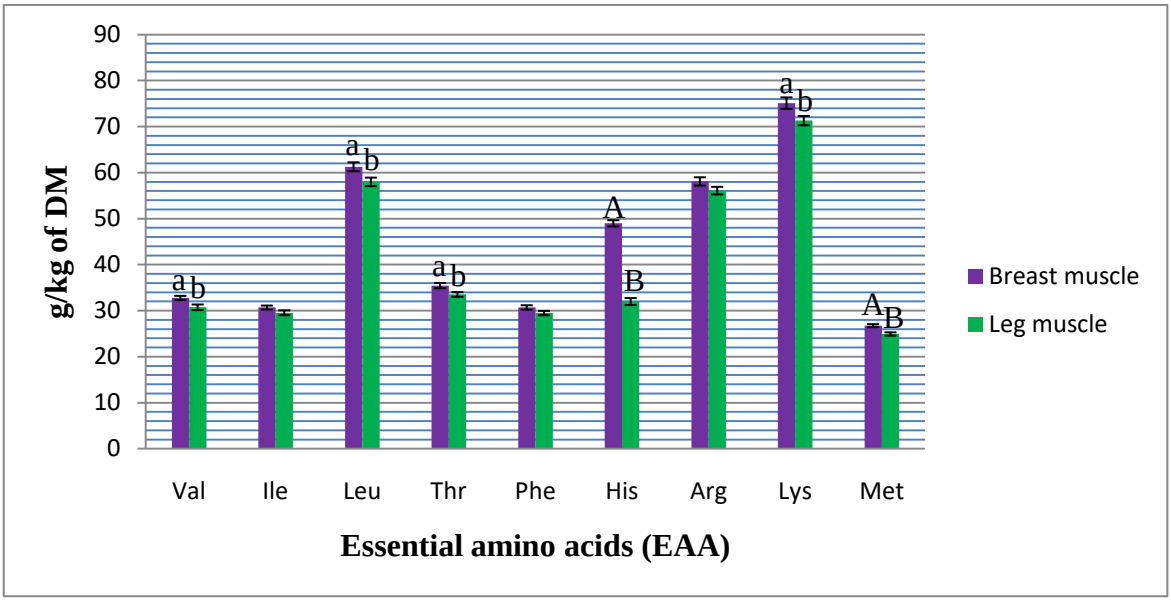
The essential AA are reported in Figure 6.5. These amino acids are key parameters in food quality assessment (Tessari et al., 2016). The usefulness of a protein feedstuff for poultry depends upon its ability to supply a sufficient amount of the essential AA that the bird requires. Dietary essentiality of some AA depends on species and developmental stage (Beski et al., 2015).

In the current research, breast muscle compared with leg muscle showed higher content of valine (Val), leucine (Leu), threonine (Thr), lysine (Lys) ($P < 0.05$) and histidine (His), as well as markedly higher values ($P < 0.01$) of histidine (His, +34.70%) and methionine (Met, +6.73%). Based on presented results, it could be concluded that breast muscles of examined turkeys were characterized by better nutritional quality. It is connected with functions of mentioned six essential AA. For example, valine plays a role in the intramembranous proteolysis (Tanii et al., 2006) that influences processes as diverse as cellular differentiation, lipid metabolism, and the response to unfolded proteins (Ye et al., 2013). Considering Leu, it has been observed that this essential AA at a very high dose can stimulate muscle protein synthesis, inhibit protein degradation in skeletal muscle, as well as in liver (Garlik, 2005). While, Thr is involved in digestion and immunity functions. A significant part of the threonine intake is used by the gut itself and for the synthesis of endogenous secretions (Primot et al., 2008). The histidine amino acid is a precursor for histamine- a multifunctional amine that regulates a multitude of cellular responses, and plays diverse roles in physiological and pathological processes (Tünde et al., 2011). Met is involved in the synthesis of body proteins and is a constituent of many body parts, including muscles, organs, and feathers. It is also involved in functions unrelated to protein synthesis, such as the synthesis of polyamines (Fanatico et al., 2010). Taking into account above mentioned Val, Leu, Thr, Lys, His and Met level, our results are partially in line with these described by Badr (2005). This research author observed higher level of Val, Leu, Thr,

Lys and Met in breast muscle in comparison to leg muscle; however for His content the opposite trend was noted.

In addition, breast muscle was slightly richer in isoleucine (Ile), phenylalanine (Phe), and arginine (Arg) than leg muscles, however the differences were not significant ($P > 0.05$). Quantitatively, the lysine was the most concentrated essential AA (71.30-75.11%). Generally, it has been observed that Lys content in poultry breast muscle is relatively higher than other AA. Lysine represents approximately 7% of the protein in breast meat (*pectoralis major* and *minor* muscles). Furthermore, dietary Lys inadequacy has been shown to reduce breast meat yield compared with other muscles. Therefore, defining dietary AA needs for optimum growth and meat yield is of utmost importance (Dozier et al., 2008).

Figure 6.5. Effect of muscle type on essential amino acids composition of turkey meat.



DM- dry matter; Val- valine; Ile- isoleucine; Leu- leucine; Thr- threonine; Phe- phenylalanine; His- histidine; Arg- arginine; Lys- lysine; Met- methionine; ^{a,b} values within different muscles differ significantly ($P < 0.05$); ^{A,B} values within different muscles differ significantly ($P < 0.01$).

6.4. CONCLUSIONS

The principal goal of presented experiment was to verify the hypotheses about the effects of muscle type on chemical composition and nutritional properties of turkey meat. A secondary objective was to assess some blood serum biochemical parameters of turkeys.

The glucose concentration in turkeys was 13.06±0.45 mM/L and ranged from 12.33-13.48 mM/L. While, the mean serum triglycerides content in birds of presented

research was 0.55±0.07 mM/L. The serum cholesterol level in examined turkeys was 3.29±0.38 mM/L and ranged from 2.60-3.88 mM/L. While, the total protein concentration in turkeys was 39.99±4.1 g/L and ranged from 34.77-47.45 g/L. Among evaluated in our study biochemical indices were also some serum mineral parameters, including calcium, phosphorus, magnesium, sodium and potassium. In our study, serum calcium concentration in turkeys was 2.86±0.11 mM/L and ranged from 2.66-3.00 mM/L. While, the mean serum phosphorus level was 2.00±0.12 mM/L and ranged from 1.78-2.14 mM/L. In the present research, serum magnesium concentration in turkeys was 0.76±0.09 mM/L and ranged from 0.62-0.97 mM/L. The level of sodium in turkey blood serum was 150.93±1.71 mM/L, and serum potassium content was 5.24±0.30 mM/L. Regarding, the activity of selected enzymes, the activity of aspartate aminotransferase in turkeys was 7.32±2.59 μkat/L and ranged from 0.77-9.28 μkat/L. While, the mean alanine aminotransferase activity was 0.25±0.03 μkat/L and ranged from 0.21-0.31 μkat/L. Among evaluated in our study enzymes is also alkaline phosphatase, that activity in turkey serum was 33.12±4.33 μkat/L.

The effect of muscle type on chemical composition of turkey meat was observed. Crude protein content was higher ($P < 0.01$) in turkeys breast muscles than in leg muscles (+8.95%), while the leg muscles were characterized by higher concentration of crude fat (+8.63%) in comparison to breast muscle. Moreover, energy level was higher (+ 8.27%; $P < 0.05$) for leg muscles than that of breast muscles. Also, intramuscular collagen concentration was higher (+39.82%) in comparison to breast muscle.

The mean mineral composition of breast and leg muscles from turkeys was also presented. The muscle type affected significantly ($P < 0.01$) two macroelements (phosphorus and magnesium) and three microelements (copper, iron, and zinc). Phosphorus content was higher ($P < 0.01$) in leg muscle in comparison to breast muscle. The opposite tendency was observed in case of magnesium concentration, that was higher in breast muscle than in leg muscle ($P < 0.01$). In presented study the leg muscle was distinguished from breast muscle by significantly higher ($P < 0.01$) content of copper, iron and zinc (+51.5%,143.6% and 178.7%, respectively).

Considering the nutritional value, except the mineral composition, also the fatty acids profile of meat plays important role. The fatty acid profile exhibits a dominance of two classes: MUFA and SFA; the third position belongs to the PUFA. Taking into account SFA, muscle type significantly affected the lauric (C12:0) and myristic (C14:0)

acids content, that was higher in leg muscle than that in breast muscle (+0.21, $P < 0.01$ and +0.09%, $P < 0.05$, respectively). Quantitatively, the palmitic acid (C16:0) was the most concentrated saturated fatty acid (24.95-25.12%). Muscle type affected the total MUFA amount; value of this trait was higher for breast muscle compare to leg muscle (+2.12%; $P < 0.05$). Regarding single MUFA concentration, breast muscle was characterized by higher ($P < 0.05$) palmitoleic acid content (C 16:1) than the other investigated muscle. The most abundant MUFA was the oleic acid (C 18:1 n-9). Total PUFA amount was higher ($P < 0.05$) in leg muscle (+1.94%) than in breast muscle. Muscle type did not markedly affect the proportion of single PUFA, except for a linoleic acid (C 18:2 n-6) content that was higher ($P < 0.05$) in leg muscle when compared to that in breast muscle. The most abundant PUFA was the linoleic acid (C 18:2 n-6). In the current research, levels of Σn-6 PUFA was higher in leg muscles in comparison to breast muscles ($P < 0.05$). The n-6/n-3 ratio was lower in white meat than that in dark meat of thighs (16.51 *versus* 17.36, respectively; $P < 0.05$).

Muscle type affected some of non-essential and essential amino acids. Taking into account non-essential amino acids, marked differences between examined muscles have been recorded for asparagine and tyrosine contents. Levels of those amino acids were higher ($P < 0.01$) in breast muscle than that in leg muscle (+7.42, and +18.38%, respectively). Moreover, turkey breast muscle was characterized by higher content of alanine in comparison to leg muscle (45.39 *versus* 42.37 g/kg of dry matter; $P < 0.05$). Breast muscle compared with leg muscle showed higher content of valine, leucine, threonine, lysine ($P < 0.05$) and histidine and methionine ($P < 0.01$).

Results of presented study completing existing knowledge regarding level of biochemical parameters of blood serum in poultry species. It is important, because limited information is available for domestic avian species, and even less has been established for turkey species. Moreover, the results of this research confirmed that muscle type markedly influences the chemical composition and nutritional value of turkey meat.

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