STUDY OF DIETARY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA*, ON MEAT QUALITY OF THE BROILER CHICKEN

Ganzaya Perenlei

Doctoral Program in Life and Food Science

Graduate School of Science and Technology

Niigata University

TABLE OF CONTENT

ABSTRACT

We evaluated effects of dietary supplementation with astaxanthin (Ax)-rich yeast, *Phaffia rhodozyma,* on the broiler chicken meat quality under normal ambient temperature and high ambient temperature condition.

I. Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on meat quality of the broiler chicken under normal ambient temperature

Experiment 1: Fourteen-day-old female Ross broilers were divided into three groups: control group: Ax-free diet, Ax 10 group: 10 mg/kg Ax diet, and Ax 20 group: 20 mg/kg Ax diet for 28 days. At 42 days old, chickens were slaughtered, and then growth performance, meat quality, and sensory attributes were analyzed. Compared with the control, a* values increased significantly just after slaughter and 48 h post-mortem storage for Ax 20 samples ($P < 0.05$) and for b* values in Ax 20 and Ax 10 groups ($P < 0.05$). Cooking loss decreased in the Ax 20 group ($P < 0.05$). After 120 h aging, contents of several free amino acids and total free amino acid content of Ax 20 group were significantly higher than the control ($P < 0.05$). In sensory evaluation, meat texture attributes improved significantly in the Ax 20 group ($P < 0.01$). No significant changes occurred in flavor attribute scores of meat soup from the Ax 20 group compared with the control even though most assessors preferred meat soup from the Ax 20 group. Overall, Ax-rich yeast in the diet improves broiler chicken meat quality.

Experiment 2: High concentration Thirty-two-day-old female Ross broilers were divided into three groups: control group: Ax-free diet, Ax 50 group: 50 mg/kg Ax diet, and Ax

100 group: 100 mg/kg Ax diet for 10 days. At 42 days old, chickens were slaughtered, and then growth performance, meat quality and sensory attributes were analyzed. Compared with the control, Ax 100 group was significantly higher daily body weight gain, and feed intake ($P < 0.05$). Ax groups had significant high a* values and b* values comparing to the control either from just after slaughter or 48 h postmortem storage ($P < 0.05$). No significant differences were observed in results of meat shear force value analysis between all groups ($P > 0.05$). However, in sensory evaluation, meat texture attributes improved significantly in the Ax 50 group ($P < 0.01$), but not in Ax 100 group. Overall, high concentration and short period feeding of Ax-rich yeast improves broiler performance and has a tendency to improve meat quality.

II. Effect of dietary Ax-rich yeast, *Phaffia rhodozyma,* on meat quality of the broiler chicken under heat stress condition

Chronic heat stress:

Thirty two-day-old female Ross broilers were divided into three groups: control group: Ax-free diet at normal temperature (24.5 ± 0.3°C), HS group: Ax-free diet at high ambient temperature (31.5 ± 1.3°C), and HS+Ax group: 20 mg/kg Ax diet at high ambient temperature (31.5 ± 1.3°C) for 10 days. Ax pre-feeding with 20 mg/kg Ax was for 18 days at normal ambient temperature. Three days ago before slaughter, blood samples were taken. At 42 days old, chickens were slaughtered, and then growth performance, meat quality, oxidative stress parameters, and sensory attributes were analyzed. However, compared to control group HS group had significantly lower daily body weight gain and feed intake,

and feed efficiency ($P < 0.05$), but HS+Ax group dramatically inhibited the heat stress effect on feed efficiency ($P > 0.05$). Under chronic heat stress, drip loss increased significantly in HS group ($P < 0.05$) but HS+Ax group was no difference were observed comparing to control ($P > 0.05$). The main indicating parameter of lipid peroxidation, malondialdehyde (MDA) content was tend to increase in HS group, but HS+Ax group successfully decreased the MDA content from HS group ($P < 0.05$). However heat stress did not influence on breast meat color from either just after slaughter or 48 h post-mortem ($P > 0.05$), Ax-rich yeast can effectively increase meat a* values and b* values in 48 h post-mortem ($P < 0.05$) and b* values in just after slaughter muscles ($P < 0.05$). Furthermore, some free amino acid contents in blood plasma and breast muscle from heat stressed groups were significantly lower than control group ($P < 0.05$), but after 48 h postmortem storage the increase of total free amino acid content from HS+Ax group had elevated more effectively than HS group but not same as control. In addition, there were not observed any significant differences in other meat quality parameter includes water binding capacity, cooking loss, shear force value, pH and in sensory evaluation revealed also no differences some sensory attributes such as tenderness, juiciness, fibrousness, and overall preference ($P > 0.05$) in three groups but first bite of meat from heat stressed groups were significantly higher than the control ($P < 0.05$). There are totally 120 compounds in control group, 122 compounds in HS group, and 123 compounds in HS+Ax group were detected in analysis of metabolites. The results revealed that comparing to control group, downregulated compounds were 58 and 52 in HS group and HS+Ax group respectively, and upregulated compounds were 11 and 28 in HS group and HS+Ax group

respectively. Comparing to HS group, 41 compounds were upregulted in HS+Ax group. The pyruvate and lactic acid which are the final products of glycolitic metabolism that is main altering metabolism during heat exposure were not altered in between all groups in chronic heat stress. However, fructose-1-6-phosphate and dihydroxyacetone phosphate was downregulated in HS group and upregulated in HS+Ax group. In TCA cycle, comparing to control group, citric acid in HS group was decreased and in HS+AX group was increased. Malic acid of HS+AX group was increased comparing to HS group. In addition, ATP was downregulated in HS group comparing to the control but in HS+Ax group it was upregulated comparing to HS group. Finally, one of the main antioxidant compounds in organism, glutathione is upregulated in HS+AX group comparing to others.

Acute heat stress:

Fourteen-day-old female Ross broilers were divided into two groups: control group: Ax-free diet and Ax group: 20 mg/kg Ax diet for 28 days at normal ambient temperature. After feeding trial, at forty-two-day old, removed feed and chickens were exposed in two ambient temperatures: 24°C (Control group) and 34°C (HS group and HS+Ax group) for two hours before slaughter. After heat exposure, blood samples were taken and chickens were slaughtered, and then body organ weights measured and meat quality, sensory attributes were analyzed. Under acute heat stress, drip loss increased significantly in HS group ($P < 0.05$) but HS+Ax group was no difference were observed comparing to control ($P > 0.05$). However acute heat stress did not influence on breast meat color from either just after slaughter or 48 h post-mortem ($P > 0.05$), Ax-rich yeast can effectively increase

meat b* values in just after slaughter and 48 h postmortem ($P < 0.05$) comparing to other groups. The ultimate pH value of HS group was significantly decreased comparing to control group ($P < 0.05$). In addition, some free amino acid contents in heat stressed groups were significantly lower than the control ($P < 0.05$). The main indicating parameter of protein oxidation, protein carbonyl content was tend to increase in HS group, but HS+Ax group significantly decreased the protein carbonyl content from HS group ($P < 0.05$) under acute heat exposure, but malondialdehyde (MDA) content was not differed significantly between all groups ($P > 0.05$)

Overall, Ax-rich yeast can improve meat quality even under heat stress, especially this influence more effective in chronic heat stress condition.

In conclusion, *Phaffia* yeast that contains high amount of astaxanthin is an effective dietary supplementation for the improvement of meat quality of broiler chicken in either normal environment or high temperature environment.

CHAPTER I. INTRODUCTION

Meat is edible flesh of animal. Meat consumption in the worldwide is various, depending on cultural or religious preference, nature and economic conditions. In recent years, there is rapid increase in world population that is expecting the world population will reach over nine billion in 2050 year. According to increase of population, the urgent problem that to supply sufficient food, especially animal protein food; is facing. For this reason, world meat production, exclusively poultry meat production is increasing dramatically.

In meat production, not only animal performance, meat quality is also important point. Meat quality is a very wide conception that is including eating quality, convenience, stability, wholesomeness, and nutritive value that is defined from two perspectives-scientific status and consumer preferences (Nollet *et al.* 2007).

The main factor influencing consumer choice, when buying meat and meat products, is eating quality, which includes meat appearance, flavor, and texture. These attributes are influenced by many factors such as preslaughter stresses, post mortem storage, rearing and crating condition, and feeding. Therefore, methods to control meat quality can be based on animal genetics and nutrition or processing techniques. Especially, dietary nutrients such as amino acids, vitamins, minerals, and antioxidants can play a significant role in determining meat quality and also the growth rate of poultry.

In normal aerobic metabolism generates reactive oxygen species (ROS) that are naturally regulated by chain breaking antioxidants and antioxidant enzymes which in balance system of reactive species and antioxidants. During times of environmental stress, particularly heat

stress and postmortem storage, ROS is released dramatically and results harmful oxidative stress includes lipid and protein oxidation and DNA damage which are main reasons of meat quality deterioration such as discoloration, off-flavors, and a reduction in water-holding capacity leading to drip loss and unacceptable texture (Decker *et al*. 2000). Therefore, for recovering insufficient antioxidant level, dietary supplementation of natural and synthetic antioxidant is an adequate method.

There are some studies about meat quality improvement and inhibition of oxidative stress by dietary natural antioxidants, vitamin E (Olivo *et al.* 2001), vitamin C (King *et al.* 1995), carotenoids (King *et al.* 1995) in normal environmental condition, and vitamin E (Vakili and Rashidi 2011; Imik *et al.* 2012a), vitamin C (Imik *et al.* 2012b) in heat stressed condition. However antioxidant effects on meat quality under heat exposure are not well studied. When oxidative stress occurred, the lipids in cell membranes are oxidized. In this case, lipid-soluble antioxidants are more effective when integrated into animal tissue through feeding than when incorporated into meat during processing (Higgins *et al*. 1998).

Ax is a lipid-soluble red-colored carotenoid that has proven to be a free radical scavenger (Naguib 2000) and has activity approximately 10 times stronger than that of other carotenoids and 100 times greater than that of α-tocopherol for singlet oxygen quenching (Miki 1991). However because of its anti-oxidative power, there have been many studies on the functional benefits of Ax in human and rat but few studies have related to in livestock. However its brilliant colorful characteristic is due to make study about pigmentation effect in animal meat, especially broiler meat, and aquaculture.

Ax occurs naturally in micro algae, yeast, fish, and most crustaceans. Ax's study in animal experiment is commonly performed as using Ax-rich algae or yeast as a source of Ax. One of them is the yeast *Phaffia rhodozyma (Xanthophyllomyces dendrorhous*) which has been studied as a dietary source of Ax to improve the pigmentation of broiler meat (Akiba *et al.* 2001; An *et al.* 2004) and egg yolk (Akiba *et al.* 2000; Yang *et al.* 2006). Unfortunately, almost no studies have investigated Ax in connection with other meat quality attributes such as meat texture and flavor. Dietary Ax-rich yeast accumulates Ax in blood, muscle and other organs of broiler chickens (Akiba *et al.* 2001; An *et al.* 2004*;* Takahashi *et al.* 2004) and this accumulation is correlated concentration of dietary *Phaffia* yeast (Akiba *et al.* 2001). However the optimum concentration or feeding period of dietary Ax supplement for improvement meat quality is not clear.

Moreover, as mentioned above, even some antioxidants alleviate heat stress deterioration of meat quality but the study about effect of Ax on meat quality under heat stress condition absolutely has not been done yet.

Therefore, the object of this study was to evaluate the effect of dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, on the meat quality of broiler chickens in normal rearing condition and under heat exposue.

CHAPTER II. LITERATURE REVIEW

2.1 Meat quality and affecting factors

After death of animal metabolic processes initiates in the muscle which alter its in vivo characteristics and gradual transformation of the muscle into meat takes place. These processes influenced fundamental effects on the final characteristics of good-quality of meat. It can be divided into three stages: pre-rigor, rigor mortis, and resolution (Isabel Guerrero-Legarreta 2010). This process is shown in Fig 2-1.

Except performance, the meat quality is important view of meat production. The meat quality is defined from two perspectives-scientific status and consumer preferences. Scientific characteristics of meat quality include: physical, chemical, morphological, microbial, technological, hygienic, culinary, and nutritional properties. Consumer preferences are linked directly to the human senses – sight, touch, smell, taste, and mouthfeel which are sensing appearance, texture, juiciness, wateriness, firmness, tenderness, odor, and flavor that are the most important and perceptible meat features. Main meat quality attributes are shown in Fig 2-2.

Intensive research on poultry meat quality started after World War II, mainly industrialized countries because of to satisfy increasing demand for animal protein in human nutrition and to increase safety of meat (Grashorn 2010). Meat quality is assessed by measuring meat pH, color, shear force value (SFV), water holding capacity (WHC), and its related.

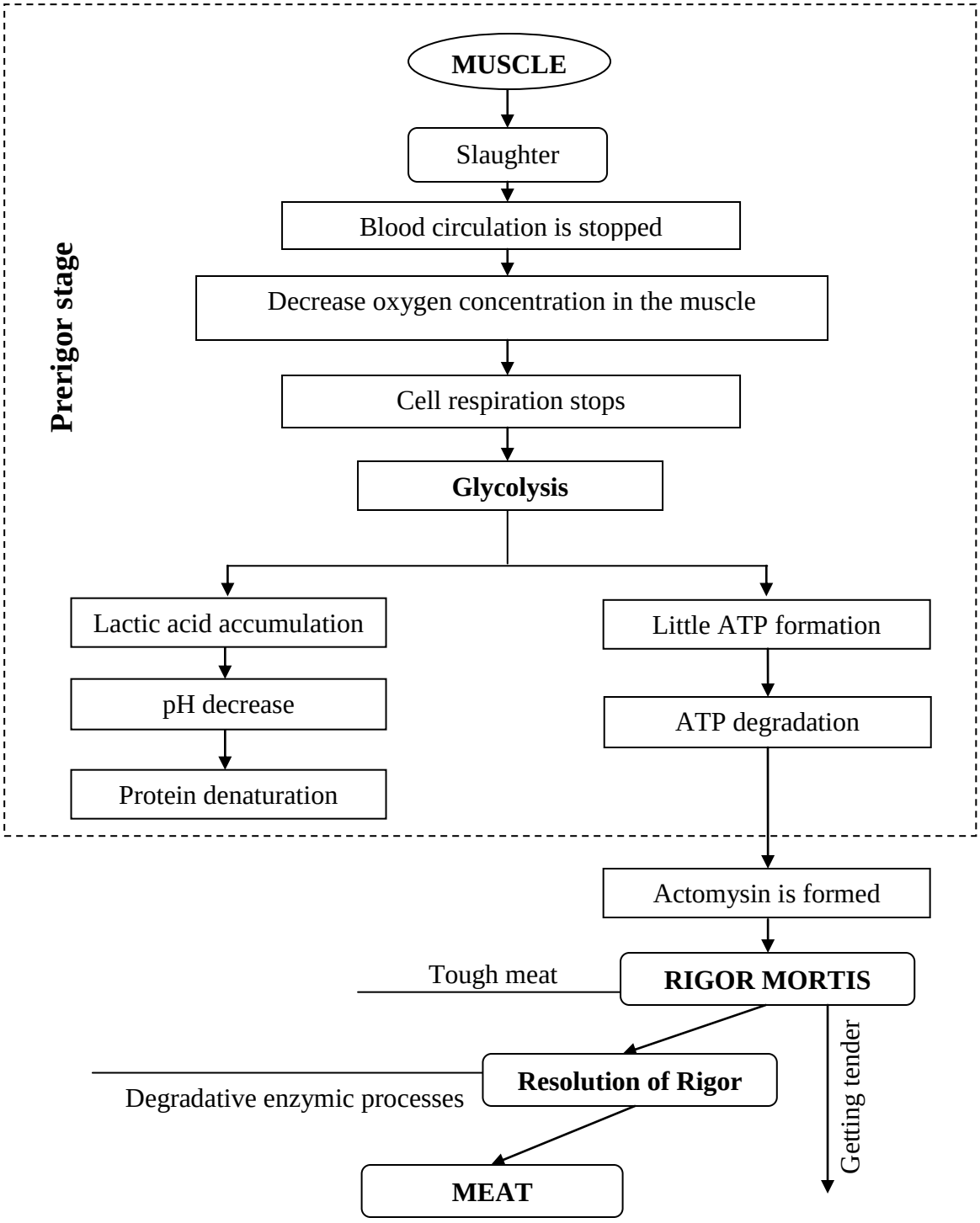


Fig 2-1 Muscle to meat (Modified from Isabel Guerrero-Ledarreta 2010)

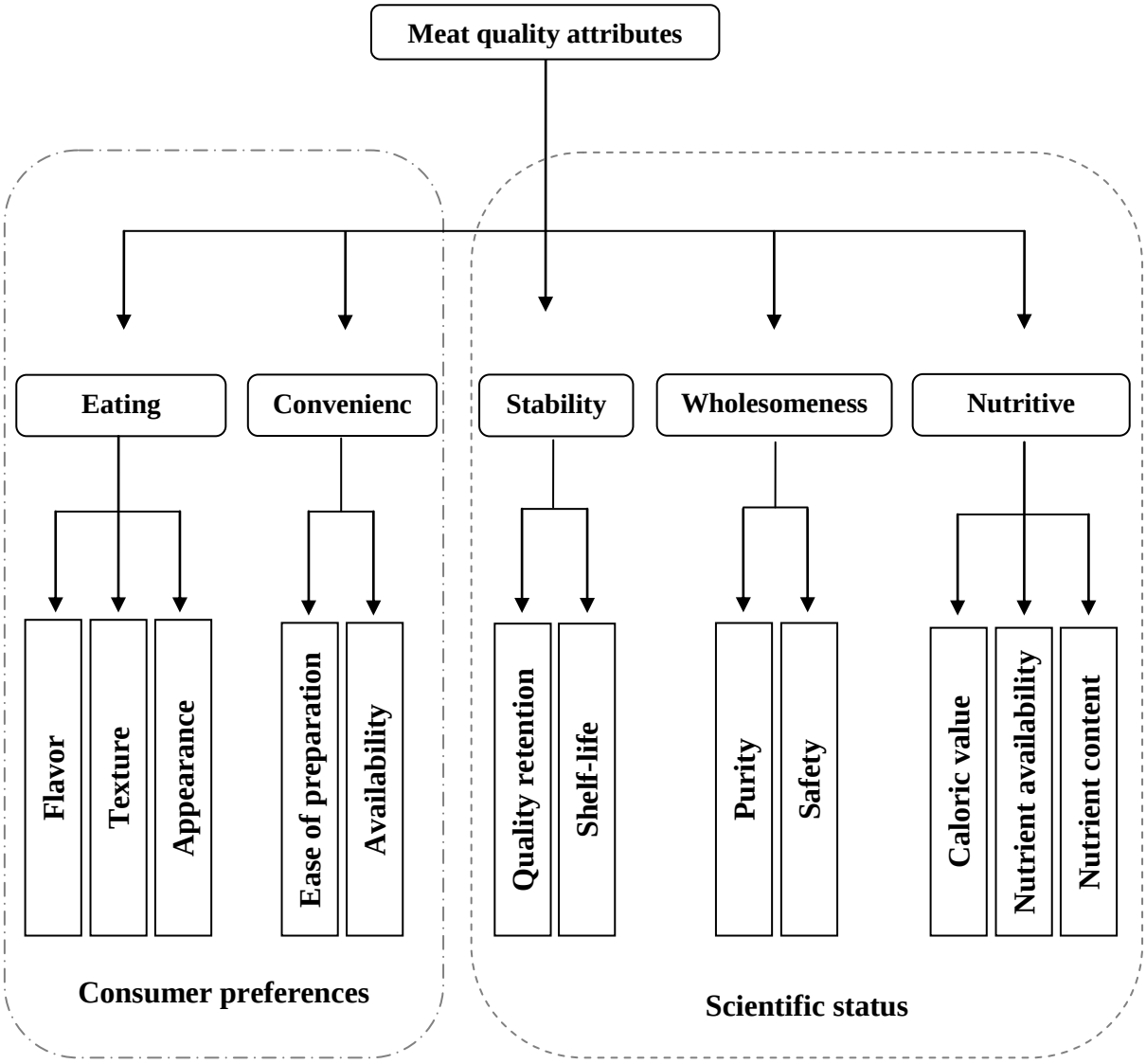


Fig 2-2 Meat quality attributes (Modified from Erdtsieck, 1989)

parameters such as drip loss and cooking loss which are main attributes of flesh meat and further processed meat products

2.1.1 Meat color

The main attributes of meat quality that influences the first impression of consumer and making choice is meat appearance including meat color. The demand by consumers for poultry meat color shows pronounced regional differences around the world. Some countries highly acceptable for meat pigmentation but some countries preferred pale meat. In the world there basically two kinds of color scales are used today: CIE (International Commission on Illumination) scale L^*, a^*, b^* and Hunter scale L, a, b. Both of them derived from first mathematically defined color spaces XYZ color space, created by the CIE in 1931.

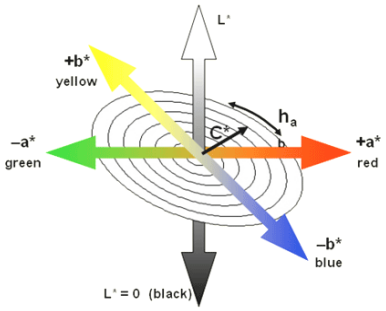


Fig 2-3 CIE scale of color, L^*, a^*, and b^*

Illuminant is C and D_{65}

In the CIE LAB, L^* value is an expression of the lightness of surface ranging from 0-100 (black to white), a^* value indicates red, ranging from negative to positive (green to red), and b^* value indicates yellow, ranging from negative to positive (blue to yellow) (Barbut 2002). The meat color is determined by the pigments those are in it. These pigments can be classified into four types:

- Biological pigments: carotenoids and hemepigments, which are accumulated or synthesized in the organism ante-mortem (Lanari *et al*. 2002).
- Pigments produced as a result of damage during manipulation or inadequate processing conditions.
- Pigments produced postmortem: from enzymatic or non-enzymatic reaction (Montero *et al.* 2001).
- Pigments resulting from the addition of natural or artificial colorants

Carotenoids, especially xanthophylls includes astaxanthin and canthaxanthin, are mainly used for pigmentation in aquaculture, but few of them are used in poultry, pork and beef. Carotenoids have naturally brilliant color availability, yellow to red or pink. Therefore accumulation in flesh is directly influence color. Meat color is due to hemopigments and their derivatives. In live animal, hemoglobin is the predominant pigment, but after slaughtered and bled animal the average amount of blood remaining in the meat joints is 0.3% (Warris and Rodes 1977). After death of animal, myoglobin accounts for some 95% of the remaining heme pigments. Myoglobin is a purple and in the presence of oxygen becomes oxygenated to form a bright-red oxymyoglobin, and it is in the air exposure oxidized to metmyoglobin, resulting in undesirable brown color.

Meat color is influenced by type of the feed the birds consumes. Poultry meat industries used various seeds and grains which all contains certain level of carotenoids. As mentioned above, the carotenoids are main regulator of meat color. In addition, dietary supplements to

feed can affect the color of muscle. The cooked meat from that fed a conjugated linoleic acid was darker and decreased redness (Du and Ahn 2002).

Another important factor influencing meat color is the meat pH value. Lower meat pH value which play main role on the higher protein denaturation associated with lower water holding capacity and light scattering increases and muscle became opaque. Higher pH value means lower protein denaturation and water molecules tightly bound, causing more light to be absorbed by the muscle, and meat appears darker color (Dadgar 2010).

Processing has a significant influence on color of meat. Feed withdraw affect meat makes lighter, less redder and more yellow (Smith *et al.* 2002). Stunning is the premortem procedure used for birds to reduce pain and stress before automated killing. After stunning birds are bled out. The delayed bleed-out has significant effect on development of discoloration (Raj *et al.* 2001). In addition, a rapid chilling time after slaughter has less muscle defects. While temperature of muscle is still high, pH value of muscle declines rapidly and causing pale, soft, and exudative (PSE) meat. Furthermore, preservation method (vacuum storage) and irradiation can influence the meat color, which reducing oxidation process during the storage and preventing loss of meat redness.

Finally, bird age is important factor for meat color. Nishida (1985) reported that as bird age, the level of myoglobin in the muscle increases, resulting in darker color.

2.1.2 Meat pH and Pale Soft Exudative, Dark Firm Dry meat

Meat pH value is crucial parameter which indicating meat quality. It associated the most other meat quality parameters such as color, tenderness, juiciness, and water holding

capacity. After slaughter, the only source of ATP is the anaerobic metabolism of glycogen, the main energy reserve of the muscle, which converts to lactic acid through postmortem glycolysis. This process results the decrease of pH value of muscle. Decrease in pH value that accompanies glycolysis approximately from 7.2 to values close to 5.3-5.7 according to handling and species. The speed of decrease pH is influenced by many factors, such as species of animal, muscle type, temperature during the postmortem process, and stress factors (Isabel Guerrero-Ledarreta 2010). Rapid contraction fibers or white fibers fast but slow contraction fibers or red fibers are relatively slow to reach ultimate pH value. The temperature of muscle has influence on speed of postmortem glycolysis, high temperature accelerate the decrease of pH. High glycogen content in muscle at the time of slaughter, high quantities of lactic acid is synthesized and pH decrease is greater than when the glycogen content is low. Rapid acidification of meat results high protein denaturation due to it drops to the isoelectric point, solubility, and water binding capacity, because of net charge absence on the protein to bind to water molecules and less space for water within myofibrils (Dadgar 2010). Reduction of solubility and water retain ability means that pale, soft, exudative meat and opposite process results dark, firm, dry (DFD) meats. Sample of pH drop in normal, PSE and DFD poultry meat is shown in Fig 2-4. PSE meat has been described for the first in 1954 by Ludvigsen in swine. PSE meat leads to huge economic losses in poultry and pork production. It has been estimated that PSE-type meat represents 5-47% of meat produced in poultry industry (Barbut 1996). PSE meat is caused mainly from two factors: genetic and environmental cause.

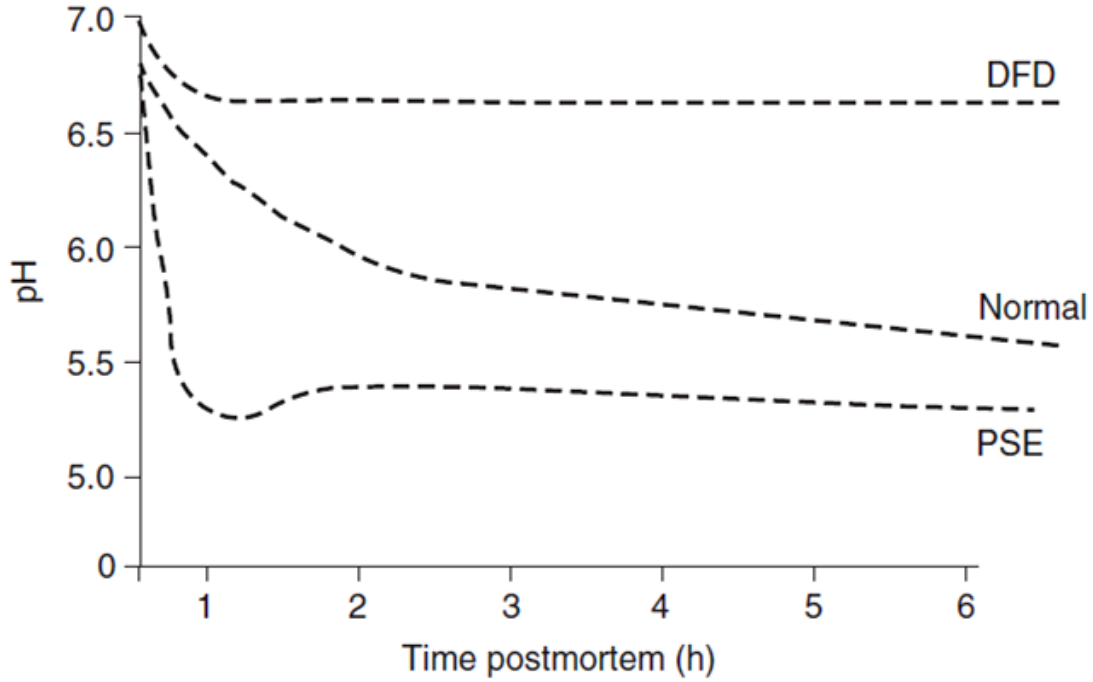


Fig 2-4 Typical pH drop of poultry meat in postmortem, (from Isabel Guerrero-Ledarreta 2010)

Environmental temperature plays main role in premortem stress which is resulting meat defect. Acute heat stress exhibits PSE meat in turkey (Mckee and Sams 1997) and chicken (Northcutt 1994). Furthermore, Zhang *et al*. (2012) revealed that chronic heat exposed chicken has significantly lower pH and higher lightness of meat in other words PSE like meat.

High consumption of ATP and glycogen premortem reveals the substances free from degradation of glycogen (CO_2 and lactic acid) are forced out of the muscle by the circulatory torrent and at the slaughter in the muscle there has low amount of glycogen and minimal or no lactic acid production is occurred and resulted incomplete pH drop (Isabel Guerrero-Ledarreta 2010). DFD is influenced from transportation (Warris *et al*. 1999), capture, loading, feed withdrawal, and cold stress (Dadgar 2010).

2.1.3 Meat water holding capacity (WHC)

The major composition of meat is water. In muscle water is held within the structure of the muscle and muscle cells. Especially, in the muscle cell, water is found within the myofibrils, between the myofibrils, and between the myofibrils and cell membrane, between muscle cells and between muscle bundles. Water is located in three patterns in muscle.

1. Bound water: Water is dipolar molecule and is attracted to charged species like protein. Therefore some of water in muscle cells is bound to protein. Bound water exists in the vicinity of non-aqueous constituents and has reduced mobility. It is a very small fraction in muscle and it is very resistant to freezing and heating. Change in bound water amount is very little.

2. Entrapped (mobilized) water: This fraction may be held either by steric effect or by attraction to the bound water. It is within the structure of muscle but not bound to protein. In early postmortem it does not flow freely from the tissue, but it can be removed by drying and easy to convert ice during freeze. This water fraction is most affected by conversion of muscle to meat (Offer and Knight 1988).
3. Free water: Flow from tissue is unimpeded. Weak surface force mainly holds this fraction. In prerigor condition no free water is seen but can develop as conditions change that allow the entrapped water to remove from the structures where it is found.

The one of the most important parameters of meat is the ability that to retain these water in. It helps meat tender, firm, especially juicy, leading to improvement of meat quality and economic value. Water binding potential is defined as the ability of the muscle proteins to retain water in excess and under the influence of external force. Free drip refers to the amount of water that is lost by the meat without the use of force other than gravity force.

Water holding capacity is affected by pH reduction which results protein denaturation, loss of protein solubility and overall reduction of reactive groups available for water binding on muscle proteins. Another important factor for WHC is the lack of space between the myofibrillar proteins that results by accumulation of actinomyosin complexes as energy source are depleted in the muscle.

2.1.4 Meat tenderness

Meat tenderness is one of the most important sensory attributes which entails many parameters to consumers. More tender muscle food is the better perception of freshness and quality resides in the mind. Peluffo and Monteiro define texture as “difficulty or facility for chewing meat”, and Kramer (1951) defined firmness/tenderness as “the main textural characteristics of all meat products”. Meat tenderness to toughness is determined by two groups of meat components: the connective tissues and muscle fiber as illustrating in Fig 2-5 (Koohmaraie *et al.* 1992). Connective tissue is fibrous structure composed of primarily collagen fibrils. There are three types of connective tissue in meat: epimysium (thick sheath connective tissue surrounding entire muscle), perimysium (thin layer enveloping muscle fibers), and endomysium (between muscle fibers). Perimysium and endomysium connective tissues present a realistic toughness problem to meat. The influence depends upon their thickness (amount of collagen present, as well as the density and type of cross-linkages between collagen fibrils) and it is affecting by animal species, age, feed nutrients (Nollet *et al.* 2007).

Postmortem metabolism in muscle plays a significant role in the myofibrillar component affect meat tenderness. The contractile state of the muscle is the most important factor affecting tenderness. Sarcomere state, when actomyosin cross-bridges form, affects meat tenderness. During rigor mortis development, there is natural shortening of the sarcomeres due to the increased number of the actomyosin bonds that are formed. However natural form of shortening does not influence on meat toughness but shortening that is induced can affect meat tenderness significantly.

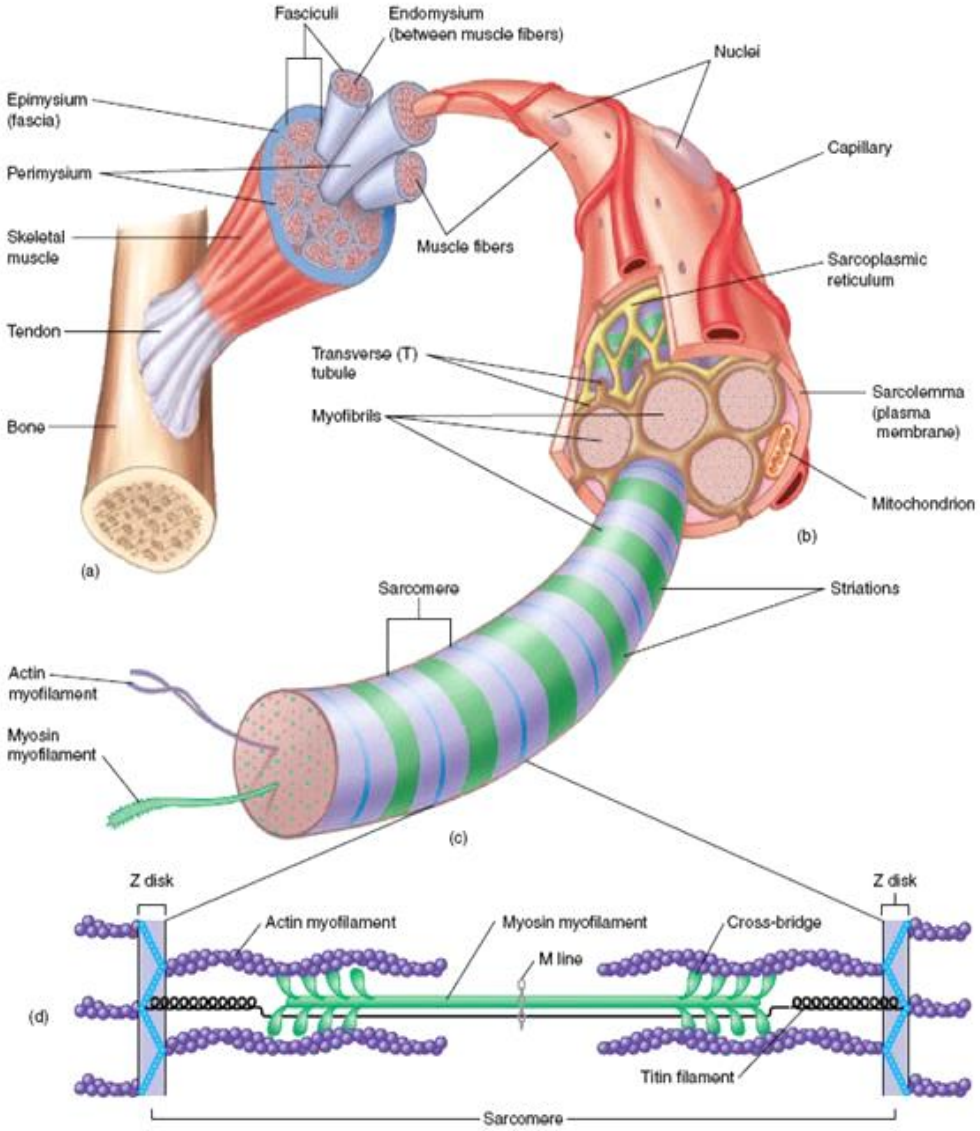


Fig 2-5 Muscle structure (from http://apbrwww5.apsu.edu/)

Prerigor excision, or early deboning prior to rigor completion, is a major cause of induced sarcomere shortening and meat toughening. The major indicating parameter for meat tenderness is shear force value (SFV).

2.1.5 Meat flavor

From the sensory evaluation of view, flavor is series of sensation perceived by two senses, taste and smell. Taste is perceived by the buds on the tongue and other parts of the mouth. There are five basic tastes: sweet, salty, bitter, sour, *umami* and other tastes (rich taste, astringency so on) can be detected. The sense of smell also performs a role in this event. Some chemicals can stimulate the olfactory receptor at the top of the nasal cavity. Odor substances can be detected before eat. Substances contributing in flavor can be divided into aroma compounds and taste compounds. Aroma compound are commonly volatile organic compounds. Until today, many volatile flavor compounds have been identified in meat: sulfur-containing compounds, nitrogen-containing compounds, furanones, lipid-derived compounds. Taste compounds are non-volatile or water-soluble substances with taste or tactile properties. Salty tastes in meat are caused by sodium chloride and some other inorganic salts, together with monosodium glutamate and monosodium aspartate (Macleod 1986). Sweetness is caused by sugars which are formed from rigor mortis glycolysis and certain amino acids. Bitter tastes are influenced by mainly amino acids and peptides (Macleod 1986). Sour tastes are caused by acids, such as lactic acid, organic acids, amino acids, and acidic phosphates (Macleod 1986). *Umami* taste is affected by monosodium glutamate, inosine monophosphate, and guanosine monophosphate, additionally, free

amino acids, especially Glu, can greatly contribute to the taste of meat (Kato & Nishimura 1987; Fujimura *et al.* 1996).

2.2 Heat stress and meat quality

Selye (1976) reported that "Stress is the nonspecific response of the body to any demand". Therefore, stress represents the reaction of the animal organism to stimuli that disturb its normal physiological equilibrium or homeostasis (Lara and Rostagno 2013). Birds are heat stressed if they have difficulty achieving a balance between body heat production and body heat loss. In thermoneutral zone birds can lose heat at a controlled rate using normal behavior. There is no heat stress and body temperature held constant. When conditions mean the upper critical temperature is exceeded, birds must lose heat actively by panting. Panting is a normal response to heat and is not initially considered a welfare problem. But as temperature increase the rate of panting increase. During the panting the loss of carbon dioxide is increased and bicarbonate ions coupled with monovalent cations trough urine, disturbed the acid base balance, in the consequences respiratory alkolysis is occurred (during respiratory alkolysis, the shift in the blood pH increasingly depressed the feed intake and adversely effected the overall performance of broiler) (Linsley and Burger 1964). Therefore dietary electrolyte balance has significant effect on poultry performance. If heat production becomes greater than max heat loss either in intensity or over long periods birds may into the death (Fig 2-6). According to increase of global surface warming in livestock animals productions, especially in poultry production, heat stress is becoming harmful and urgent problem results significant economical loss in producers.

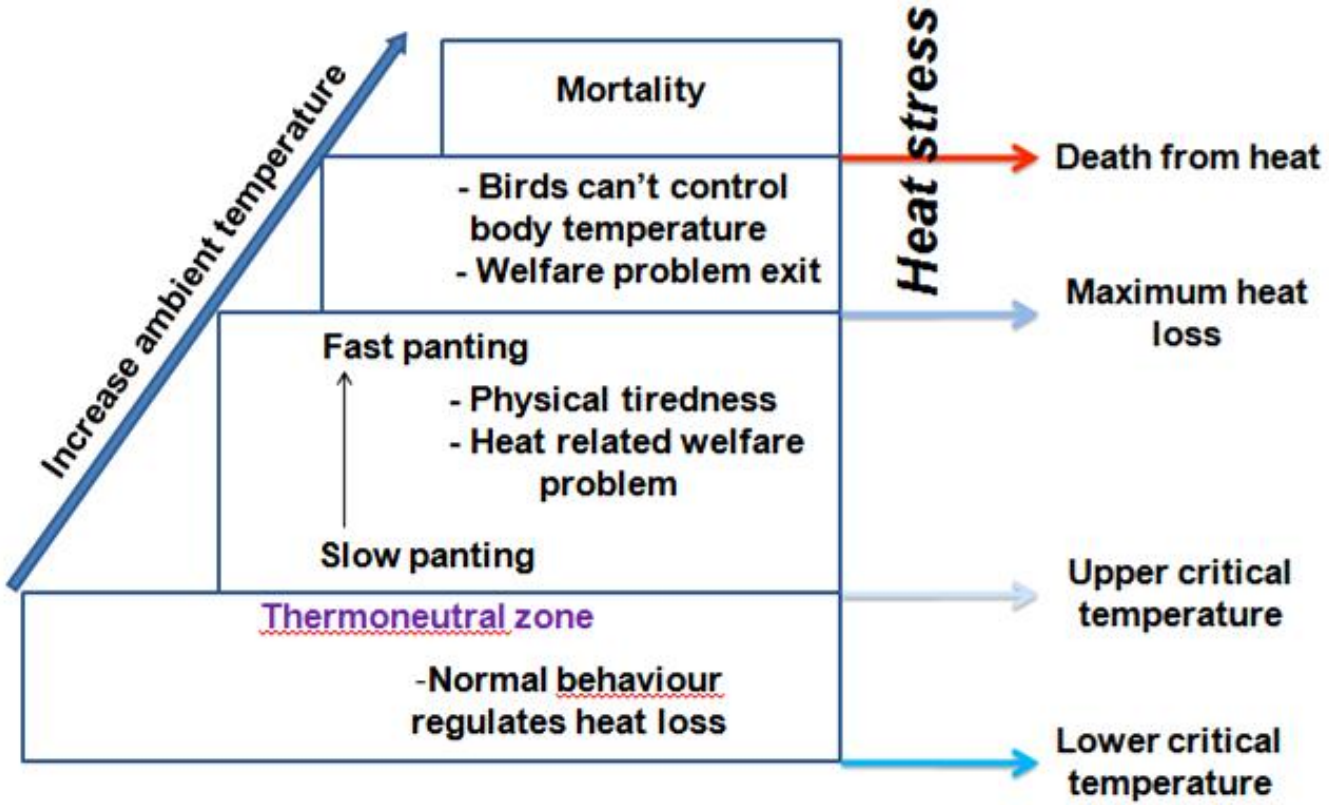


Fig 2-6 Initiation of heat stress

Particularly, genetic improvement program continue to emphasize production traits, and this may inadvertently decrease heat tolerance (Rhoads *et al*. 2013). Heat stress could be occurred when boilers transporting (acute heat stress) and during the rearing (chronic heat stress). During the heat stress various metabolic changes occurs in organism (Rhoads *et al.* 2013), from these postmortem glycolysis is influenced crucially and it deteriorates meat quality as decrease ultimate pH, increase lightness and increase WHC in chicken meat, PSE-like meat (Northcutt *et al*, 1994; Mckee and Sams 1997; Sandercock *et al.* 2001).

Furthermore, heat stress increases oxygen radicals, possibly by the disruption of the electron transport assemblies of the membrane and it affects on molecular changes in DNA, proteins, and lipids which means oxidative stress. Therefore, there is some studies to inhibit heat stress effects on meat quality by dietary antioxidants, vitamin C (Imik *et al*. 2012b), vitamin E (Imik *et al*. 2012a).

2.3 Oxidative stress and meat quality

Normal aerobic metabolism generates reactive species. These formed ROS is naturally regulates by chain breaking antioxidants by terminating the free radical chain reaction by donating hydrogen atoms to free radical species and forming less reactive products. Due to this process, systemic manifestation of reactive oxygen species and antioxidant defense system are in balance.

During times of environmental stress (*e.g.,* UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures that are oxidative stress including lipid oxidation and protein oxidation.

2.3.1 Lipid oxidation

Lipid oxidation is the process by which molecular oxygen reacts with unsaturated lipids to form lipid peroxides. It is a free radical chain reaction mechanism involving initiation/branching, propagation and termination stages (Fig 2-7).

In initiation stage the free radical is formed by initiator, ROS. Then in propagation stage free radicals are oxidized by molecular oxygen forming lipid peroxy radical and it can abstract a hydrogen atom from another unsaturated fatty acid and propagate the chain reaction. In branching step lipid hydroperoxides formed may undergo homolytic scission to form alkoxyl and hydroxyl radicals, which are capable of propagating further oxidation and lead to chain branching and final stage, termination stage is the reaction between free radicals to form non initiating and non propagating products. In consequence of lipid peroxidation, meat quality is lowered that has characterized off flavor, discoloration and toxic compound formation (Decker *et al.* 2000). Lipid oxidation can be regulated successfully by dietary antioxidants, vitamin E (Jensen *et al.* 1995), β-carotene and zeaxanthin (Woodall *et al.* 1996), and improve meat quality of chicken.

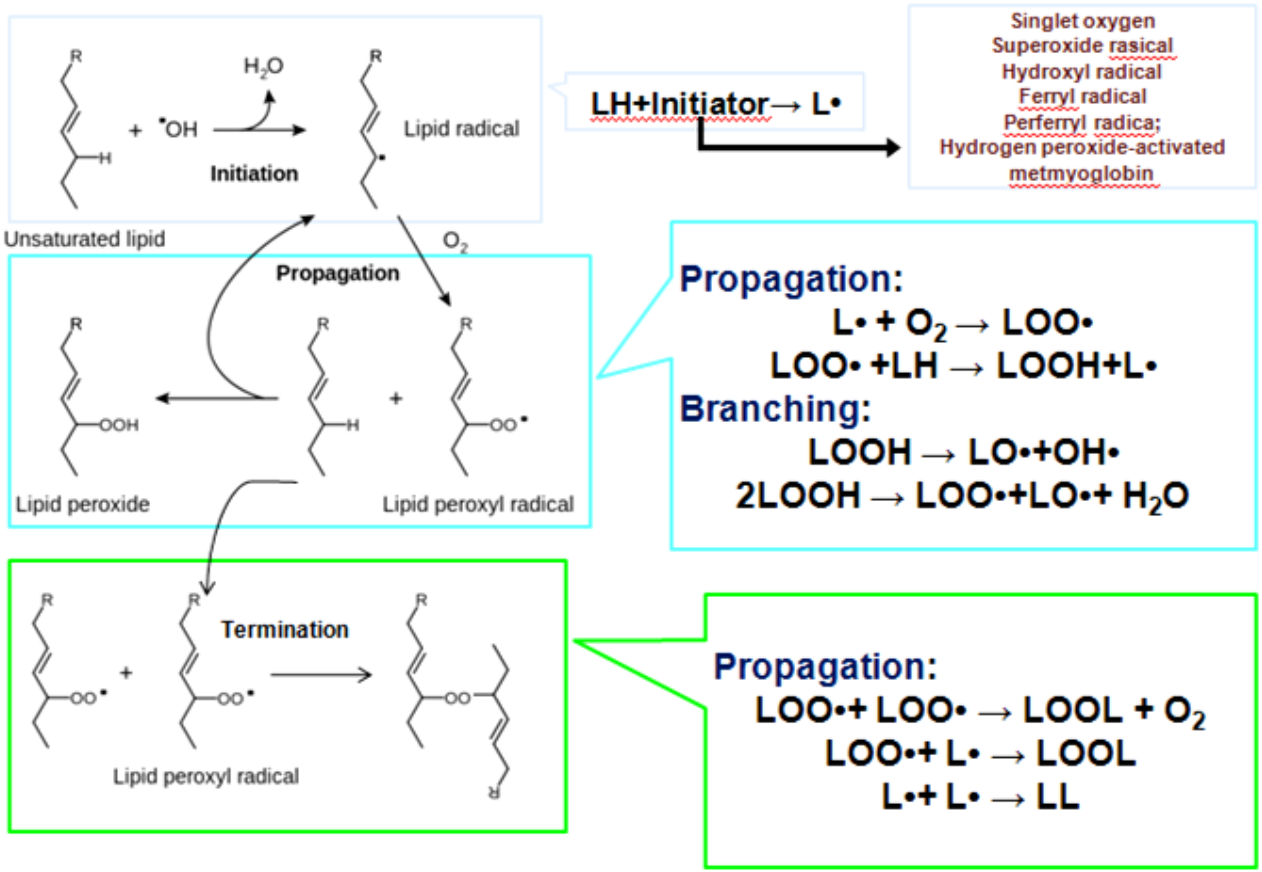


Fig 2-7 Mechanism of lipid oxidation

2.3.2 Protein oxidation

In presence of oxidizing lipids, protein oxidation is manifested by free radical chain reactions similar to those for lipid oxidation, which involve invitation, propagation and termination (Fig 2-8).

In result of protein oxidation, protein cross-linking is formed. The intra and inter-molecular cross-linking of muscle proteins involves the formation of a variety of cross-linked oxidation products and subsequently polymerization of the proteins including disulfide cross-links and dityrosine.

Some amino acids from myofibril protein are oxidized in presence of ferric ion and hydrogen peroxide to yield some aldehydes and ketones.

Oxidized proteins have amino acid destruction, decrease in protein solubility, loss of enzyme activity, formation of amino acid derivatives including carbonyls, and increase in protein digestibility (Meucci *et al.* 1991; Stadtman and Oliver 1991; Agarwal and Sohal 1994). In consequence of protein oxidation, meat quality is lowered that has characterized decrease EAA, decrease WHC, forming gel matrix, protein lipid complex formation, insoluble protein aggregates, fat globule membrane, decrease tenderness, toxic compounds formation. The influence of antioxidant strategies (dietary and processing) on the functionality and quality of the muscle protein is still unclear (Decker *et al.* 2000).

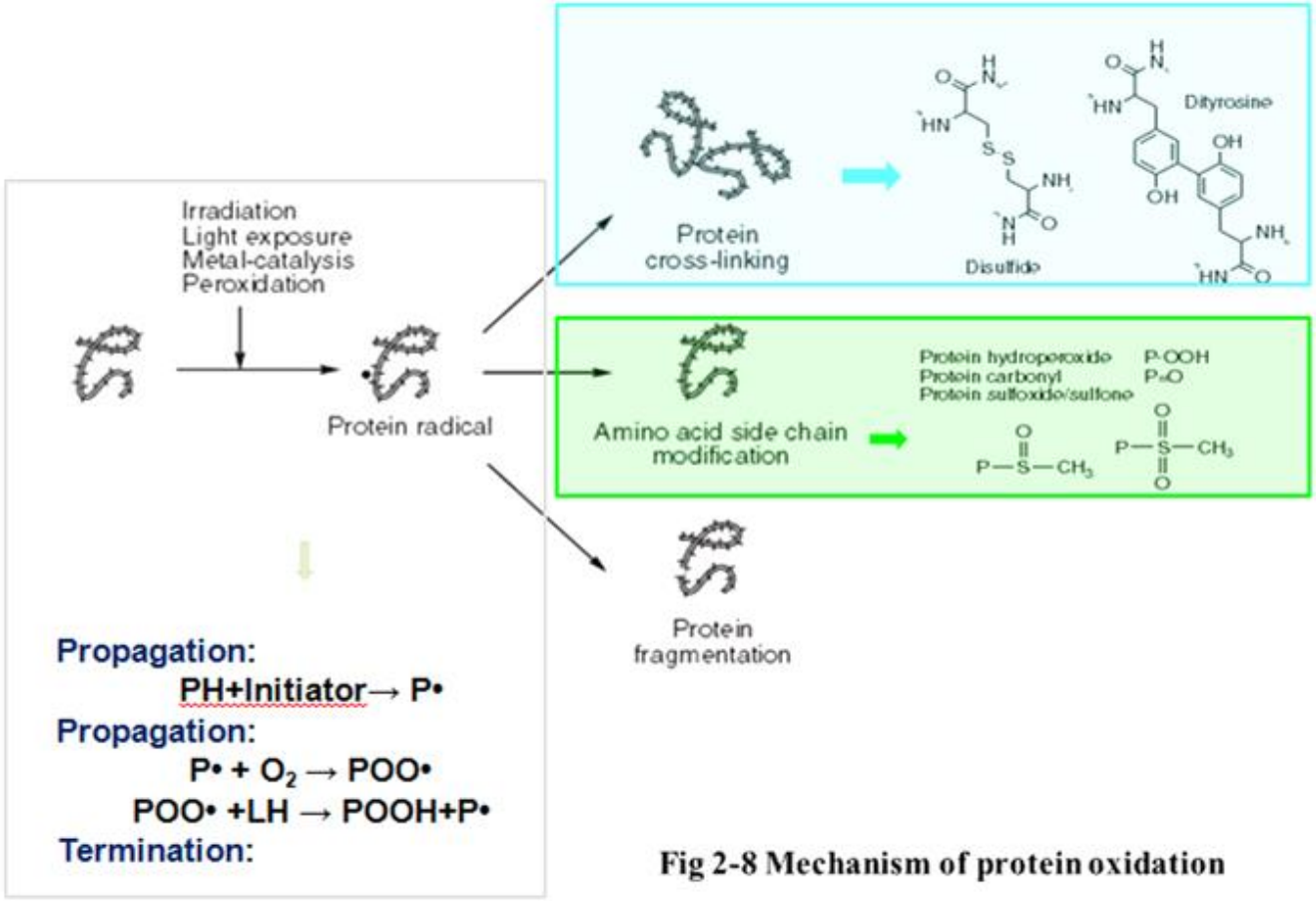


Fig 2-8 Mechanism of protein oxidation

from Lund *et al.* (2011)

2.4 Astaxanthin

Ax is in xanthophylls family which is oxygenated derivatives of carotenoid that belongs to a larger class of phytochemicals known as terpenes.

IUPAC name: (6*S*)-6-Hydroxy-3-[(1*E*,3*E*,5*E*,7*E*,9*E*,11*E*,13*E*,15*E*,17*E*)-18-[(4*S*)-4-hydroxy-2,6,6-trimethyl-3-oxo-1-cyclohexenyl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-2,4,4-trimethyl-1-cyclohex-2-enone.

Ax has red pigmenting properties occurring in yeasts, algae, crustaceans, and predator fish like salmonids. Unlike several carotenoids, is not converted to vitamin A and is not made in the human body (Wikipedia, 2013).

It is a metabolite of zeaxanthin and cantaxanthin, containing both hydroxyl and ketone functional groups and like other carotenoids, contains extended chain of conjugated double bonds that is responsible for the color and also for the antioxidant function, at the center of the compound. Due to its antioxidative prowess, it has a many functional benefits including extraordinary potential for protecting cardiovascular disease, cancers, and some disease of immunological system of human, animals and aquaculture. Therefore, Ax has been attracting scientists' interest effectively and more studies have been focused on the identification, production, and utilization of Ax all over the world.

2.4.1 Chemical structure

Ax was found by German Professor Richard Kuhn in 1938, but Great Britain professor, Basil Weedon was the first to map the its structure.

The structure is derived from lycopene which majority is hydrocarbons of 40 carbon atoms containing two terminal ring systems joined by a chain of conjugated double bonds or poliene system. The poliene system gives Ax its distinctive molecular structure, the chemical properties, and its light absorption characteristics. Each double bond from the poliene may exist in two configurations; as geometric isomers *cis* or *trans*. *Cis* isomers are thermodynamically less stable than the *trans* isomers (Britton 1995), and in addition it also has two chiral centers in C-3 and C-3', therefore it may present three configurationally isomers: two enantomers (3R, 3'R and 3S, 3'S) and a meso form (3R, 3'S) (Turujman *et al*. 1997) (Fig 2-9).

Depending on their source, Ax can be found in free or in association with other compounds; esterified in one or both hydroxyl groups with different fatty acids, or forming chemical complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins) (Johnson and An 1991). One example is lobster, shrimp, and some crabs turn red when cooked because the Ax which bound to the protein becomes free as a protein denatures and unwinds.

2.4.2 Sources

Synthethic source

Synthetic Ax is obtained by Wittig reaction (Fig 2-10). A condensation of C10-dialdehyde with 2 equiv. of C15-phosphonium salt and subsequent thermal isomerization afforded Ax and is used commercially. Synthetic Ax is an identical molecule to that produced in living

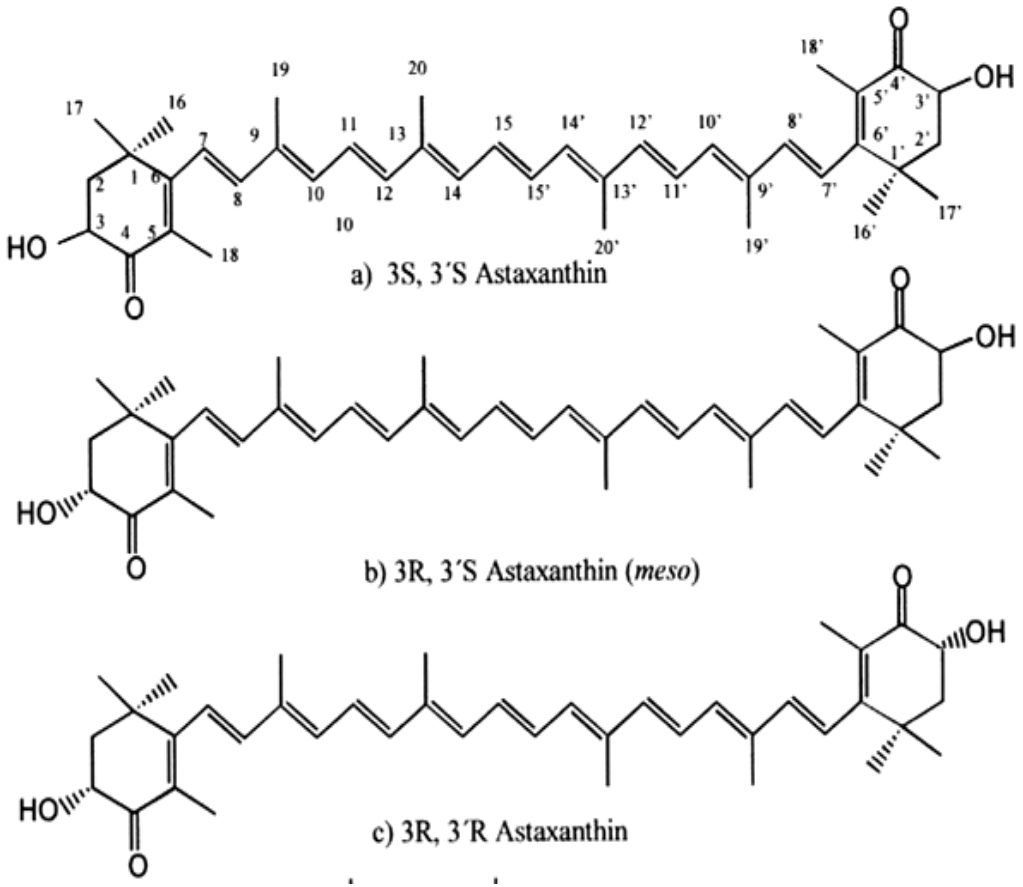


Fig 2-9 Configurational isomers of Astaxanthin

organisms and it consists of a mixture 1:2:1 of isomers (3S, 3'S), (3R, 3'S), and (3R, 3'R) respectively. Nearly all commercial Ax for aquaculture is produced synthetically in the worldwide and annual turnover over 200 million dollars. Price of 1kg synthetic Ax is about 5000-6000$ in 2012 (Wikipedia 2013).

Natural sources

In recent years there has been growing tend toward using natural ingredients in all forms of food nutrients, resulting from increasing concerns for consumer safety and regulatory issues over the introduction of synthetic chemicals into the human food chain. This is also true for the nutraceutical and cosmeceutical markets. There are some sources that are used for the commercial production of Ax: Salmonids – 5ppm, Arctic shrimp – 1200 ppm, *Phaffia* yeast – 10000 ppm, *Haematococcus pluvialis* – 40000 ppm Ax is contained respectively. Natural Ax is expensive ingredient, but few sources of microbial origin can compete economically with synthetic Ax: the green microalgae *Haematococcus pluvialis* and the red yeast *Phaffia rhodozyma*.

***Phaffia rhodozyma* yeast** is a the yeasts *Xanthophyllomyces dendrorhous* (teleomorph) and *Phaffia rhodozyma* (anamorph) are of basidiomycetous affinity and have the unique property among yeasts of producing the carotenoid pigment Ax. For more than two decades, the red yeast, *Phaffia rhodozyma,* has been widely studied focusing on the effect of form Ax. This yeast exhibits 100% free, non-esterified Ax which is considered advantageous because it is readily absorbable and need not to be hydrolyzed in the digestive tract.

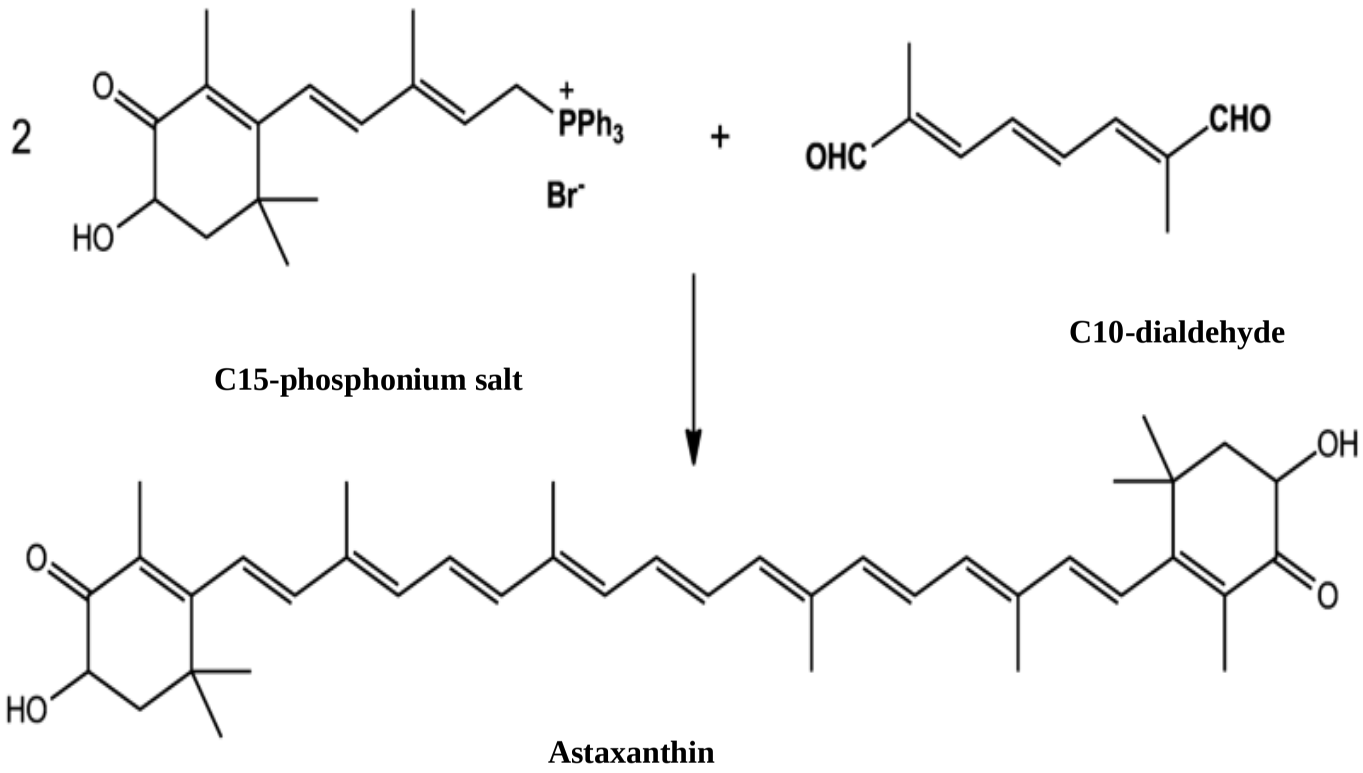


Figure 2-10 Wittig reaction for synthetic Ax formation

It is also consist all in 3R, 3'R enantiomeric form and 100% trans-isomer that have higher bioavailability than cis-isomer. In the worldwide, there are some companies make a production of Ax-rich yeast, *Phaffia rhodozyma,*

Aquasta®

Aquasta® is a natural Ax source from inactivated dried yeast, *Phaffia rhodozyma*, made in Naturxan that is world's leading provider of naturally sourced Ax. Naturxan is a joint venture between the Archer Daniels Midland Company and Igene Biotechnology. Aquasta® is made in Aska pharmaceutical Co., Ltd in Japan.

Aquasta® is like synthetic source (Ax concentration – 10000ppm), exhibit 100% free, non-esterified Ax which is readily absorbable and need not be hydrolyzed in the digestive tract. It consists virtually all in 3R, 3'R form, an important Ax source in nature and the all-E geometrical isomer, that has greater bioavailability, is higher in Aquasta®. It is high level of stability that during the 180 days at 20^0C storage of feed that is containing Aquasta®, Ax concentration decreases by10% in maximum.

2.4.3 Absorption and metabolic transformation

In human after ingestion of esterified Ax, only unesterified Ax appears in the blood (Coral-Hinostroza *et al.* 2004). This is due to breaking the ester bonds by digestive enzymes via their hydrolytic activity. Absorption into the intestinal lining cells is through to occur by passive diffusion and is facilitated in the presence of fat or other lipids (Okada Y *et al.* 2009).

Table 2-1 Composition of Aquasta® yeast

Description	Amount, %
Moisture	Max 8.0
Total carbohydrate	40.5
Protein, N*6.25	17.4
Phosphorus	0.24
Fat (acid hydrolysis)	37.9
Ash (acid hydrolysis)	1.11
Estimated Caloric Value	573cal/100g
Astaxanthin	10000 ppm
Disrupted yeast	> 90 %

More detailed information is available in "Technical bulletin" for Aquasta® of Archer Daniels Midland Company and Igene Biotechnology.

The enterocytes then incorporate the unesterified Ax into chylomicrons, which transport it to the liver. The liver doesn't convert this molecule to vitamin A or otherwise biochemically transform it. Instead it becomes incorporated into low-density lipoprotein (LDL) and high - density lipoprotein (HDL), which then distribute into the tissues via the circulation.

In the cell membrane, Ax locates as the polar end groups overlap the polar boundary overlap the polar boundary zones of the membrane, while the non polar middle fits the membrane's non polar interior. This position has a ability to conduct electrons along the Ax molecule, possibly to other antioxidants located outside the membrane (Pashkow, Watumull, and Campbell *et al*. 2008) (Fig 2-11).

Ax is metabolized in fish through oxidative and reductive pathways. In salmonids and trout, Ax's metabolic process leading to idoxanthin then to adonixanthin and zeaxanthin. No cleavage of the polyene chain is observed. The conversion of zeaxanthin to Ax does not occur (Schiedt *et al.* 1985). In broiler Ax cannot esterify and also no oxidative degradation is observed. Instead, it is degraded reductively to idoxanthin and crustaxanthin, which are yellowish carotenoids (Schiedt *et al.* 1985).

2.4.4 Usage and Benefits

Astaxanthin in aquaculture

Salmonid and crustacean coloring is perceived as a key quality attributes by consumers. The reddish-orange color characteristic of such organisms originates in the carotenoids obtained from their feeds which are deposited in their skin, muscle, exoskeleton, and

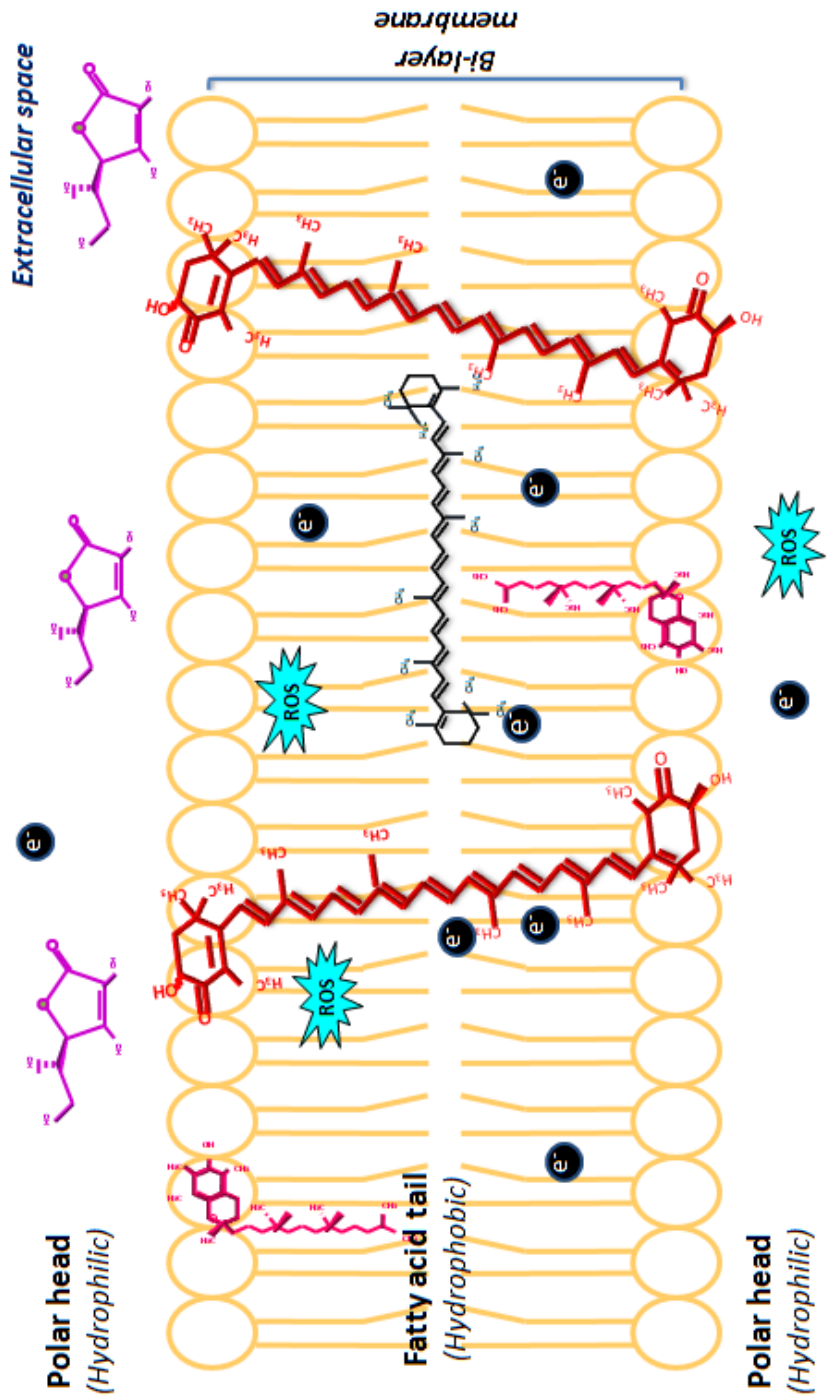


Fig 2-11 Astaxanthin location in cell membrane

gonads. The predominant carotenoid in most crustacea and salmonids, is Ax. In aquatic environment, the microalgae biosynthesize Ax which are consumed by zooplankton, insects, and crustacean, and later it is ingested by fish, thereby getting the natural coloration. Farmed fish do not have access to natural sources of Ax hence the total Ax intake must be derived from their feed. From 1984, the synthetic source of Ax became available in the market and was started to use in feed of fish.

Beside the pigmentation effect in fish, Ax is beneficial antioxidant for fish and shellfish. Reported functions of Ax in fish range from a general enhancement of performance to specific functions in reproduction and metabolism including improvement of reproduction and brood quality, reduced embryonic mortality, effects on photoresponse, behavior and respiration, improvement of health and immunstatus and functions as antioxidants (Torrissen 1995).

Astaxanthin in human

The in vivo metabolic study of Ax in primary human hepatocytes allowed the identification of 3-hydroxy-4-oxo-beta-ionol and 3-hydroxy-4-oxo-beta-ionone as the main free metabolites (Kistler *et al.* 2002). Ax is a common component in the food chain, being found in various fish and crustaceans that are consumed as foods, and in particular salmon which contains from 2 – 37 mg/kg of Ax. Several companies have also been marketing the use of microalgae *Haematococcus pluvialis* as a dietary supplement for use in human. Due to their high antioxidant properties these supplements have been attributed with potential properties against many diseases.

Anticancer activity: Even several research groups have studied the effect of Ax supplementation on various cancer types showing that oral administration Ax inhibits carcinogenesis in mice urinary bladder (Tanaka *et al.* 1994), in the oral cavity (Tanaka *et al.* 1995a) and rat colon (Tanaka *et al*. 1995b), but the effects of Ax and other carotenoids on proliferation of human breast cancerous cells study shows that Ax is less effective than other carotenoids in inhibiting the proliferation of MCF-7 cell line *in vitro*.

Booster and modulator of the immunological system: Jyonouchi *et al.* 1996 has performed the large majority of investigations regarding the potential activity of Ax as a booster and modulator of the immunological system. Ax increases the production of T-helper cell antibody and increases the number of antibody secretory cells from primed spleen cells (Jyonouchi *et al.* 1996).

Astaxanthin in chicken

Study about Ax related to chicken is commonly focused on meat and skin color improvement. Except pigmentation effect, Takahashi *et al.* 2011 revealed that an addition of Ax from *Phaffia* yeast to corn–enriched diet did not show anti-inflammatory effects in broiler. Furthermore, Ax influenced splenocyte proliferation and drug metabolizing activity but had no effect on lipid peroxidation in liver, spleen, heart, and plasma of broiler (Takimoto *et al.* 2007).

CHAPTER III. EFFECT OF LOW CONCENTRATION DIETARY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA*, ON MEAT QUALITY, SENSORY ATTRIBUTES, AND GROWTH PERFORMANCE OF THE BROILER CHICKEN DURING LONG PERIOD FEEDING

3.1 ABSTRACT

We evaluated effects of dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, on broiler chicken meat quality. Fourteen-day-old female Ross broilers were divided into three groups: control group: Ax-free diet, Ax 10 group: 10 mg/kg Ax diet, and Ax 20 group: 20 mg/kg Ax diet for 28 days. At 42 days old, chickens were slaughtered, and then growth performance, meat quality, and sensory attributes were analyzed. Compared with the control, a* values increased significantly after slaughter and 48 h postmortem for Ax 20 meat ($P < 0.05$) and for b* values in Ax 20 and Ax 10 groups ($P < 0.05$). Cooking loss decreased in the Ax 20 group ($P < 0.05$). After 120 h aging, contents of several free amino acids and total free amino acid content of Ax 20 group were significantly higher than the control ($P < 0.05$). In sensory evaluation, meat texture attributes improved significantly in the Ax 20 group ($P < 0.01$). No significant changes occurred in flavor attribute scores of meat soup from the Ax 20 group compared with the control even though most assessors preferred meat soup from the Ax 20 group. Overall, Ax-rich yeast in the diet improves broiler chicken meat quality.

3.2 INTRODUCTION

Dietary supplementation merits investigation as a potential method for improving meat quality. There are numerous researches about improvement of poultry meat quality by dietary natural antioxidants, vitamin E, vitamin C, carotenoid but in relation with Ax is very few. Most of them only focused on meat pigmentation. The study about effect of Ax for poultry used mainly Ax-rich yeast *Phaffia rhodozyma* or Ax-rich algae *Haematococcus pluvialis* because of their economical benefits. Studies investigated that dietary Ax-rich yeast can accumulate Ax in muscle of broiler chicken and it is correlated with dietary Ax concentration. Example: Akiba *et al.* 2001 revealed that 15 mg/kg Ax containing diet for 21 days accumulated 0.17 μg/g in breast meat, Takahashi *et al*. 2004 reported that 50 mg/kg Ax containing diet for 14 days accumulated 0.2 μg/g in breast muscle and An *et al*. 2004 resulted that 22.5 mg/kg Ax containing diet for 28 days accumulated 860 ng/ml in blood and 1490 ng/g in skin of broiler chicken, and also they found that meat redness is relatively dependent on dietary Ax concentration (Therefore we chose Ax concentration as 10 and 20 mg/kg at experimental design). From these results, it is clear that however other natural antioxidants are used in high amount (vitamin E over 50 mg/kg, vitamin C over 100 mg/kg, and β-carotene also over 100 mg/kg for adequate concentration of usage in feed) in feeding of poultry for meat quality improvement, but dietary Ax has an ability to influence on meat color even in low concentration. Unfortunately, no studies have investigated effect of Ax on other meat quality such as meat tenderness, water holding abilities, pH, and sensory attributes. Therefore, the object of this study was to evaluate the effect of dietary

supplementation with low concentration of Ax-rich yeast, *Phaffia rhodozyma*, on the meat quality of broiler chickens.

3.3 MATERIALS AND METHODS

3.3.1 Birds and housing

One-day-old female Ross strain broilers were purchased from a commercial hatchery (Ohnuma Co. Ltd., Niigata, Japan). From 1 to 14 days, the broilers were housed and kept warm in a brooder and fed a commercial starter diet based on corn and soybean meal (crude protein (CP), 22%; metabolizable energy (ME), 3.10 kcal/g). At 14 days of age, they were separated into individual cages and started feeding on the experimental diets. Feed and water were provided *ad libitum*. The ambient temperature was gradually decreased from 36 to 24°C over the period from 1 to 14 days during which lighting was given for 15 h from 04:00 to 19:00 each day. All procedures were performed using the 'Management manual' for Ross broiler chickens and all experimental protocols were approved by the Niigata University Animal Care Committee.

3.3.2 Diets and experimental design

Fourteen-day-old broilers were allocated to three groups: control group: broilers fed an Ax-free diet, Ax 10 group: broilers fed a diet containing 10 mg/kg Ax; and Ax 20 group: broilers fed a diet containing 20 mg/kg Ax. Twelve birds in each group. The formulation

and nutritional values of the experimental diets are shown in Table 3-1. All nutrition levels fulfilled the requirements of the National Research Council (1994).

3.3.3 Measurements and analysis

3.3.3.1 Sample collection and growth performance

At the end of the experimental period, the 42-day-old broilers were slaughtered by cutting the neck carotid arteries. After slaughter, the breast muscles (*M. Pectoralis superficialis*), liver, and abdominal fat were dissected immediately from the carcass then weighed. The muscle samples were immediately frozen in liquid nitrogen and stored at −80°C for free amino acid analysis. For each experimental group, the initial and final body weights and feed amount were measured. Then, the body weight gain per day, daily feed intake, and feed efficiency were calculated individually. The breast muscle, abdominal fat, and liver yields were expressed as percentages of the overall body weight.

3.3.3.2 Meat quality

pH and meat color: The sample pH was measured using an electronic pH meter TPX-90i (Toko Chemical Laboratories Co. Ltd., Tokyo, Japan) with a needle-type electrode (CE201S-SR; Toko Chemical Laboratories Co. Ltd.) after slaughter (initial pH, pH_i) and 48 h postmortem at 4°C (ultimate pH, pH_u). Each sample was measured at four points and their average value was taken as the final result. Then, the CIE parameters, the L^* (lightness), a^* (redness) and b^* (yellowness) values from the breast muscle sample surface on the dorsal side were determined initially and at 48 h postmortem using a Colorimeter

Table 3-1 Composition and nutritional content of the experimental diets

Ingredients (%)	Control		Ax 10[1]		Ax 20[2]	
	14–21d	21–42d	14–21d	21–42d	14–21d	21–42d
Soybean meal	50.68	44.13	50.68	44.13	50.68	44.13
Corn starch	37.82	46.76	37.82	46.76	37.82	46.76
Soybean oil	7.24	5.46	7.24	5.46	7.24	5.46
$CaCO_3$	1.28	1.34	1.28	1.34	1.28	1.34
$CaHPO_4$	1.55	1.10	1.55	1.10	1.55	1.10
NaCl	0.47	0.35	0.47	0.35	0.47	0.35
Vitamin and mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Aquasta[4]	0.00	0.00	0.10	0.10	0.20	0.20
Agar	0.21	0.21	0.11	0.11	0.01	0.01
L-Methionine	0.26	0.155	0.26	0.155	0.26	0.155
Calculated analysis						
CP (%)	23.00	20.00	23.00	20.00	23.00	20.00
ME (kcal/g)	3.20	3.20	3.20	3.20	3.20	3.20

[1] Ax 10: broilers fed 10 mg/kg astaxanthin in diet.

[2] Ax 20: broilers fed 20 mg/kg astaxanthin in diet.

[3] Vitamin and mineral premix provided the following per kg of diet: vitamin A 300000 IU, vitamin D 40000 IU, vitamin E 2 g, vitamin K_3*$3H_2O$ 0.37 g, vitamin B_1 0.36 g, vitamin B_2 0.72 g, vitamin B_6 0.7 g, vitamin B_{12} 2 mg, pantothenic acid 2.17 g, nicotinic acid 6.94 g, biotin 0.03 g, folic acid 110 mg, choline 299.81 g, $MnSO_4$ 32.98 g, $FeSO_4$ 43.52 g, $CuSO_4$ 4.02 g, $ZnSO_4$ 19.75 g, $Ca(IO_3)_2$ 0.11 g, and MgO 198.95 g.

[4]Aquasta®: Red yeast, *Phaffia rhodozyma*, providing astaxanthin at 10000 mg/kg (Aska Pharmaceutical Co. Ltd, Tokyo, Japan).

CR-400 (Konica Minolta Sensing Inc., Osaka, Japan).

*Water holding capacity (WHC) and cooking loss***:** Measurement of WHC was performed using two different traditional methods; the Honikel gravimetric method (Honikel 1998) illustrated as drip loss and the centrifugation method (Bertram *et al.* 2001) illustrated as water binding capacity (WBC). Samples were placed (the muscle fiber direction was horizontal to gravity) in plastic bags filled with air and left at 4°C, then the final breast muscle weight was determined after 48 h storage. The percentage of drip loss was calculated as: (initial breast muscle weight – final breast meat weight)/initial breast muscle weight × 100%. The WBC was determined by the centrifugation method (Bertram *et al.* 2001) with a slight change. After slaughter, breast meat samples were cut into 1 cm cubes weighing approximately 1.00 ± 0.5 g. The samples were weighed and placed in centrifuge tubes with glass beads in the bottom to separate the meat from the expelled liquid. Then the samples were centrifuged at 1000 × g for 15 min at a temperature of 4°C. After centrifugation, the samples were reweighed and WBC was calculated as: 100 − (initial sample weight – final sample weight)/initial sample weight × 100%.

Cooking loss was measured according to the method of Honikel (1998) with a slight change. After determining the drip loss, the breast meat was weighed and put into another plastic bag and cooked for 60 min at 70°C in a water bath. Following cooking, the sample was cooled in running water for 30 min until reaching room temperature. Then sample was reweighed and the percentage of cooking loss calculated as: (initial breast meat weight− final breast meat weight)/initial breast meat weight × 100%.

Shear force value (SFV): The samples used for measuring cooking loss were also used to measure SFV. This was performed according to the procedure described by Sasaki *et al.* (2010) with a slight change. The samples were cut parallel to the fiber orientation into pieces measuring 1 × 1 × 4 cm from the thickest portion of the cooked meat and the SFV (kg) was determined using a Rheometer (Fudoh-Rheo Meter RT-2005J; Rheotech Ltd., Tokyo, Japan) fitted with a 5 kg compression load cell with a crosshead speed of 30 cm/min. The peak force values, measuring the shearing of the centers of the cores perpendicular to the fibers, were used to determine the instrumental SFV of the samples. The overall value was obtained from 12 measurements of each sample.

Free amino acid content: Extracts from muscle samples immediately after slaughter and from samples stored for 48 and 120 h at 4°C were prepared for free amino acid analysis. The free amino acid extraction and determination procedure were performed according to the method described by Imanari *et al.* (2008). The frozen samples were weighed, approximately 4.00 ± 0.01 g, and after adding 20 mL 10% perchloric acid mixture were homogenized for 2 min with a high-speed homogenizer (Ultra-Turrax T25 Basic, Ikawerke, Staufen, Germany). The homogenate was centrifuged and the supernatant adjusted to pH = 6.5 ± 0.05 using 10 N potassium hydroxide. After removing potassium crystals by filtration, the filtrate volume was adjusted to 50 mL using DDW (deionized distilled water). Extracts were kept at −20 °C until analysis. Before analysis, the samples were filtered through a polyvinylidene fluoride membrane filter (SJHV004 NS; Millipore, Billerica, MA, USA). The free amino acid contents in the extracts were measured using an amino acid analyzer (JLC-500/V; JEOL, Tokyo, Japan).

3.3.3.3 Sensory attributes

As well as instrumental analysis, human sensory evaluation is very important for determining meat quality. We performed two different sensory evaluations to compare the control group samples with the Ax 20 group samples, that were evaluated the texture attributes of the breast meat and the flavor characteristics of breast meat soup. The sensory assessment of breast meat was carried out by 14 panelists and of meat soup by 16 panelists consisting of trained students (aged 20–29 years) from Niigata University. The paired comparison test was used to evaluate the results from the assessors (Stone & Sidel 2004). The samples were prepared according to the methodology which is reported in "Sensory evaluation method and assessment of broiler and native chicken meat" (Japan Poultry Breeders and Hatcheries Association. 2008). 7-grade scale (-3 to +3) was used to estimate residual particles in the mouth, tenderness, first bite, fibrousness, juiciness, and meat flavor characteristics: aroma (odor), *umami* taste (pleasant savory taste), sourness, sweetness, bitterness, after taste (taste intensity of sample that is perceived immediately after sample is removed from the mouth), chicken-like taste, *koku* taste (rich taste), and taste intensity (intensity of taste that while perceiving taste of sample). Sensory evaluation were conducted in testing room which has shadow-free illumination at 750-850 lx and air conditioned at 22-24℃ and 45-55% relative humidity. Assessors were separated a simple booths consisting of dividers placed on tables. Evaluating samples serving order, coding, size and number of samples to each assessors is performed with same methods which described in "Sensory evaluation method and assessment of broiler and native chicken meat".

3.3.4 Statistical analysis

Means and standard errors were calculated for chicken samples in each group. For statistical analysis, a one-way analysis of variance was used with the GLM procedure in SAS 8 (SAS Institute 1999). Except for the sensory evaluation data, significant differences between means were determined by the Tukey HSD test at a significance level of $P < 0.05$ or $P < 0.01$. For the sensory evaluation data, differences between means were analyzed by Scheffe's test at a significance level of $P < 0.05$ or $P < 0.01$.

3.4 RESULTS

3.4.1 Growth performance

Table 3-2 shows the growth performance of the broiler chickens. Diets neither with higher nor lower concentrations of Ax-rich yeast, influenced body weight gain, feed intake, feed efficiency, and organ yields ($P > 0.05$).

3.4.2 Meat quality

Meat texture: The results of the instrumental meat water holding ability measurements are shown in Table 3-4. The Ax-rich yeast significantly decreased cooking loss: those from the Ax 20 group were lower than those from the control group ($P < 0.05$). However, the meat drip loss and WBC were not influenced by Ax-rich yeast ($P > 0.05$). SFV of Ax 10 and Ax

20 groups were 17% and 23.7% lower than the control group, respectively, not significantly (Table 3-3).

Meat pH and color: The pH values from just after slaughter and 48 h postmortem meat are illustrated in Table 3-5. Significant differences were observed in neither pH_i nor pH_u values between all groups ($P > 0.05$). The color changes in the broiler breast meat are shown in Table 3-6. There were no significant differences in L* values between the three groups. However, compared with the control group, the a* values were significantly higher in meat samples from the Ax 20 group after slaughter and 48 h post mortem samples ($P < 0.05$), but not from the Ax 10 group ($P > 0.05$). For after slaughter samples, significant increases in b* values ($P < 0.05$) were seen in samples from the Ax 20 and Ax 10 groups compared with the control group. In addition, the b* values of 48 h postmortem samples from the Ax 20 group were higher ($P < 0.05$) than the control group, but not from the Ax 10 group.

Free amino acid content: The free amino acid analysis results are shown in Table 3-7. Analysis revealed that after 120 h of aging, the content of several free amino acids and the total free amino acid content of meat samples from the Ax 20 group were significantly higher than the control ($P < 0.05$). However, there was a tendency for free amino acid contents to increase in samples from the Ax 20 and Ax 10 groups at 48 h postmortem compared with the control group but not significantly. In addition, the increase in total free amino acid content in breast meat from just after slaughter to 120 h postmortem were 31.09%, 75.5%, and 62.5% for the control, Ax 10, and Ax 20 groups, respectively.

Table 3-2 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma,* on growth performance of broiler chickens

Growth performance	Control	Ax 10	Ax 20
Body weight gain, g/day	61.39 ± 2.07	63.92 ± 2.08	65.33 ± 1.64
Feed intake, g/day	168.96 ± 6.70	178.09 ± 4.58	173.96 ± 4.31
Feed efficiency	0.37 ± 0.02	0.36 ± 0.02	0.38 ± 0.01
Body organs (% of whole body weight)			
Breast muscle	16.87 ± 0.57	16.30 ± 0.21	16.99 ± 0.44
Liver	1.97 ± 0.11	2.09 ± 0.12	2.17 ± 0.20
Abdominal fat	0.85 ± 0.14	1.00 ± 0.11	0.89 ± 0.15

Values are mean ± SE.

n = 12 birds.

Table 3-3 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on the breast meat SFV of broiler chickens

Parameters	Control	Ax 10	Ax 20
Shear force value$_{48}$, kg	2.34 ± 0.16	1.94 ± 0.22	1.79 ± 0.23

Values are mean ± SE.

$_{48}$ 48 h postmortem meat sample.

n = 12 birds.

Table 3-4 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on the breast meat water holding ability of broiler chickens

Parameters	Control	Ax 10	Ax 20
Drip loss$_{48}$, %	0.61 ± 0.10	0.54 ± 0.06	0.63 ± 0.07
Cooking loss$_{48}$, %	11.63 ± 0.24^{a}	10.87 ± 0.34^{ab}	10.63 ± 0.34^{b}
Water binding capacity, %	77.69 ± 1.74	78.28 ± 1.31	77.02 ± 0.72

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$_{48}$ 48 h postmortem meat sample.

n = 12 birds.

Table 3-5 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat pH values of the broiler chickens, initially and at 48 h postmortem

Parameter	Aging, h	Control	Ax 10	Ax 20
pH	0	6.66 ± 0.16	6.59 ± 0.13	6.66 ± 0.10
	48	5.79 ± 0.06	5.93 ± 0.07	6.00 ± 0.05
	Decrease	0.87 ± 0.19	0.67 ± 0.13	0.58 ± 0.14

Values are mean ± SE.

n = 12 birds.

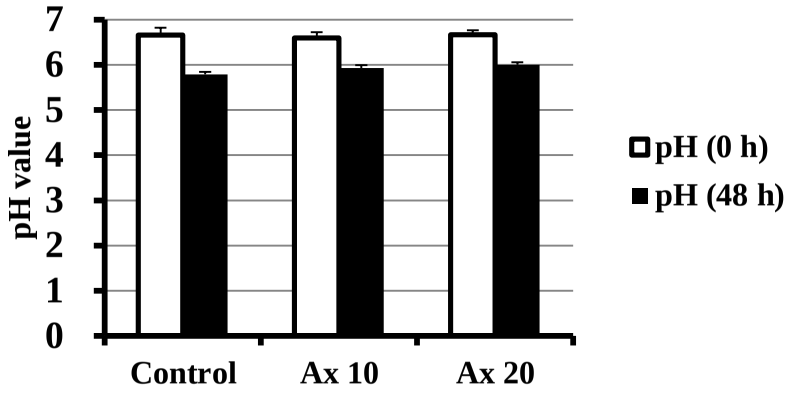


Fig 3-1 pH values of breast meat from just after slaughter and 48 h postmortem

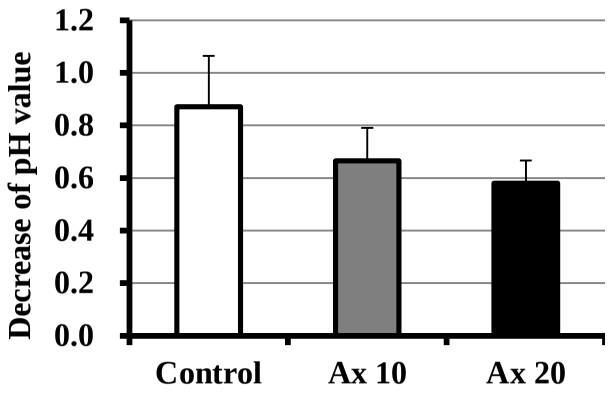


Fig 3-2 pH value decrease of breast meat during aging (from 0 h until 48 h)

Table 3-6 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat color of broiler chickens, initially and at 48 h post mortem

Parameters	Control	Ax 10	Ax 20
L* value	41.20 ± 0.66	40.43 ± 0.86	41.94 ± 1.02
L* value$_{48}$	52.14 ± 0.58	50.40 ± 0.90	50.99 ± 0.82
a* value	2.96 ± 0.19^{b}	3.12 ± 0.29^{b}	4.11 ± 0.33^{a}
a* value$_{48}$	2.65 ± 0.17^{b}	2.87 ± 0.25^{b}	4.00 ± 0.21^{a}
b* value	0.80 ± 0.25^{c}	2.74 ± 0.34^{b}	4.10 ± 0.41^{a}
b* value$_{48}$	2.42 ± 0.49^{b}	3.83 ± 0.47^{ab}	5.22 ± 0.38^{a}

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$_{48}$ 48-h postmortem meat sample.

Values are mean ± SE.

n = 12 birds.

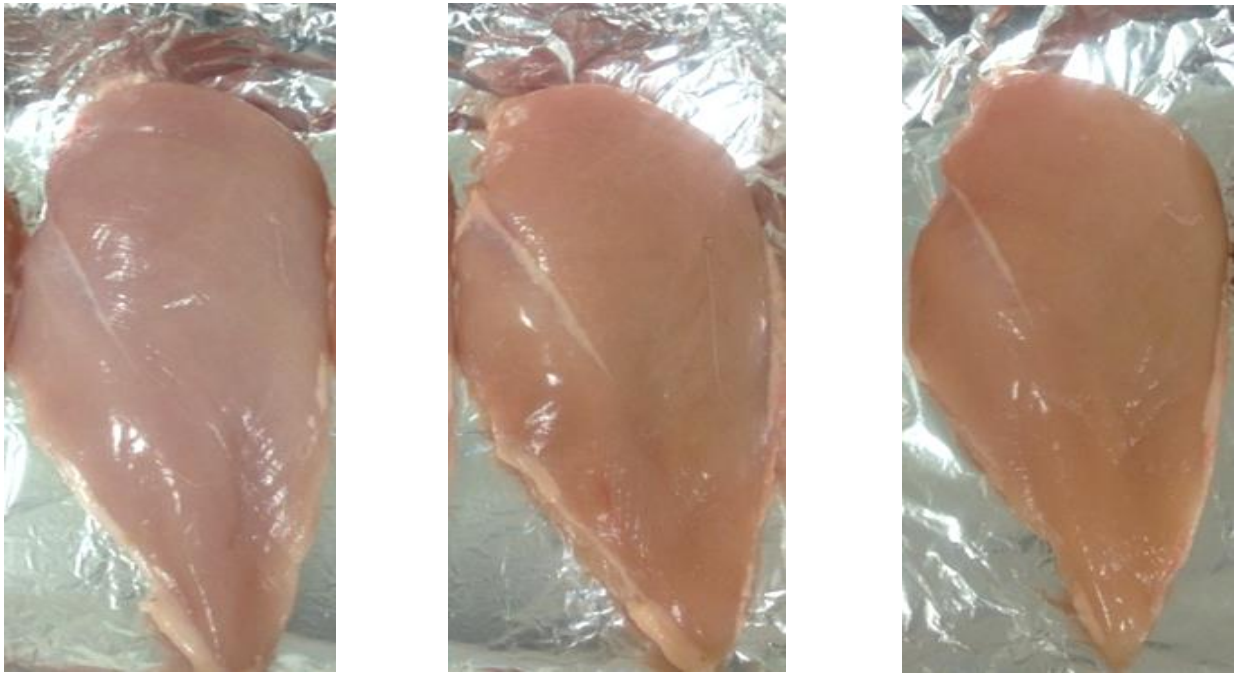


Fig 3-3 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast muscle color from just after slaughter samples.

From the left side Control group, Ax 10 group and Ax 20 group

There were no significant differences between the three groups in samples taken just after slaughter.

3.4.3 Sensory attributes

Meat soup: The results from the sensory evaluation of breast meat soup are shown in Table 3-8. A paired comparison test of breast meat soup for flavor assessment showed that there was a tendency for flavor attributes — aroma, *umami* taste, sweetness, sourness, *koku* taste, chicken-like taste, after taste, and taste intensity with the exception of bitterness — to improve, but using Scheffe's test, these improvements were not shown to be significant ($P > 0.05$).

Breast meat: Sensory evaluation of breast meat texture results are shown in Table 3-9. Furthermore, a paired comparison test of breast meat for texture assessment reveals that samples from the Ax 20 group had significantly improved sensory attributes for tenderness, residual particles in mouth, juiciness, first bite, and fibrousness ($P < 0.01$) compared with the control group and additionally, in preference test 12 assessors from 16 panelists chose meat soup from the Ax 20 group.

Table 3-7 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on the free amino acid content of broiler chicken breast meat

	Amino acid content, μg/g	Aging, h	Groups		
			Control	Ax 10	Ax 20
Sweet tasting amino acids	Gly	0	77.5 ± 3.0	76.8 ± 2.1	82.4 ± 12.3
		48	70.0 ± 8.5	72.1 ± 6.4	88.7 ± 14.9
		120	80.3 ± 6.6	98.6 ± 1.5^{ab}	117.4 ± 13.0^{a}
	Ala	0	136.7 ± 20.8	122.4 ± 15.1	145.8 ± 20.2
		48	159.1 ± 16.7	159.7 ± 17.6	197.6 ± 20.8
		120	191.8 ± 15.5^{b}	232.6 ± 9.7^{ab}	267.2 ± 26.9^{a}
	Thr	0	67.9 ± 6.1	64.4 ± 3.8	79.4 ± 12.8
		48	74.8 ± 1.2	69.5 ± 3.9	87.5 ± 13.2
		120	96.6 ± 3.1^{b}	102.9 ± 5.9^{ab}	136.9 ± 16.2^{a}
	Ser	0	115.3 ± 15.6	125.1 ± 4.1	138.1 ± 6.6
		48	125.8 ± 12.5	142.0 ± 2.2	142.8 ± 10.0
		120	166.9 ± 12.4^{b}	200.1 ± 6.5^{ab}	221.2 ± 17.8^{a}
	Lys	0	56.9 ± 7.2	48.1 ± 7.6	46.8 ± 5.4
		48	66.3 ± 9.0	63.9 ± 7.9	68.3 ± 6.7
		120	99.5 ± 7.4	109.9 ± 9.5	131.9 ± 11.3
	Gln	0	269.2 ± 33.4	237.5 ± 23.7	298.9 ± 49.1
		48	254.5 ± 29.5	226.0 ± 22.1	291.8 ± 52.6
		120	271.5 ± 20.3^{ab}	252.5 ± 18.9^{b}	356.3 ± 42.9^{a}
Bitter tasting amino acids	Phe	0	20.7 ± 3.1	17.0 ± 1.5	21.4 ± 1.5
		48	27.7 ± 2.5	27.1 ± 1.2	32.9 ± 2.8
		120	46.4 ± 1.9^{b}	52.1 ± 2.8^{ab}	60.1 ± 3.1^{a}
	Arg	0	55.0 ± 6.4	52.8 ± 8.2	42.8 ± 5.3
		48	74.6 ± 5.1	74.1 ± 10.7	75.0 ± 7.0
		120	120.7 ± 4.7	133.6 ± 14.2	155.5 ± 12.3
	Ile	0	13.7 ± 2.8	10.4 ± 0.8	14.1 ± 1.1
		48	20.8 ± 2.2	20.6 ± 1.5	24.5 ± 2.2
		120	35.4 ± 1.5^{b}	42.4 ± 2.1^{a}	47.8 ± 1.4^{a}
	Val	0	22.4 ± 3.5	17.6 ± 1.3	23.6 ± 1.8
		48	31.0 ± 3.6	27.8 ± 1.5	37.6 ± 3.9
		120	56.1 ± 4.4^{b}	59.8 ± 4.0^{b}	72.8 ± 1.9^{a}
	Leu	0	20.8 ± 5.6	14.2 ± 0.8	19.9 ± 1.6
		48	31.5 ± 8.8	35.7 ± 1.3	44.1 ± 4.2
		120	77.5 ± 5.6^{b}	89.1 ± 5.4^{ab}	96.3 ± 4.2^{a}
	Met	0	5.6 ± 3.0	2.3 ± 0.3	3.8 ± 1.1
		48	14.3 ± 1.9	16.2 ± 0.7	16.1 ± 2.8
		120	35.0 ± 1.7	41.3 ± 2.0	41.7 ± 2.7
	His	0	3.9 ± 1.4	4.9 ± 1.1	5.7 ± 1.5
		48	8.8 ± 1.8	12.4 ± 1.8	16.7 ± 3.6
		120	27.1 ± 3.4^{b}	35.5 ± 3.2^{ab}	41.1 ± 2.3^{a}
Sour	Asp	0	48.0 ± 9.9	57.0 ± 15.9	70.6 ± 15.7
		48	111.8 ± 23.6	102.5 ± 17.8	166.1 ± 24.0
		120	129.8 ± 21.1	148.5 ± 21.0	203.9 ± 28.1
Umami	Glu	0	148.5 ± 19.2	133.8 ± 14.0	185.4 ± 20.1
		48	78.6 ± 15.7	68.8 ± 14.0	75.3 ± 7.9
		120	125.0 ± 19.3^{ab}	108.2 ± 9.7^{b}	154.3 ± 10.9^{a}
Total free AA, mg/g		0	1.19 ± 0.05	1.06 ± 0.06	1.29 ± 0.1
		48	1.20 ± 0.1	1.21 ± 0.05	1.42 ± 0.1
		120	1.56 ± 0.05^{b}	1.86 ± 0.08^{ab}	2.09 ± 0.1^{a}
Increase in free AA, %		0-120	31.1	75.5	62.5

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

n = 6 birds.

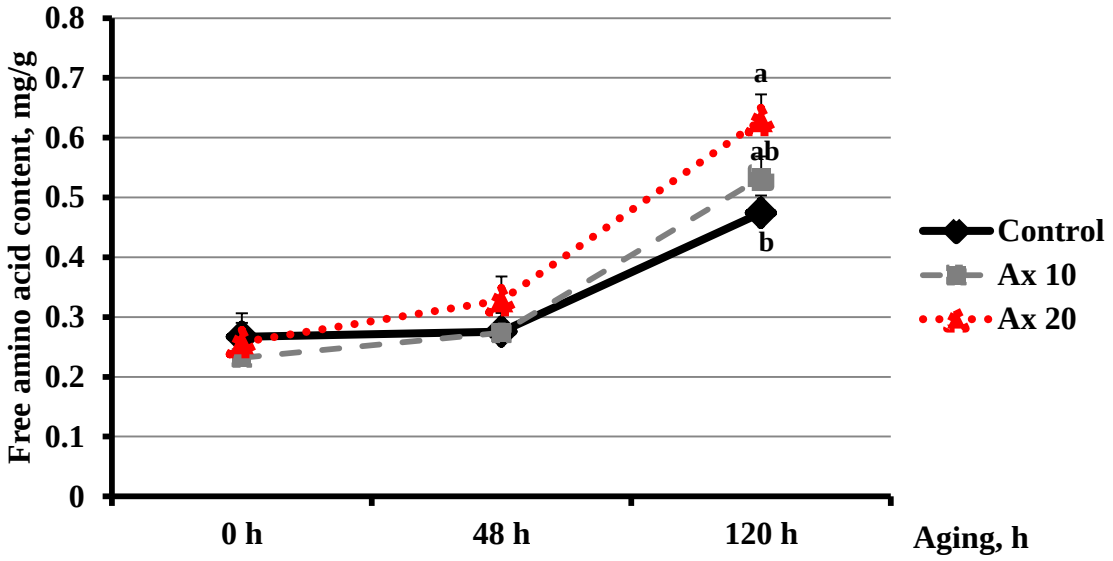


Fig 3-4 Total free essential amino acid content in breast meat during the aging

(a,b $P < 0.05$)

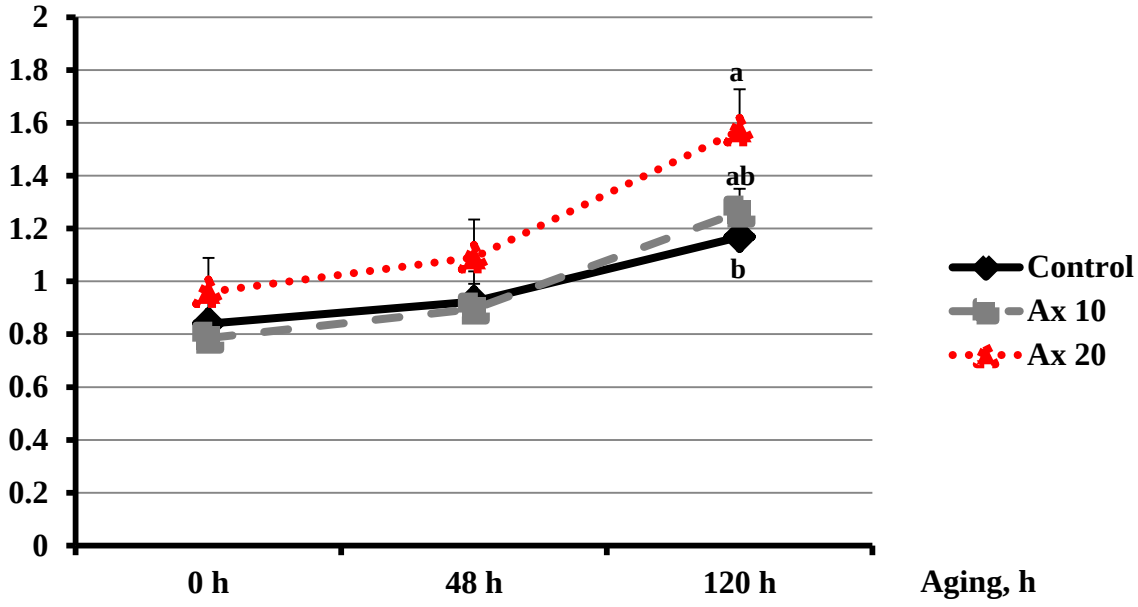


Fig 3-5 Total free non essential amino acid content in breast meat during the aging

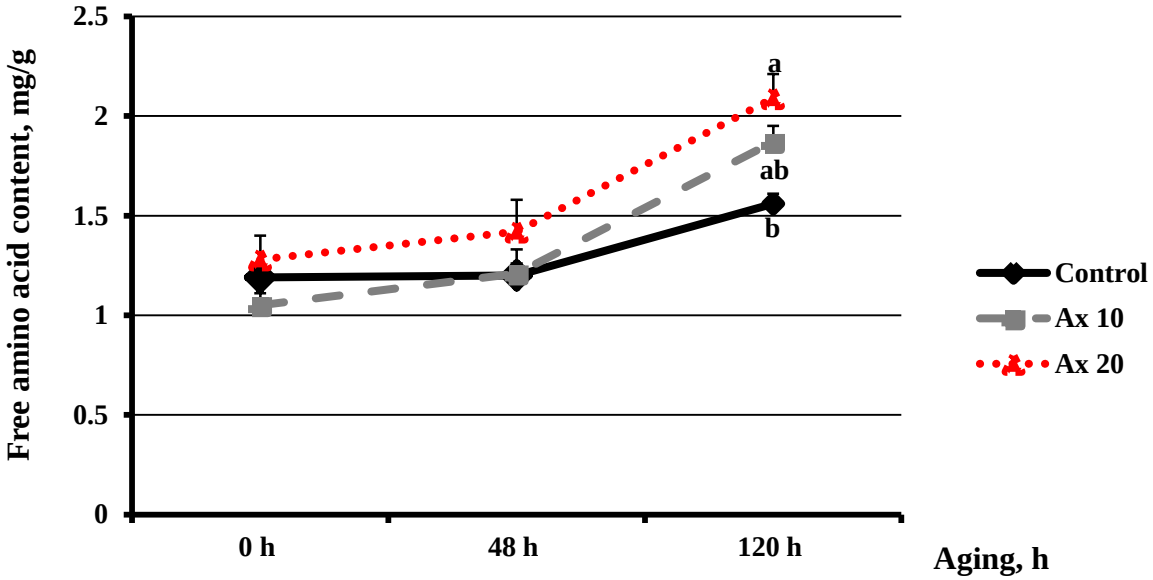


Fig 3-6 Total free amino acid content in breast meat during the aging

(a,b $P < 0.05$)

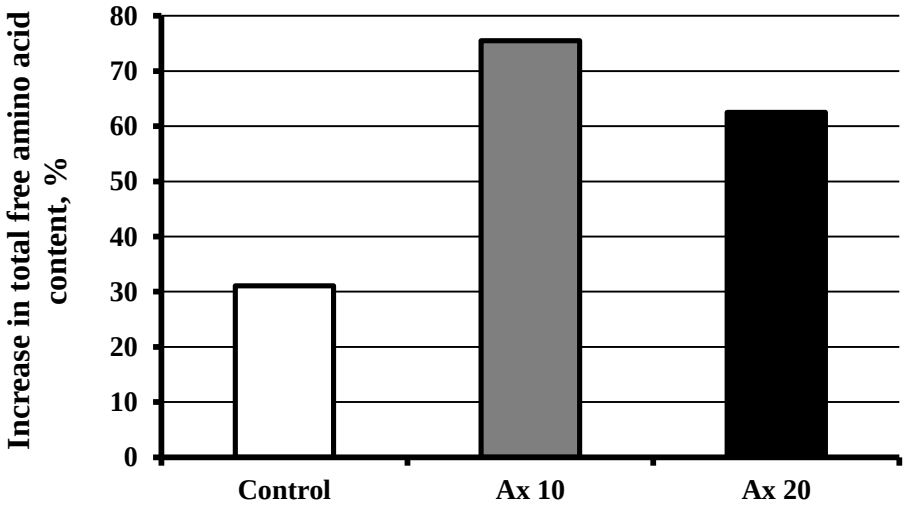


Fig 3-7 Increase in total free amino acid content in breast meat during aging

(from 0 h until 120 h)

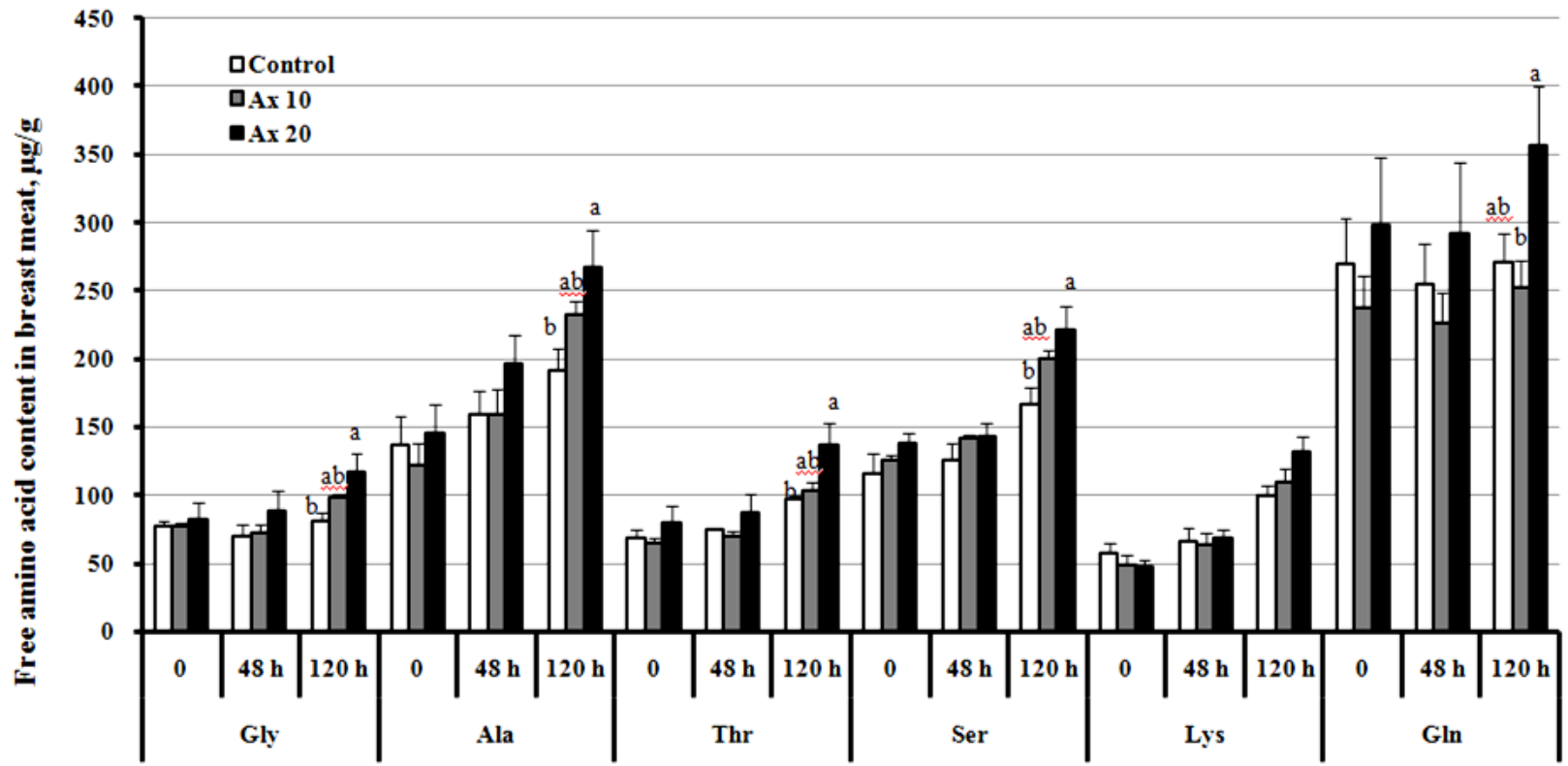


Fig 3-8 Sweet tasting free amino acid content in breast meat during aging (0 – just after slaughter sample, 48 h – 48 h postmortem samples, 120 h – 120 h postmortem samples)

[a, b] $P < 0.05$

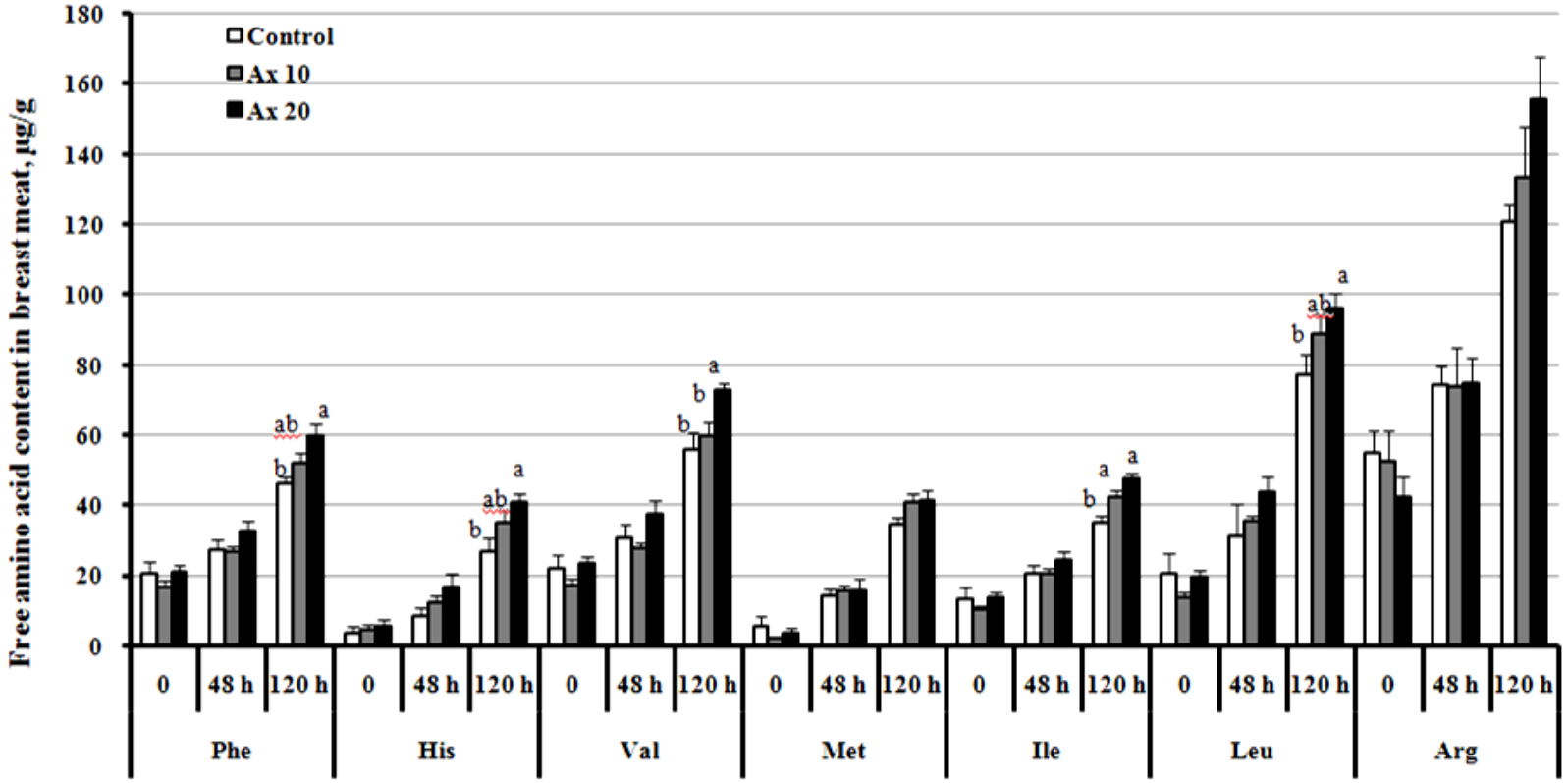


Fig 3-9 Bitter tasting free amino acid content in breast meat during aging (0 – just after slaughter sample, 48 h – 48 h postmortem samples, 120 h – 120 h postmortem samples)

[a, b] $P < 0.05$

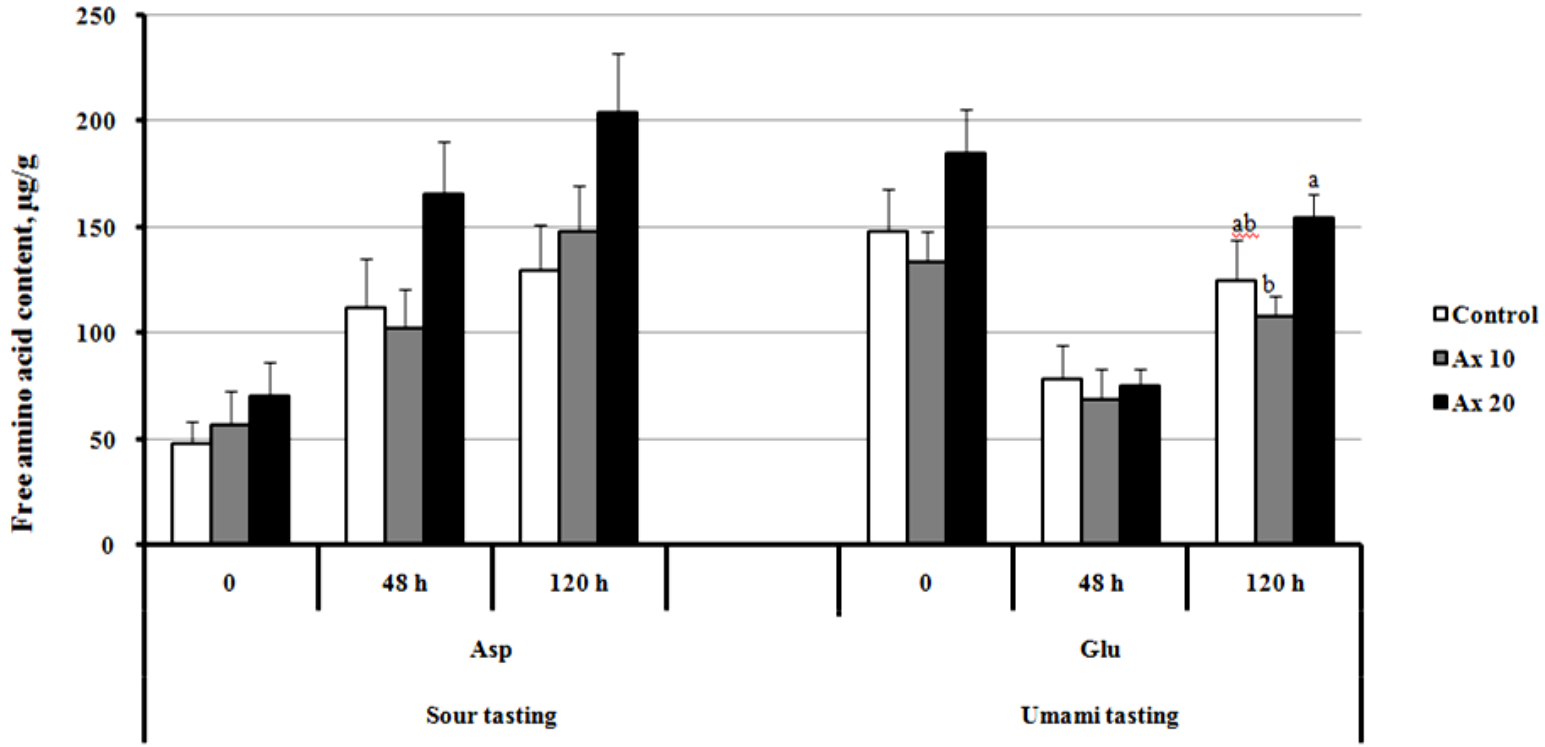


Fig 3-10 Sour and *umami* tasting free amino acid content in breast meat during aging (0 – just after slaughter sample, 48 h – 48 h postmortem samples, 120 h – 120 h postmortem samples)

[a, b] $P < 0.05$

3.5 DISCUSSION

3.5.1 Growth performance

There was a tendency for growth performance to increase but the analysis revealed no significant difference between the three groups (Table 3-2). This result agreed with reports from several studies (Takahashi *et al.* 2004; An *et al.* 2004; Akiba *et al.* 2001) that Ax-rich yeast did not influence broiler growth performance and chicken meat production. Furthermore, Inborr and Lignell (1997) revealed that algal meal, with a high concentration of Ax fed to broiler chickens, improved growth performance more rapidly, increased breast muscle weight, and used feed more efficiently. These previous studies and the present study imply that Ax may not be the main factor affecting growth performance: performance may also be influenced by the source of Ax.

3.5.2 Meat quality

Meat texture: An important parameter for measuring meat tenderness is SFV, which gradually decreased as Ax concentration increased. Although these changes were not statistically significant, the sensory evaluation results suggested that meat tenderness improved as the diet became richer in Ax-rich yeast. This comparison suggests that the human sensory assessment of meat texture may be more sensitive than instrumental analysis. In addition, the drip loss and water binding capacity of muscles from the Ax-rich yeast diet groups were not significantly different from the control group.

Table 3-8 Sensory evaluation of flavor attributes of broiler chicken breast meat soup

Parameters	Control: Ax 20[1]	F value	Significance
Aroma	0.07	0.12	NS
Umami taste	0.43	2.77	NS
Sour taste	0.5	3.87	NS
Bitter taste	-0.06	0.06	NS
Sweet taste	0.36	4.17	NS
Chicken-like taste	0.21	0.54	NS
Koku taste	0.36	1.47	NS
Taste intensity	0.14	0.26	NS
After taste	0.43	2.51	NS

[1] Average Ax 20 group score of each subject when the contrrol group score is 0.00.

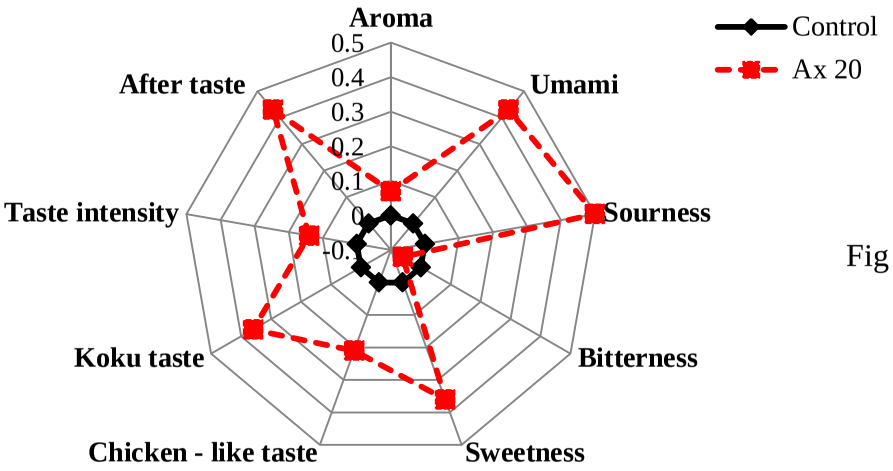


Fig 3-11 Paired comparison test of breast meat soup

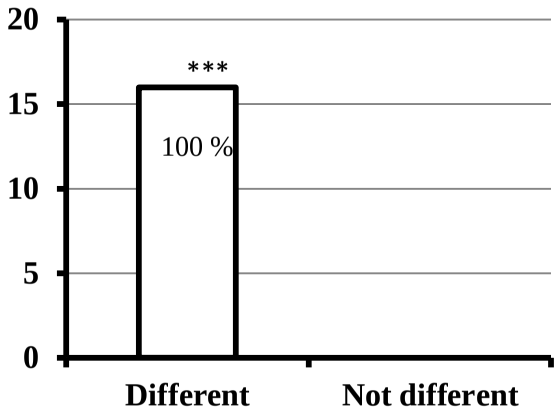


Fig 3-12 Paired difference test of breast meat soup from control and Ax 20 group

*** $P < 0.001$

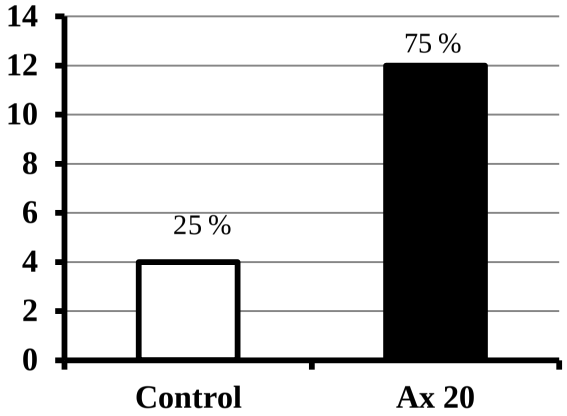


Fig 3-13 Paired preference test of breast meat soup from control and Ax 20 group

Table 3-9 Sensory evaluation of the texture of broiler chicken breast meat

Parameters	Control: Ax 20[1]	F value	Significance
First bite[2]	1.36	14.44	**
Tenderness[3]	1.14	15.06	**
Fibrousness[4]	1.14	17.45	**
Juiciness[5]	0.79	7.26	**
Residual particles[6]	1.0	9.33	**

[1] Average Ax 20 group score of each subject when the control group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that amount of loose particles left in the mouth after swallowing is less.

[**] $P < 0.01$ F value > 9.33

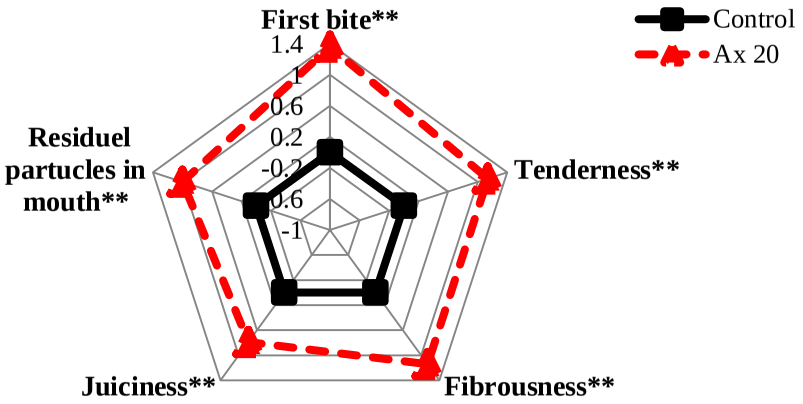


Fig 3-14 Paired comparison test of breast meat

** $P < 0.01$

*** $P < 0.001$

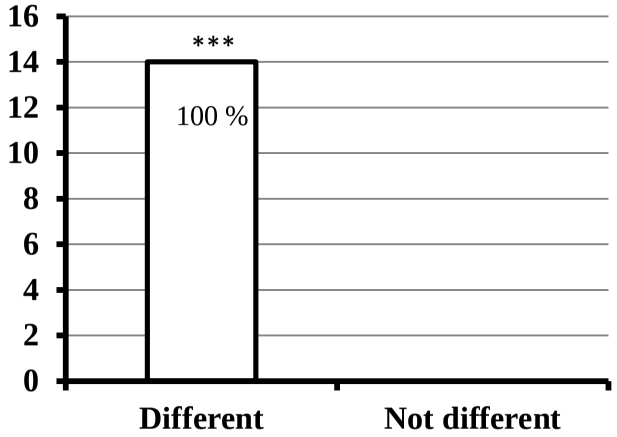


Fig 3-15 Paired difference test of breast meat from control and Ax 20 group

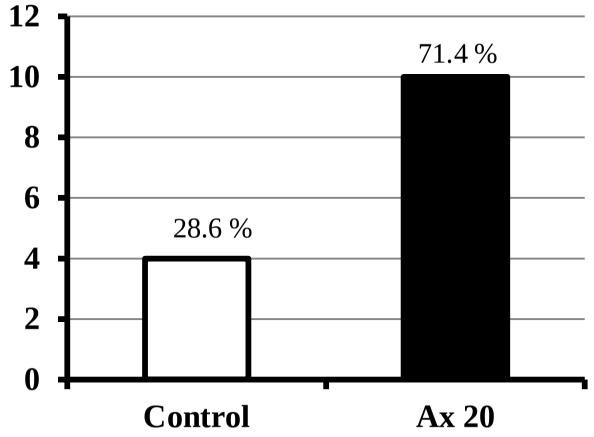


Fig 3-16 Paired preference test of breast meat from control and Ax 20 group

Until now there have been no data regarding the effect of Ax-rich yeast on poultry meat quality attributes such as meat tenderness, WBC, cooking loss, and drip loss, but Carr *et al*. (2010) and Yang *et al.* (2006) have reported that Ax supplementation had no effect on drip loss from samples of lean pork muscle. Furthermore, there was a significant decrease in cooking loss for samples from the Ax 20 group compared with the control group. This newly demonstrates that Ax-rich yeast can improve the ability of meat to retain water during cooking. High cooking losses lead to low juiciness in meat (Toscas *et al*. 1999); therefore this result suggests that Ax-rich yeast can increase meat juiciness. However, higher cooking losses were associated with high drip losses and low WBC, but Aaslyng *et al.* (2003) reported that WBC did not influence cooking losses in pork; it was more a question of an area or a threshold of influence than a linear relationship within the whole area of variation. Therefore, this recent study may not reveal a specific correlation between WBC and cooking loss. During the heat, denaturing protein amount affects meat cooking loss, higher protein denaturation results higher cooking loss (Aaslyng *et al.* 2003). In this study Ax group may have a low rate of protein denaturation during heat. Cooking loss is of interest because it influences the appearance of the meat and is also of great economic importance to the catering industry.

Meat pH and color: Ax-rich yeast has no effect on pH values from just after slaughter samples and 48 h post mortem storage samples. Postmortem rapid glycolysis leads to rapid decline in pH values because of effective formation of lactic acid. The rapid glycolysis can be result from premortem stress. A strong relationship between ultimate pH and lightness of breast meat in chicken is reported. There were no significant differences in L* values

between the three groups. Meat pH and lightness are the main indicators of defective meat conditions such as PSE (pale, soft, and exudative) and DFD (dark, firm, and dry) meat. The normal postmortem pH in broiler meat is around 5.2–6.2 but the pH of PSE broiler meat is less than 5.2 (Isabel Guerrero-Legarreta 2010). In our study, postmortem pH from all groups was 5.8–6.0. This means that there was no stress or adverse effects during the rigor mortis process in the Ax-rich yeast diet groups. In addition, a* values significantly increased in meat samples from Ax 20 for after slaughter and 48 h postmortem samples. Dietary Ax-rich yeast accumulates Ax in blood, muscle and other organs of broiler chickens (Akiba *et al.* 2001; An *et al.* 2004; Takahashi *et al*. 2004). Even we did not measured Ax content in muscle, but our results agreed with some studies that a* values in the edible meat of broiler chickens is increased by feed supplemented with *Phaffia* yeast, which contains a high concentration of Ax (Akiba *et al.* 2001; An *et al.* 2004). Furthermore, a* values of meat samples from the Ax 10 group were higher than from the control group but were not significantly different. From these, it is clear that a higher concentration of dietary *Phaffia* yeast is more effective at increasing meat redness and this results also affirmed with some studies that a* value of breast meat is intensified with the increase of dietary Ax (Akiba *et al.* 2001). Ax, which is contained in *Phaffia* yeast, is itself a red pigment. Therefore, a higher concentration in the diet means a greater accumulation in muscles of broilers. For this reason, meat redness is directly affected by Ax content in the diet. Additionally, the range of color for meat is influenced predominately by the content of myoglobin which is one of the major proteins in the sarcoplasm, and is the main pigment in meat (Coggins 2007). The discoloration of meat is due to the oxidation of myoglobin to

metmyoglobin during the storage. Therefore color stability is enhanced by the addition of some antioxidants to meat, vitamin E and ascorbate (Yin *et al.* 1993). Ax also has antioxidant property because of it may inhibit heme pigments oxidation in meat during storage. However, after slaughter, samples from the Ax-rich yeast diet groups had notable increases in b* value compared with samples from the control group, but after 48 h aging only meat samples from the Ax 20 group had significantly higher values than the control group. This result agreed with An *et al.* (2004) that teleomorph *Phaffia* yeast, *Xanthophyllomyces dendrorhous,* can dramatically affect the yellowness of broiler meat. Others have found no significant changes in b* value (Akiba *et al.* 2001). Interestingly, even though Ax is red pigment, a recent study results revealed that Ax-rich yeast can increase meat yellowness as well as its redness. In the body of a chicken, Ax cannot esterify and also no oxidative degradation is observed. Instead, it is degraded reductively to idoxanthin and crustaxanthin, which are yellowish carotenoids (Schiedt *et al.* 1985). These alterations may increase meat yellowness or other carotenoids found in *Phaffia* yeast, which may affect meat yellowness, but the exact reason is unclear. This is an interesting topic for further study.

Free amino acid content: Analysis revealed that after 120 h aging, the content of several free amino acids, and the total free amino acid contents of meat samples from the Ax 20 group were significantly higher than the control group even though there were no statistically significant differences between groups for the samples taken after slaughter and at 48 h postmortem. This is the first demonstration that Ax-rich yeast can increase the content of free amino acid during the aging of broiler chicken meat and this increase is

more effective using diets containing a higher concentration of Ax. Free amino acids, especially Glu, can greatly contribute to the taste of meat (Kato & Nishimura 1987; Fujimura *et al.* 1996). Therefore the current results suggest that *Phaffia* yeast can influence meat aging during storage to improve the taste of meat. Even today there is no study about effect of dietary carotenoids on free amino acid content alteration of broiler meat during post mortem storage, but the increase in free amino acids during the post-mortem storage of meat is caused by the action of amino-peptidases and proteases (Migita & Nishimura 2006). The enzymatic reactions in proteolytic system are affected by meat pH decline (Moya *et al.* 2000), but in this study no significant differences in pH values and in pH decline were observed. Thus it may be not affected from meat pH values. Furthermore we hypothesized that the reason for the increased free amino acid content, is that as Ax is a powerful antioxidant, it may inhibit enzymes (perform the main role in protein degradation and free amino acid formation) oxidation. In other words, free amino acid formation effectively occurs in the meat of the Ax-rich yeast groups during aging. Another hypothesis is that Ax can inhibit the oxidation of amino acids that are formed by protein degradation or by protein side-chain amino acids oxidizing into carbonyl compounds.

3.5.3 Sensory attributes

Meat soup: The sensory analysis of meat soup from the Ax 20 group indicated no significant difference in flavor attributes compared with those from the control group using Scheffe's test. However, there was a tendency to increase in scores for *umami* taste, sourness, sweetness, *koku* taste, and after taste and 75% of the assessors preferred the flavor

of meat soup from the Ax 20 group. As mentioned above, free amino acids, especially Glu, effectively improve meat *umami* taste. The 48 h postmortem meat samples, used in the sensory analysis, had free amino acid contents not significantly different between the two groups but were higher in the Ax group. Therefore, significant differences in the scores of meat flavor attributes may not have been detected using the paired comparison test.

Breast meat: Meat has many kinds of texture attribute and we chose the main five: tenderness, residual particles in the mouth, juiciness, first bite, and fibrousness for the sensory evaluation of breast meat. The results revealed that the Ax 20 group significantly increased these sensory attributes of meat texture compared with the control group. The main components of muscle considered to affect meat tenderness are myofibrillar protein, muscle cytoskeleton, and intramuscular connective tissue (Harris 1976; Silva et *al.* 1993) and also intra-fiber water (Currie & Wolfe 1980). When myofibrillar protein is oxidized, the oxidation process promotes the aggregation and cross-linkage of protein so meat becomes tough (Estévez 2011; Lonergan *et al.* 2010). In this reason, we suggested that Ax may prevent myofibrillar proteins from oxidizing.

The juiciness of meat depends on the quality of the raw meat and on the cooking procedure (Aaslyng *et al*. 2003). Furthermore, Dransfield *et al*. (1985) discovered a quadratic correlation between juiciness and pH_u with a minimum at pH_u= 6.1. According to our study, even though there were no significant differences between all groups, the ultimate pH of Ax-rich yeast groups was typically close to the value above. Breast meat is the driest part of edible chicken meat. After cooking, juiciness increased compared with the

control group, a very significant point for improving the eating quality of meat. Our results indicate that *Phaffia rhodozima* yeast plays an important role in improving meat texture. In a preference test, 71.4% of the sensory assessors preferred the meat from Ax-rich yeast fed chickens to the control group meat. From these results, we can assume that meat from chickens fed with Ax-rich yeast is highly acceptable to the consumer.

In conclusion, astaxanthin rich yeast, *Phaffia rhodozyma*, even in low concentration in diet, is an effective dietary supplement for improving meat quality in broiler chickens.

CHAPTER IV. EFFECT OF HIGH CONCENTRATION DIETARY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA*, ON MEAT QUALITY AND GROWTH PERFORMANCE OF THE BROILER CHICKEN DURING SHORT PERIOD FEEDING

4.1 ABSTRACT

We investigated in this study the effects of high concentration dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, on broiler chicken meat quality. Thirty-two-day-old female Ross broilers were divided into three groups: control group: Ax-free diet, Ax 50 group: 50 mg/kg Ax diet, and Ax 100 group: 100 mg/kg Ax diet for 10 days. At 42 days old, chickens were slaughtered, and then growth performance, meat quality and sensory attributes were analyzed. Compared with the control, Ax 100 group was significantly higher daily body weight gain, and feed intake ($P < 0.05$). Ax groups had significant high a* values and b* values comparing to the control either from just after slaughter or 48 h postmortem storage ($P < 0.05$). No significant differences were observed in results of meat texture analysis between all groups ($P > 0.05$). However, in sensory evaluation, meat texture attributes improved significantly in the Ax 50 group ($P < 0.01$), but not in Ax 100 group. Overall, high concentration and short period feeding of Ax-rich yeast improves effectively broiler performance but has tendency to improve meat quality.

4.2 INTRODUCTION

In previous study, we investigated that low concentration and long period feeding of Ax-rich yeast diet has very effective influence on meat quality of broiler chicken. For further investigation of Ax-rich yeast's optimum concentration in broiler diet for meat quality, this time we aimed to check high concentration Ax supplemented diet. When using dietary supplement in high concentration, the adverse effects must be considered carefully. Up to date, many researchers have been studying about toxicity and safety of Ax in animal and human. It has demonstrated safety in numerous human clinical trials and has investigated that Ax-rich yeasts has no mutagenic, toxic and clastogenic property. Ax is approved at European Union level as feed additive for salmon and trout at 100 mg/kg complete feed from six months of age onwards without time limit (European Commission of Health and Consumer Protection Directorate-General). For the adequate dose study, some researchers reported that the response to increased dietary dose is linear for low dietary doses, levels off for higher and finally reaches a plateau (from 100 mg/kg Ax diet) where no further increased flesh pigmentation is obtained by increases in dietary Ax level in Atlantic salmon (Torrissen *et al.* 1995; Olsen and Mortensen 1997). In addition, Takahashi *et al.* (2004) fed broiler chickens 50 mg/kg and 100 mg/kg Ax containing diet for 14 days and revealed that Ax content in breast muscle and liver of broiler chicken increased with dietary Ax concentration. Thus in this study we chose 50 mg/kg and 100 mg/kg Ax containing diet. Furthermore, Ax is powerful antioxidant, thus it has possibility to loss its valuable property during the storage even it is said that shelf-life of yeast is long. Furthermore it is an expensive supplement. Therefore it is beneficial to use in low amount as low as possible.

Therefore, our purpose of this study is to evaluate effect of high concentration Ax-rich yeast containing diet on meat quality and growth performance of the broiler chickens on short period of feeding.

4.3 MATERIALS AND METHODS

4.3.1 Birds and housing

One-day-old female Ross strain broilers were purchased from a commercial hatchery (Ohnuma Co. Ltd., Niigata, Japan). From 1 to 32 days, the broilers were housed and kept warm in a brooder and fed a commercial starter diet based on corn and soybean meal (crude protein (CP), 22%; metabolizable energy (ME), 3.10 kcal/g). At 32 days of age, they were separated into individual cages and started feeding on the experimental diets. Feed and water were provided *ad libitum*. The ambient temperature was gradually decreased from 36 to 24°C over the period from 1 to 14 days during which lighting was given for 15 h from 04:00 to 19:00 each day. All procedures were performed using the 'Management manual' for Ross broiler chickens and all experimental protocols were approved by the Niigata University Animal Care Committee.

4.3.2 Diets and experimental design

Table 4-1 Composition and nutrient content of experimental diets (32 - 42 day of age)

Ingredients (%)	Control	Ax 50[1]	Ax 100[2]
Soybean meal	44.14	44.14	44.14
Corn starch	45.40	45.40	45.40
Soybean oil	6.00	6.00	6.00
$CaCO_3$	1.34	1.34	1.34
$CaHPO_4$	1.10	1.10	1.10
NaCl	0.35	0.35	0.35
Vitamin and mineral premix[3]	0.50	0.50	0.50
Aquasta[4]	0.00	0.50	1.00
Agar	1.01	0.51	0.01
L-Methionine	0.16	0.16	0.16
Calculated analysis			
CP, %	20.00	20.08	20.17
ME, kcal/kg	3.2	3.2	3.2

[1] Ax 50: broilers fed 50 mg/kg astaxanthin in diet.

[2] Ax 100: broilers fed 100 mg/kg astaxanthin in diet.

[3] Vitamin and mineral premix provided the following per kg of diet: vitamin A 300000 IU, vitamin D 40000 IU, vitamin E 2 g, vitamin K_3*$3H_2O$ 0.37 g, vitamin B_1 0.36 g, vitamin B_2 0.72 g, vitamin B_6 0.7 g, vitamin B_{12} 2 mg, pantothenic acid 2.17 g, nicotinic acid 6.94 g, biotin 0.03 g, folic acid 110 mg, choline 299.81 g, $MnSO_4$ 32.98 g, $FeSO_4$ 43.52 g, $CuSO_4$ 4.02 g, $ZnSO_4$ 19.75 g, $Ca(IO_3)_2$ 0.11 g, and MgO 198.95 g.

[4]Aquasta®: Red yeast, *Phaffia Rhodozyma*, providing astaxanthin at 10000 mg/kg (Aska Pharmaceutical Co. Ltd, Tokyo, Japan).

Thirty-two-day-old broilers were allocated to three groups: control group: broilers fed an Ax-free diet, Ax 50 group: broilers fed a diet containing 50 mg/kg Ax; and Ax 100 group: broilers fed a diet containing 100 mg/kg Ax. The formulation and nutritional values of the experimental diets are shown in Table 4-1. All nutrition levels fulfilled the requirements of the National Research Council (1994).

4.3.3 Measurements and analysis

Sampling, calculation of growth performance and meat quality analysis were performed the same methods which are described in Chapter 3.

4.4 RESULTS

4.4.1 Growth performance

Table 4-2 shows the growth performance of the broiler chickens. However, there were no significant differences on feed efficiency and organ yields between all groups ($P > 0.05$), but Ax 100 group was significantly high daily body weight gain and feed intake comparing to control ($P < 0.05$) but Ax 50 group not significantly ($P > 0.05$).

4.4.2 Meat quality

Meat texture: The results of the meat SFV measurements are shown in Table 4-3 and water holding ability analysis in Table 4-4. The high content of Ax-rich yeast diet for short period

feeding had no effect on SFV, cooking loss, drip loss, and water binding capacity those from the control group ($P > 0.05$).

Meat color: The color changes in the broiler breast meat are shown in Table 4-5. There were no significant differences in L* values between the three groups. However, compared with the control group, the a* values were significantly higher in meat samples from the Ax 50 group and Ax 100 group from just after slaughter and 48 h post mortem samples ($P < 0.05$), but between Ax groups no significant differences were observed ($P > 0.05$). For just after slaughter samples and 48 h aged samples from Ax groups significant increases in b* values ($P < 0.05$) were seen compared with the control group and increase was correlation in Ax concentration. The visual color of breast muscle is displayed in Fig 4-1.

The thigh muscle color is shown in Table 4-6. Thigh muscle a* values and the b* values from Ax groups were also significantly higher than control group ($P < 0.05$, $P < 0.01$) and the increase of b* values linearly increased with increase in dietary Ax concentration.

4.4.3 Sensory attributes

The results from the sensory evaluation of breast meat for texture attributes are shown in Table 4-7 and Table 4-8. A paired comparison test of breast meat for texture assessment reveals that samples from the Ax 50 group had significantly improved sensory attributes for meat texture: tenderness, residual particles in mouth, juiciness, first bite, and fibrousness ($P < 0.01$) compared with the control group, but in paired preference test 11 assessors from 14 panelists chose breast meat from the Ax 50 group. But Ax 100 group did not differed from the control significantly.

Table 4-2 Effect of dietary high concentration Ax-rich yeast, *Phaffia rhodozyma*, on growth performance of broiler chickens

Growth performance	Control	Ax 50	Ax 100
Body weight gain, g/day	75.01 ± 6.18[b]	79.11 ± 3.80[ab]	92.31 ± 4.29[a]
Feed intake, g/day	133.59 ± 5.20[b]	137.40 ± 8.07[ab]	146.40 ± 3.31[a]
Feed efficiency	0.57 ± 0.04	0.58 ± 0.01	0.63 ± 0.03
Body organs (% of whole body weight)			
Breast muscle	15.71 ± 0.37	15.51 ± 0.58	15.21 ± 0.46
Liver	2.23 ± 0.10	2.22 ± 0.12	2.45 ± 0.07
Abdominal fat	1.11 ± 0.08	1.20 ± 0.13	1.54 ± 0.15

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

n = 6 birds

Table 4-3 Effect of dietary high concentration Ax-rich yeast, *Phaffia rhodozyma,* on meat SFV of broiler chickens

Parameters	Groups		
	Control	Ax 50	Ax 100
Shear force value$_{48}$, kg	2.58 ± 0.57	2.50 ± 0.47	2.75 ± 0.51

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

n = 6 birds

Table 4-4 Effect of dietary high concentration Ax-rich yeast, *Phaffia rhodozyma,* on meat water holding ability of broiler chickens

Parameters	Groups		
	Control	Ax 50	Ax 100
Drip loss$_{48}$, %	0.37 ± 0.05	0.41 ± 0.07	0.37 ± 0.04
Cooking loss$_{48}$,%	9.79 ± 0.60	10.14 ± 0.80	10.00 ± 0.42
Water binding capacity, %	79.9 ± 2.37	77.40 ± 2.82	79.40 ± 2.10

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

n = 6 birds

Table 4-5 Effect of dietary high concentration Ax-rich yeast, *Phaffia rhodozyma*, on breast meat color of broiler chickens, initially and at 48 h post mortem

Groups	Aging	Control	Ax 50	Ax 100
L* value	0 h	40.57 ± 0.76	39.52 ± 1.50	39.57 ± 0.63
	48 h	49.21 ± 0.57	47.21 ± 1.58	47.13 ± 0.98
a* value	0 h	4.18 ± $0.36^{B,b}$	5.82 ± $0.51^{A,a}$	6.03 ± 0.44^{A}
	48 h	3.18 ± $0.41^{B, b}$	5.50 ± $0.55^{A, a}$	6.33 ± 0.67^{A}
b* value	0 h	2.26 ± 0.08^{C}	5.96 ± 0.60^{B}	8.28 ± 0.78^{A}
	48 h	5.12 ± 0.5^{C}	9.05 ± 0.4^{B}	11.70 ± 0.89^{A}

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$^{A, B, C}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

n = 6 birds.

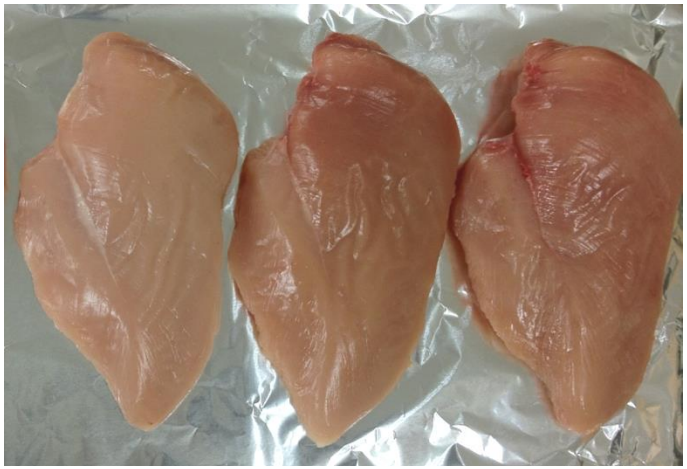


Fig 4-1 Effect of dietary high concentration Ax-rich yeast, *Phaffia Rhodozyma,* on breast meat color from just after slaughter sample From the left side Control group, Ax 50 group and Ax 100 group

Table 4-6 Effect of dietary high concentration Ax-rich yeast, *Phaffia rhodozyma,* on thigh muscle color of broiler chicken from just after slaughter sample

Groups	L*	a*	b*
Control	44.19 ± 0.24	4.66 ± 0.18^{B,b}	1.99 ± 0.42^{C}
Ax 50	42.27 ± 1.18	6.78 ± 0.78^{A,a}	4.65 ± 0.25^{B}
Ax 100	43.61 ± 0.36	6.34 ± 0.40^{A}	7.42 ± 0.60^{A}

$^{a, b}$ Means in a column within an effect with no common superscript differ significantly ($P < 0.05$).

$^{A, B, C}$ Means in a column within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

n = 6 birds.

Table 4-7 Sensory evaluation of broiler chicken breast meat for texture attributes assessment from control and HS groups

Parameters	Control: Ax 50[1]	F value	Significance
First bite[2]	1.36	17.75	**
Tenderness[3]	1.50	24.05	**
Fibrousness[4]	1.21	11.12	**
Juiciness[5]	0.79	11.71	**
Overall preference[6]	0.93	13.00	**

[1] Average Ax 50 group scores of each subject when the control group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that overall preference is more acceptable.

** $P < 0.01$ F value > 9.33

Table 4-8 Sensory evaluation of broiler chicken breast meat for texture attributes assessment from control and HS+Ax groups

Parameters	Control: Ax 100[1]	F value	Significance
First bite[2]	-0.07	0.04	NS
Tenderness[3]	-0.14	0.15	NS
Fibrousness[4]	-0.14	0.12	NS
Juiciness[5]	-0.07	0.08	NS
Overall preference[6]	-0.29	1.41	NS

[1] Average Ax 100 group scores of each subject when the control group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that overall preference is more acceptable.

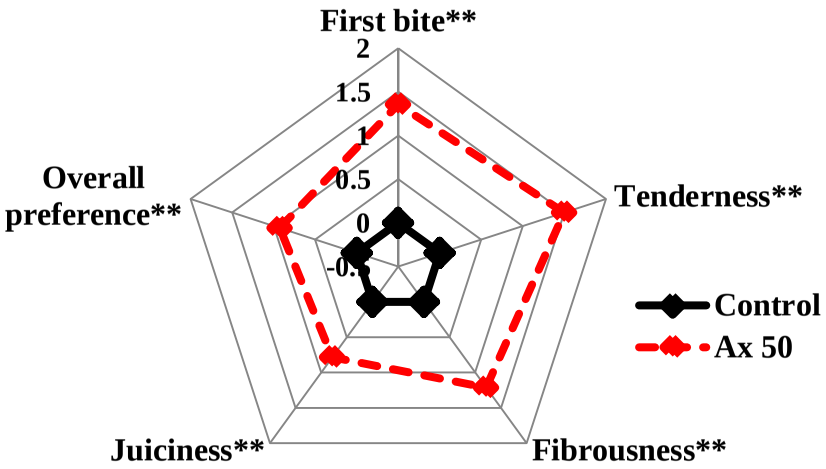


Fig 4-2 Paired comparison test of breast meat between control and Ax 50 groups

** $P < 0.01$

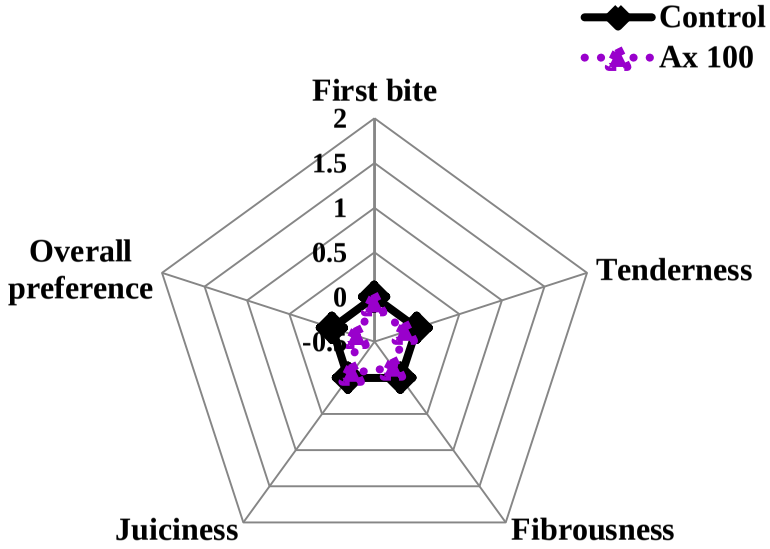


Fig 4-3 Paired comparison test of breast meat between control and Ax 100 groups

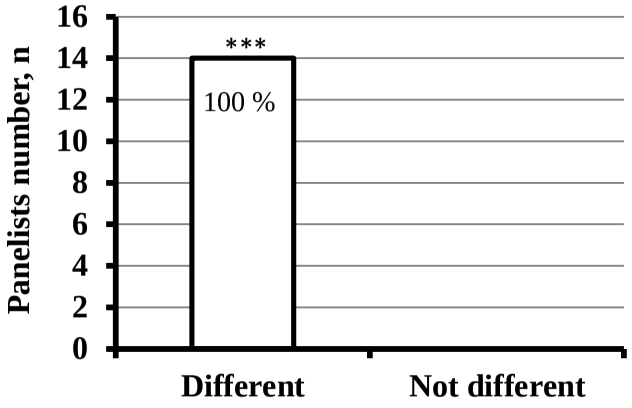


Fig 4-4. Paired difference test of control to Ax 50 group

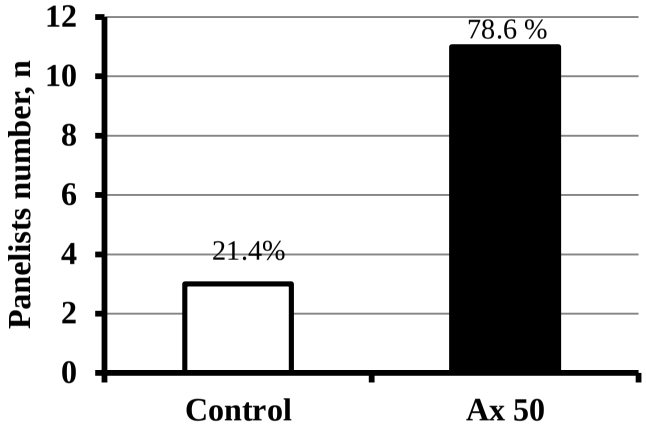


Fig 4-5. Paired preference test of control to Ax 50 group

*** $P < 0.001$

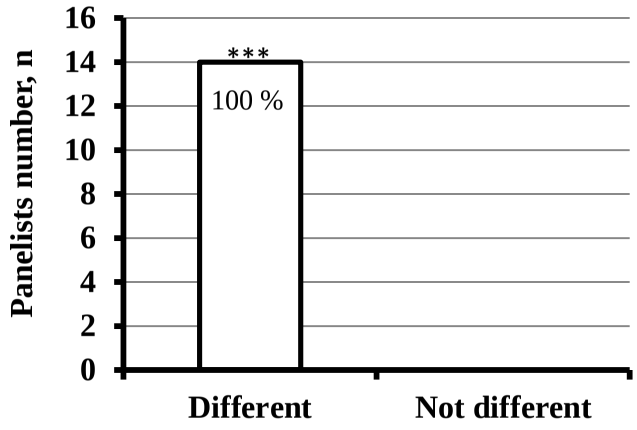


Fig 4-6. Paired difference test of control to Ax 100 group

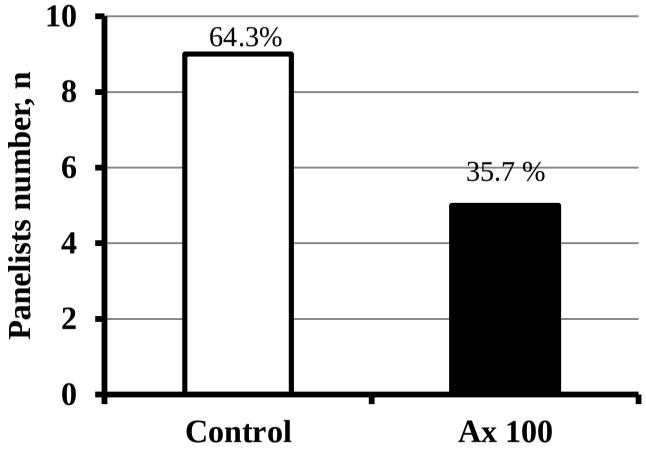


Fig 4-7. Paired preference test of control to Ax 100 group

4.5 DISCUSSION

4.5.1 Growth performance

There was a significant increase on daily body weight gain and feed intake from 100 mg/kg Ax containing diet group comparing to control group (Table 4-2). *Phaffia* yeast has a good nutritional quality (Johnson and An 1991). Therefore, in our study it significantly increased performance of broiler chicken. However, this result did not agreed with reports from Takimoto *et al. (*2007) that 100 mg/kg dietary Ax-rich yeast for 14 days did not influence broiler growth performance and chicken meat production. Difference of feeding composition may was resulted a different results.

4.5.2 Meat quality

Meat texture: Long period feeding trial Ax-rich yeast had effect on meat SFV but in short period feeding high concentration Ax-rich yeast had no effect on SFV. Although these SFV were not statistically different, the sensory evaluation results revealed that meat tenderness improved in Ax 50 group significantly. This comparison proved the previous suggestion that the human sensory assessment of meat tenderness may be more sensitive than instrumental analysis. In addition, drip loss, cooking loss and water binding capacity of muscles from the Ax-rich yeast diet groups were not significantly different from the control group. In precedent study low concentration long period feeding of Ax-rich yeast significantly decreased cooking loss. From these results, it can be implied that short period feeding is not adequate for meat cooking loss.

Meat color: There were no significant differences in L* values between the three groups and a* values significantly increased in meat samples from Ax 50 group and Ax 100 group either in just after slaughter samples and 48 h postmortem samples. However no significant correlation was observed between Ax groups in a* values but there had a tendency that higher concentration of dietary Ax is higher redness. Moreover, meat b* value had significantly increased in evident correlation with dietary Ax concentration. From these results, we suggest that a higher concentration of dietary *Phaffia* yeast is more effective at increasing meat color and it is not dependent on feeding period.

4.5.3 Sensory attributes

Meat texture is one of the most important attributes of meat quality that are influencing on consumer choice. In this study we chose the main four attributes of meat texture: tenderness, juiciness, first bite, and fibrousness for the sensory evaluation of breast meat. The results revealed that the Ax 50 group significantly increased these sensory attributes of meat texture compared with the control group. Meat texture of Ax 100 group was not differed from control group either in results from SFV or results from sensory evaluation. It implies that overmuch concentration of Ax-rich yeast in diet may affect adverse effect on meat tenderness in spite of Ax's non pro-oxidant effect.

In conclusion, dietary high concentration Ax-rich yeast in short period feeding for broiler chicken is possible to improve meat productivity but is not convenient for the meat quality improvement except meat color.

CHAPTER V. EFFECT OF DIETARY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA*, ON MEAT QUALITY, OXIDATIVE STRESS, AND GROWTH PERFORMANCE OF THE BROILER CHICKENS UNDER CHRONIC HEAT STRESS

5.1 ABSTRACT

We evaluated effects of dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, on broiler chicken meat quality under chronic heat stress. Thirty two-day-old female Ross broilers were divided into three groups: control group: Ax-free diet at normal temperature (24.5 ± 0.3°C), HS group: Ax-free diet at high ambient temperature (31.5 ± 1.3°C), and HS+Ax group: 20 mg/kg Ax diet at high ambient temperature (31.5 ± 1.3°C) for 10 days. Ax pre-feeding, 20 mg/kg was for 18 days at normal ambient temperature. At 42 days old, chickens were slaughtered, and then growth performance, meat quality, and sensory attributes were analyzed.

However, compared to control group HS group had significantly lower daily body weight gain and feed intake, and feed efficiency ($P < 0.05$), but HS+Ax group has dramatically inhibit the heat stress effect on feed efficiency. Under chronic heat stress, drip loss increased significantly in HS group ($P < 0.05$) but HS+Ax group was no difference were observed comparing to control ($P > 0.05$). Heat stress did not influence on breast meat color from either just after slaughter or 48 h post-mortem ($P > 0.05$) and Ax-rich yeast can effectively increase meat a* values and b* values in 48 h post-mortem ($P < 0.05$) and b* values in just after slaughter samples ($P < 0.05$). In addition, there were not observed any

significant differences in other meat quality parameters include water binding capacity, cooking loss, shear force value, pH values. Sensory evaluation of meat texture attributes such as tenderness, juiciness, fibrousness, and overall preference revealed no differences in three groups ($P > 0.05$) and first bite was significant higher ($P < 0.05$) but sensory evaluation of breast meat soup resulted that HS group had significantly decreased aroma and sweetness of meat soup but HS+Ax group had significantly increased taste intensity, after taste, and sourness comparing to the control ($P < 0.05$). The main indicating parameter of lipid peroxidation, malondialdehyde (MDA) content was tend to increase in HS group, but HS+Ax group successfully degreased the MDA content comparing to HS group ($P < 0.05$) under chronic heat exposure.

In conclusion, *Phaffia* yeast, which contains a high amount of astaxanthin, is an effective dietary supplement for reducing heat stress-related influences and can improve meat quality of the broiler chicken.

5.2 INTRODUCTION

The global surface temperature increase about 0.8°C since 1980s. Due to the common occurrence of environmental stressors worldwide, many studies have investigated the detrimental effects of heat stress on poultry production. High ambient temperature has deleterious effects on poultry that has a negative balance between the net amount of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal. The negative impact of heat stress on poultry welfare has recently attracted increasing public awareness and concern. Poultry seems to be particularly sensitive to temperature – associated environmental challenges, especially heat stress (Lara *et al.* 2013). Continuous selection for increased growth rate may have increased sensitivity of broilers to high ambient temperature (Cahaner *et al.* 1995). At hot environment, the chemical composition of chicken is changed and meat sensory quality is decreased (Osman *et al.* 1989). Acute heat stress (occurring in transportation of broiler) has been proved to reduce the chicken meat quality (Holm and Fletcher 1997; Petracci *et al.*2001) but few researchers investigated the effect of chronic heat stress on the meat quality of broilers (Lu *et al.* 2007; Quinteiro-Filho *et al*. 2012). Even though, some researchers reported that heat stress during rearing (chronic heat stress) is one of the prominent ante-mortem stressors that result in faster pH decline and pale color in the breast meat of turkeys (McKee and Sams 1997). This meant that understanding and controlling environmental conditions is crucial to successful poultry production and welfare. Several methods are available to alleviate the negative effects of high environmental temperature. Due to the cooling of poultry house is very expensive because dietary manipulation is focused method in poultry production

(Sahin *et al.* 2001). Many studies have been conducted to investigate the effect of thermal environment and dietary nutrient level on the growth performance of broilers. Some of them are related to dietary anions and cations content for regulation of electrolyte balance of broiler (Ahmad *et al.* 2006) but several is related to antioxidants. Heat stress leads to oxidative stress. It increases the generation of free radicals, which in return react with proteins, carbohydrates, fats, and cellular structures and disrupt their structure and functions. Chicken meat has high amount of unsaturated fatty acid which increase the concerns regarding oxidative deterioration (Xiao *et al.* 2011). It has been known that lipid oxidation affects the quality of raw and cooked meats and leads to deterioration in the flavor and color quality, nutritive value, and safety of muscle foods (Pearson *et al.* 1983; Gray and Pearson 1987).

In addition, recent year, protein oxidation in muscle food is becoming attractive topic. Studies in the biomedical science have discovered that intracellular and membrane proteins in muscle can be modified by reactive oxygen species generated via lipid oxidation, metal- or enzyme-catalyzed oxidative reactions, and other chemical and biological processes (Wolf *et al.* 1986; Butterfield and Stadtman 1997). Also some findings revealed that relationship between protein oxidation and meat quality. Oxidized proteins have amino acid destruction, decrease in protein solubility, loss of enzyme activity, formation of amino acid derivatives including carbonyls, and increase in protein digestibility (Meucci *et al.* 1991; Stadtman and Oliver 1991; Agarwal and Sohal 1994). There are some studies about acute stress on protein oxidation in broiler meat but almost no study has been related to chronic stress condition.

Antioxidant vitamins and minerals as a part of a nutritional manipulation tool are commonly added to the diets of birds reared under heat stress. Although it is known that antioxidants regulate metabolism and performance, which are disrupted in the case of exposure to heat stress, not much information is available on their effects on meat quality. Some researcher reported that vitamin E alleviated the adverse effect of chronic heat stress in male broilers as inhibiting lipid peroxidation (Imik *et al.* 2012a) and Imik *et al.* (2012b) reported that ascorbic acid and lipoic acid improved the performance of the animals and the pH and color parameters in chronic heat stress. Chronic heat stress enhanced lipid oxidation can be regulated by some antioxidants but the influence of antioxidative strategies (supplementation of antioxidant, treatment of muscle food with antioxidant, and processing meat under antioxidative conditions) on the quality and functionality of muscle proteins is poorly understood.

As said before, Ax is very powerful antioxidant that is hundred times stronger that vitamin E for singlet oxygen quenching (Miki 1991). In previous study we investigated that dietary Ax-rich yeast significantly improve meat quality at normal ambient temperature, even it has such a beneficial property but no studies about Ax in relation to meat quality from heat stressed chicken have been investigated.

Therefore, in this study we purposed to evaluate effect of dietary Ax-rich yeast, *Phaffia rhodozyma,* on broiler meat quality and oxidative stress under chronic heat stress condition.

5.3 MATERIALS AND METHODS

5.3.1 Birds and housing

One-day-old female Ross strain broilers were purchased from a commercial hatchery (Ohnuma Co. Ltd., Niigata, Japan). From 1 to 14 days, the broilers were housed and kept warm in a brooder and fed a commercial starter diet based on corn and soybean meal (crude protein (CP), 22%; metabolizable energy (ME), 3.10 kcal/g). At 14 days of age, they were separated into individual cages and started feeding on the experimental diets: control – Ax free diet and Ax – 20 mg/kg Ax containing diet. Feed and water were provided *ad libitum*. The ambient temperature was gradually decreased from 36 to 24°C over the period from 1 to 14 days during which lighting was given for 15 h from 04:00 to 19:00 each day. All procedures were performed using the 'Management manual' for Ross broiler chickens and all experimental protocols were approved by the Niigata University Animal Care Committee.

5.3.2 Diets and experimental design

Fourteen-day-old broilers were allocated to two parts: control - broilers fed an Ax-free diet, and Ax - broilers fed a diet containing 20 mg/kg Ax at normal ambient temperature for pre-feeding. From thirty-two-day-old broilers were divided into three groups: Control group - Ax free diet at normal ambient temperature (24°C); HS group - Ax free diet at high ambient temperature; and HS+Ax group – 20 mg/kg Ax containing diet at high ambient temperature (32°C) for ten days. Experimental environment condition shown in Table 5-1 and the formulation and nutritional values of the experimental diets are shown in Table 5-2.

All nutrition levels fulfilled the requirements of the National Research Council (1994).

5.3.3 Measurements and analysis

5.3.3.1 Sample collection and growth performance

Three days ago slaughter blood samples were taken and centrifuged at 12000 rpm for 3 minutes at room temperature. Blood plasma stored at -80°C until further analysis for MDA content. For free amino acid analysis it precipitated protein by 3% sulfosalicylic acid and centrifuged for three minutes at 12000 rpm. Supernatant was stored at -80 °C until further analysis. At the end of the experimental period, the 42-day-old broilers were slaughtered by cutting the neck carotid arteries. After slaughter, sampling and growth performance was performed same method with Chapter 3.

5.3.3.2 Meat quality

Meat quality analysis and meat sensory evaluation were performed same methods with Chapter 3.

Table 5-1 Environment control protocol of heat exposure for ten days before slaughter

Groups	Experimental diets	Ambient t°, C
Control	Basal diet	24.5 ± 0.3
HS	Basal diet	31.5 ± 1.3
HS + Ax	Basal diet + 20 mg/kg Ax	31.5 ± 1.3

Table 5-2 Composition and nutritional content of the experimental diets

Ingredients (%)	Control		HS[1]		HS+Ax[2]	
	14–21d	21–42d	14–21d	21–42d	14–21d	21–42d
Soybean meal	50.68	44.13	50.68	44.13	50.68	44.13
Corn starch	37.82	46.76	37.82	46.76	37.82	46.76
Soybean oil	7.24	5.46	7.24	5.46	7.24	5.46
$CaCO_3$	1.28	1.34	1.28	1.34	1.28	1.34
$CaHPO_4$	1.55	1.10	1.55	1.10	1.55	1.10
NaCl	0.47	0.35	0.47	0.35	0.47	0.35
Vitamin and mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Aquasta[4]	0.00	0.00	0.00	0.00	0.20	0.20
Agar	0.21	0.21	0.21	0.21	0.01	0.01
Methionine	0.26	0.155	0.26	0.155	0.26	0.155
Calculated analysis						
CP (%)	23.00	20.00	23.00	20.00	23.00	20.00
ME (kcal/g)	3.20	3.20	3.20	3.20	3.20	3.20

[1] HS: broilers fed basal diet at high ambient temperature from 32 to 42 days.

[2] HS+Ax: broilers fed 20 mg/kg astaxanthin in diet at high ambient temperature from 32 to 42 days.

[3] Vitamin and mineral premix provided the following per kg of diet: vitamin A 300000 IU, vitamin D 40000 IU, vitamin E 2 g, vitamin K_3*$3H_2O$ 0.37 g, vitamin B_1 0.36 g, vitamin B_2 0.72 g, vitamin B_6 0.7 g, vitamin B_{12} 2 mg, pantothenic acid 2.17 g, nicotinic acid 6.94 g, biotin 0.03 g, folic acid 110 mg, choline 299.81 g, $MnSO_4$ 32.98 g, $FeSO_4$ 43.52 g, $CuSO_4$ 4.02 g, $ZnSO_4$ 19.75 g, $Ca(IO_3)_2$ 0.11 g, and MgO 198.95 g.

[4]Aquasta®: Red yeast, *Phaffia rhodozyma*, providing astaxanthin at 10000 mg/kg (Aska Pharmaceutical Co. Ltd, Tokyo, Japan).

5.3.3.4 Oxidative stress

Malondialdehyde (MDA) content: MDA content was measured by TBARS assay kit of Cayman Chemical Company.

Tissue homogenates: Weight out approximately 25 mg of tissue into a 1.5 ml centrifuge tube then add 250 µl of RIPA buffer with protease inhibitors. After sonicate for 15 seconds at 40V over ice, centrifuged the tube at 1600 × g for 10 minutes at 4°C. Supernatant was stored at -80°C until analysis. The sample will be stable for one month.

Preparation of standard solution: 25 µl of the MDA standard was diluted with 975 µl of water to obtain a stock solution of 12.5 µM. Standard solutions were prepared following concentrations which illustrated in Table 5-3.

Performing assay: 100 µl of sample and standard solutions were added into 5 ml vial then 100 µl of SDS solution was added to vial and swirled to mix. The following 4 ml of the Color Reagent added forcefully down side of each vial and capped vials and place vials in foam or some other holder to keep the tubes upright during boiling. It was boiled in vigorously boiling water bath for an hour.

After one hour, removed the vials immediately and place in ice bath to stop reaction for 10 minutes. Then centrifuged the vials for 10 minutes at 1600 × g at 4°C and loaded 150 µl (in duplicate) from each vial to either the clear plate. Read the absorbance at 532 nm wavelength.

Table 5-3 Preparation of MDA standard solution

Tube	MDA(µl)	Water(µl)	MDA conc (µM)
A	0	1000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

Calculation: Calculate the average absorbance of each standard and sample.

$$\text{MDA } (\mu M) = \frac{(Corrected\ absorbance) - (y - intercept)}{Slope}$$

Protein carbonyl content: Protein carbonyl content was measured by Protein carbonyl assay kit of Cayman Chemical Company.

Tissue homogenates: 200 mg of tissue was rinsed with a phosphate buffered saline solution to remove any red blood cells or clots. Tissue samples were thoroughly and finely homogenized with 1 ml of cold buffer (50 mM MES or phosphate, pH 6.7, containing 1 mM EDTA). The fluid was centrifuged at 10,000 × g for 15 minutes at 4°C and then removed supernatant was checked for contaminating nucleic acid that absorbance at 280 nm and 260 nm.

Removal of nucleic acid: Samples incubated with streptomycin sulfate at a final concentration of 1% in the sample (A 10% streptomycin sulfate stock solution should be made in 50 mM potassium phosphate, pH 7.2) at room temperature for 15 minutes and then centrifuged at 6,000 × g for 10 minutes at 4°C.

Performing assay: Transferred 200 µl of sample to two 2 ml plastic tubes. One for sample tube and the other was for control tube. To one tube 800 µl of DNPH was added (sample tube), while to other tube 800 µl of 2.5 M HCl was added (control tube). Tubes are left in the dark at room temperature for one hour and vortexed each tube briefly every 15 minutes during the incubation. Then 1 ml of 20% TCA solution was added to each tube and vortexed then placed tubes on ice and incubate for five minutes. Centrifuged tubes at 10,000 × g for 10 minutes at 4°C in a micro centrifuge and discarded the supernatant and re-suspended the pellet in 1 ml of 10% TCA. Incubated tubes on ice and left sit for five minutes. Again centrifuged tubes at 10,000 × g for 10 minutes at 4°C in a micro centrifuge and discarded the supernatant and re-suspended the pellet in 1 ml of (1:1) Ethanol/Ethyl acetate mixture and centrifuged tubes at 10,000 × g for 10 minutes at 4°C. The pellets were washed three times with Ethanol/Ethyl acetate mixture. Final precipitates were dissolved in 500 µl of guanidine hydrochloride by vortex and insoluble materials were removed by centrifugation. Finally 220 µl of supernatant from the sample tube and control tube were transferred in to two wells of the 96-well plate respectively and measured the absorbance at a wavelength between 370 nm using plate readers.

Calculation:

Protein carbonyl (nmol/ml) = $\frac{(CA)}{0.011\mu M} \times \frac{500\ \mu l}{200\ \mu l}$;

5.3.4 Statistical analysis

Means and standard errors were calculated for chicken samples in each group. For statistical analysis, a one-way analysis of variance was used with the GLM procedure in SAS 8 (SAS Institute 1999). Except for the sensory evaluation data, significant differences between means were determined by the Tukey HSD test at a significance level of $P < 0.05$ and $P < 0.01$. For the sensory evaluation data, differences between means were analyzed by Scheffe's test at a significance level of $P < 0.05$ or $P < 0.01$.

5.4 RESULTS

5.4.1 Growth performance

Table 5-4 shows the growth performance of the broiler chickens. However, comparing to HS group, the control was significantly high daily BWG, FI, FE ($P < 0.05$), but FE did not significantly differ in HS+Ax group comparing to the control ($P > 0.05$) even daily BWG and FI was significantly lower ($P < 0.05$). The main edible meat breast muscle weight from HS+Ax group had a tendency to increase comparing to HS group but not statistically significant.

5.4.2 Meat quality

Meat texture: The results of the meat SFV measurement are shown in Table 5-5 and water holding abilities are shown in Table 5-6. Heat stress increased drip loss significantly comparing to the control ($P < 0.05$) but the Ax-rich yeast significantly decreased drip loss: those from the HS+Ax group was not significant from the control group ($P > 0.05$). However, the meat cooking loss, WBCs, and SFV were not significant different in either heat stress or Ax-rich yeast groups comparing to the control ($P > 0.05$).

Meat color: The color changes in the broiler breast meat are shown in Table 5-7 and thigh muscles are illustrated in Table 5-8. There were no significant differences in L* values between the three groups. The a* values were significantly higher in meat samples from the HS+Ax group 48 h post mortem samples comparing to control group ($P < 0.05$) and HS group ($P < 0.01$), but not from the after slaughter sample ($P > 0.05$). For just after slaughter samples and 48 hours post mortem, significant increases in b* values ($P < 0.01$) were seen in samples from the HS+Ax group compared with the control and HS groups. In addition b* values of thigh muscles from HS+Ax group was significantly higher than control and HS groups. The pictures of breast muscle from three groups were displayed in Fig 5-1.

*pH***:** No significant differences were observed in pH values between all groups ($P > 0.05$) even there were higher decrease in pH values for HS groups comparing to other groups (Table 5-9).

Free amino acid content: The free amino acid contents in plasma is shown in Table 5-10 and breast meat that from just after slaughter and 48 h aged samples are shown in Table 5-11. Analysis revealed that in blood plasma the contents of Ile, Val, Leu, Asp, and Glu acid

from HS+Ax group were significantly lower than control group ($P < 0.05$). Furthermore, Thr, Gln, and Val content in just after slaughter breast muscle samples from HS and HS+Ax groups were significantly lower than the control ($P < 0.05$). Some free amino acid content (Ala, Thr, Phe, Asp) and total free amino acid content in 48 h postmortem storage sample from HS and HS+Ax groups were significantly lower than control group ($P < 0.05$). In addition to, the increase of total free amino acid content from 0 h to 48 h storage of control group – 180 µg/g, HS group - 60 µg/g, and HS+Ax - 100 µg/g.

5.4.3 Sensory analysis

Meat soup: The results from the sensory evaluation of breast meat soup for meat flavor are shown in Table 5-12 and Table 5-13. A paired comparison test of breast meat soup from HS group comparing to control group for flavor assessment showed that there was a tendency to decrease flavor attributes in HS group. Especially, aroma ($P < 0.01$) and sweetness ($P < 0.05$) decreased significantly in HS group. Also in paired difference test all panelists can detect the differences between two groups (Fig 5-11) and in paired preference test ten assessors from 16 panelists chose the control (Fig 5-12).

Furthermore, taste intensity, after taste, and sourness of HS+Ax group increased significantly in comparison to the control ($P < 0.05$). Further, in difference test all panelists can detect the paired differences between two groups (Fig 5-13) and in paired preference test nine assessors from 16 panelists preferred the HS+Ax group (Fig 5-14).

Table 5-4 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on growth performance of the broiler chickens under chronic heat exposure

Growth performance	Control	HS	HS+Ax
Body weight gain, g/day	69.25 ± 2.29[a]	40.14 ± 5.73[b]	50.63 ± 4.25[b]
Feed intake, g/day	122.1 ± 2.21[a]	101.92 ± 4.37[b]	106.09 ± 5.26[b]
Feed efficiency	0.57 ± 0.01[a]	0.40 ± 0.06[b]	0.47 ± 0.03[ab]
Body organs (% of whole body weight)			
Breast muscle	15.51 ± 0.3	14.94 ± 0.44	15.68 ± 0.44
Liver	2.12 ± 0.04	2.09 ± 0.09	1.95 ± 0.09
Abdominal fat	1.18 ± 0.08	1.22 ± 0.08	1.16 ± 0.14

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

Table 5-5 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat SFV of the broiler chickens under chronic HS

Parameters	Control	HS	HS+Ax
Shear force value$_{48}$, kg	2.93 ± 0.20	3.01 ± 0.39	2.91 ± 0.42

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

Table 5-6 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat water holding ability of the broiler chickens under chronic heat exposure

Parameters	Control	HS	HS+Ax
Drip loss$_{48}$, %	0.29 ± 0.04^{a}	0.45 ± 0.04^{b}	0.33 ± 0.05^{ab}
Cooking loss$_{48}$, %	11.34 ± 0.38	12.56 ± 0.51	11.46 ± 0.79
Water binding capacity, %	86.00 ± 2.30	87.68 ± 2.20	86.69 ± 1.68
Water binding capacity$_{48}$, %	78.85 ± 0.84	78.67 ± 1.67	77.70 ± 1.35

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

Table 5-7 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma,* on breast meat color of broiler chickens, initially and at 48 h post mortem under chronic heat exposure

Parameters	Control	HS	HS+Ax
L* value	40.65 ± 0.43	40.65 ± 0.37	40.31 ± 0.42
L* value$_{48}$	50.42 ± 0.65	51.45 ± 1.13	49.82 ± 1.15
a* value	3.50 ± 0.25	3.68 ± 0.21	4.13 ± 0.16
a* value$_{48}$	3.09 ± 0.25^{b}	2.81 ± 0.28^{B}	3.98 ± 0.22^{A,a}
b* value	1.08 ± 0.20^{B}	0.85 ± 0.18^{B}	3.90 ± 0.18^{A}
b* value$_{48}$	4.37 ± 0.34^{B}	4.16 ± 0.44^{B}	6.92 ± 0.41^{A}

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$^{A, B}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

$_{48}$ 48-h postmortem meat sample.

Values are mean ± SE.

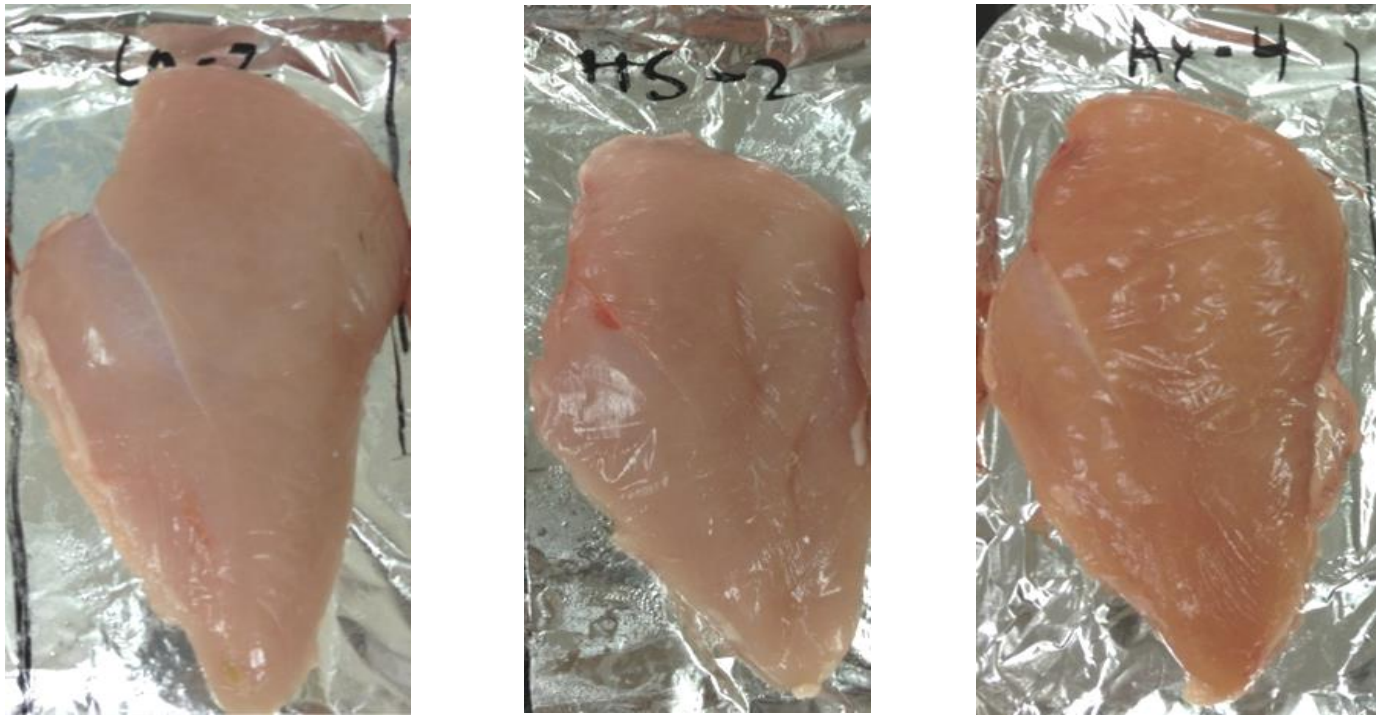


Fig 5-1 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat color from just after slaughter samples under chronic heat stress

From the left side Control group, HS group and HS+Ax group

Table 5-8 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on thigh muscle color of the broiler chickens from just after slaughter samples under chronic heat stress

Parameters	Control	HS	HS+Ax
L* value	45.79 ± 0.56	44.91 ± 1.02	45.14 ± 0.66
a* value	4.79 ± 0.29	4.76 ± 0.48	4.89 ± 0.28
b* value	1.65 ± 0.30^{B}	1.29 ± 0.25^{B}	4.76 ± 0.41^{A}

$^{A, B}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

Table 5-9 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat pH values of the broiler chickens under chronic heat exposure, initially and at 48 h postmortem

Parameter	Aging, h	Control	HS	HS+Ax
pH	0	6.51 ± 0.17	6.69 ± 0.18	6.62 ± 0.18
	48	5.66 ± 0.02	5.75 ± 0.04	5.80 ± 0.05
	Decrease	0.84 ± 0.17	0.94 ± 0.15	0.82 ± 0.22

Values are mean ± SE.

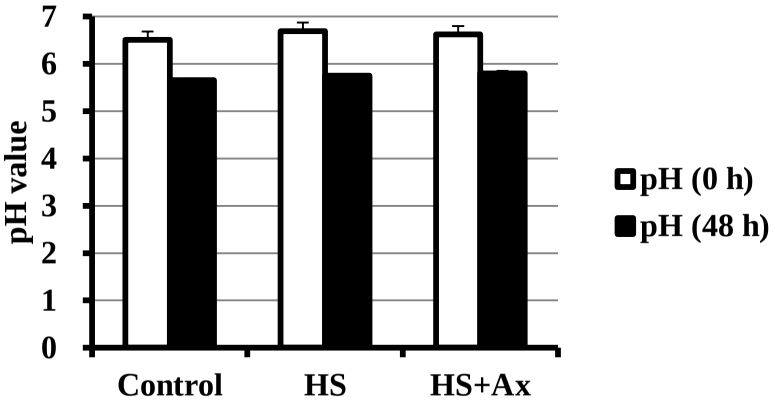


Fig 5-2 pH values of breast meat from just after slaughter and 48 h postmortem

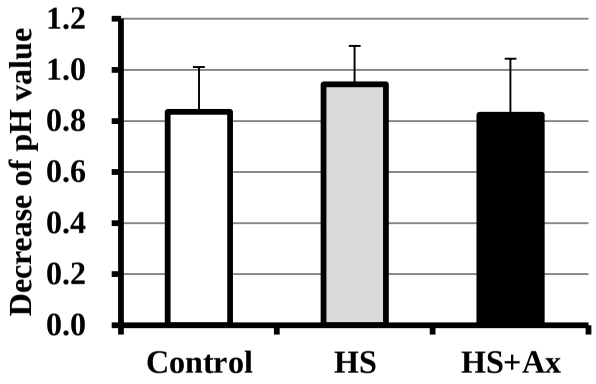


Fig 5-3 pH value decrease of breast meat during aging (from 0 h until 48 h)

Table 5-10 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on free amino acid content of blood plasma under chronic heat stress

Free amino acid content, mmol/l	Groups		
	Control	HS	HS+Ax
Gly	0.66 ± 0.02[ab]	0.71 ± 0.02[a]	0.57 ± 0.03[b]
Ala	0.94 ± 0.06[ab]	0.96 ± 0.03[a]	0.79 ± 0.04[b]
Thr	0.53 ± 0.02	0.57 ± 0.04	0.48 ± 0.02
Ser	0.8 ± 0.05	0.87 ± 0.04	0.69 ± 0.06
Tyr	0.19 ± 0.01	0.17 ± 0.004	0.17 ± 0.01
Lys	0.3 ± 0.03	0.3 ± 0.01	0.33 ± 0.03
Gln	0.93 ± 0.04	0.89 ± 0.04	0.84 ± 0.03
Phe	0.12 ± 0.001	0.12 ± 0.005	0.11 ± 0.005
Arg	0.45 ± 0.02	0.49 ± 0.02	0.51 ± 0.02
Ile	0.15 ± 0.005[A]	0.14 ± 0.006[AB]	0.12 ± 0.007[B]
Val	0.25 ± 0.01[a]	0.23 ± 0.009[ab]	0.21 ± 0.01[b]
Leu	0.21 ± 0.006[a]	0.2 ± 0.008[ab]	0.18 ± 0.01[b]
Met	0.057 ± 0.004	0.055 ± 0.004	0.052 ± 0.002
His	0.073 ± 0.007	0.075 ± 0.01	0.064 ± 0.002
Asp	0.067 ± 0.003[a]	0.063 ± 0.004[ab]	0.051 ± 0.006[b]
Glu	0.22 ± 0.007[a]	0.21 ± 0.008[ab]	0.17 ± 0.02[b]
Total free EAA, mmol/l	1.71 ± 0.07	1.70 ± 0.08	1.59 ± 0.09
Total free NEAA, mmol/l	4.27 ± 0.17	4.41 ± 0.08	3.80 ± 0.18

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

[A, B] Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

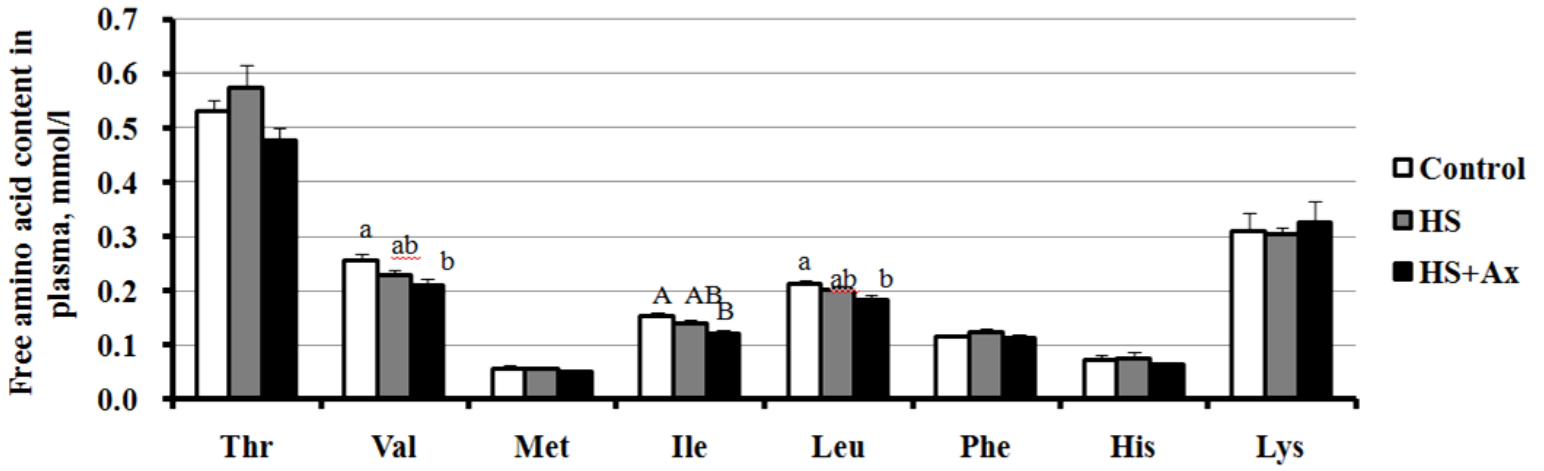


Fig 5-4 Essential free amino acid content in plasma of broiler chicken.

$^{a.b}$ $P < 0.05$

^{A,B} $P < 0.01$

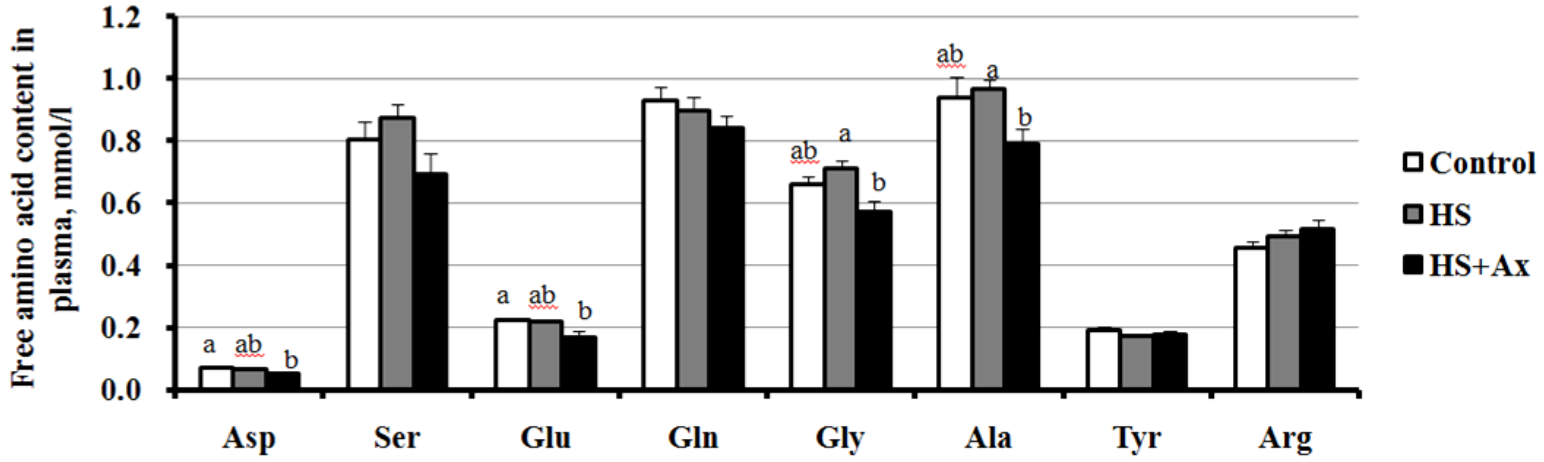


Fig 5-5 Non essential free amino acid content in plasma of broiler chicken.

Table 5-11 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat free amino acid content of the broiler chicken under chronic heat exposure

Free amino acid content, μg/g		Aging, h	Groups		
			Control	HS	HS+Ax
Sweet tasting amino acids	Gly	0	98.6 ± 11.7[a]	69.8 ± 6.0[ab]	55.9 ± 8.3[b]
		48	102.8 ± 6.6	75.2 ± 4.8	76.1 ± 12.7
	Ala	0	186.7 ± 13.6[a]	153.6 ± 5.5[ab]	143.6 ± 15.3[b]
		48	215.0 ± 13.8[a]	159.9 ± 21.1[b]	152.0 ± 18.4[b]
	Thr	0	86.1 ± 1.8[A]	63.1 ± 6.3[B]	57.5 ± 6.3[B]
		48	101.1 ± 4.0[a]	75.6 ± 7.7[b]	78.0 ± 9.5[b]
	Ser	0	156.2 ± 16.7	124.2 ± 10.3	111.8 ± 12.4
		48	184.0 ± 13.2	156.4 ± 8.0	133.7 ± 18.3
	Lys	0	55.3 ± 9.4	52.5 ± 4.0	47.0 ± 6.8
		48	78.8 ± 5.7	69.9 ± 5.2	64.4 ± 6.6
	Gln	0	246.7 ± 9.3[a]	182.2 ± 15.8[b]	180.7 ± 17.1[b]
		48	260.5 ± 16.1	186.9 ± 24.5	205.5 ± 32.8
Bitter tasting amino acids	Phe	0	14.4 ± 1.4	12.7 ± 2.4	11.6 ± 1.5
		48	32.5 ± 2.1[a]	23.5 ± 2.4[b]	24.8 ± 2.6[b]
	Arg	0	98.9 ± 16.4	103.5 ± 13.3	118.8 ± 12.2
		48	112.5 ± 9.5	132.4 ± 22.2	77.9 ± 4.5
	Ile	0	11.1 ± 0.9	10.9 ± 1.3	9.4 ± 0.9
		48	23.6 ± 1.6	18.6 ± 2.2	19.0 ± 2.6
	Val	0	23 ± 2.7[A]	15.5 ± 1.4[B]	14.1 ± 1.1[B]
		48	32.2 ± 1.5	23.5 ± 1.7	25.1 ± 2.8
	Leu	0	13.3 ± 5.6	12.8 ± 0.5	13.2 ± 0.4
		48	41.6 ± 2.7	29.9 ± 4.8	31.7 ± 4.4
	Met	0	0.0	0.0	0.0
		48	19.6 ± 2.1	17.3 ± 3.4	15.7 ± 2.0
	His	0	2.6 ± 0.4	4.0 ± 0.9	4.4 ± 0.7
		48	13.6 ± 1.9	8.7 ± 2.4	9.4 ± 1.1
Sour	Asp	0	33.7 ± 4.7	29.4 ± 2.0	24.9 ± 1.7
		48	23.7 ± 1.5[a]	15.7 ± 0.6[b]	16.9 ± 1.8[b]
Umami	Glu	0	174.4 ± 17.0	155.0 ± 9.8	158.0 ± 20.6
		48	94.7 ± 18.1	96.4 ± 5.0	105.0 ± 18.8
Total free AA, mg/g		0	1.22 ± 0.08	1.00 ± 0.06	0.98 ± 0.07
		48	1.40 ± 0.06[a]	1.06 ± 0.05[b]	1.08 ± 0.1[b]
Increase in free AA, %			14.0	6.0	10.0

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

[A, B] Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

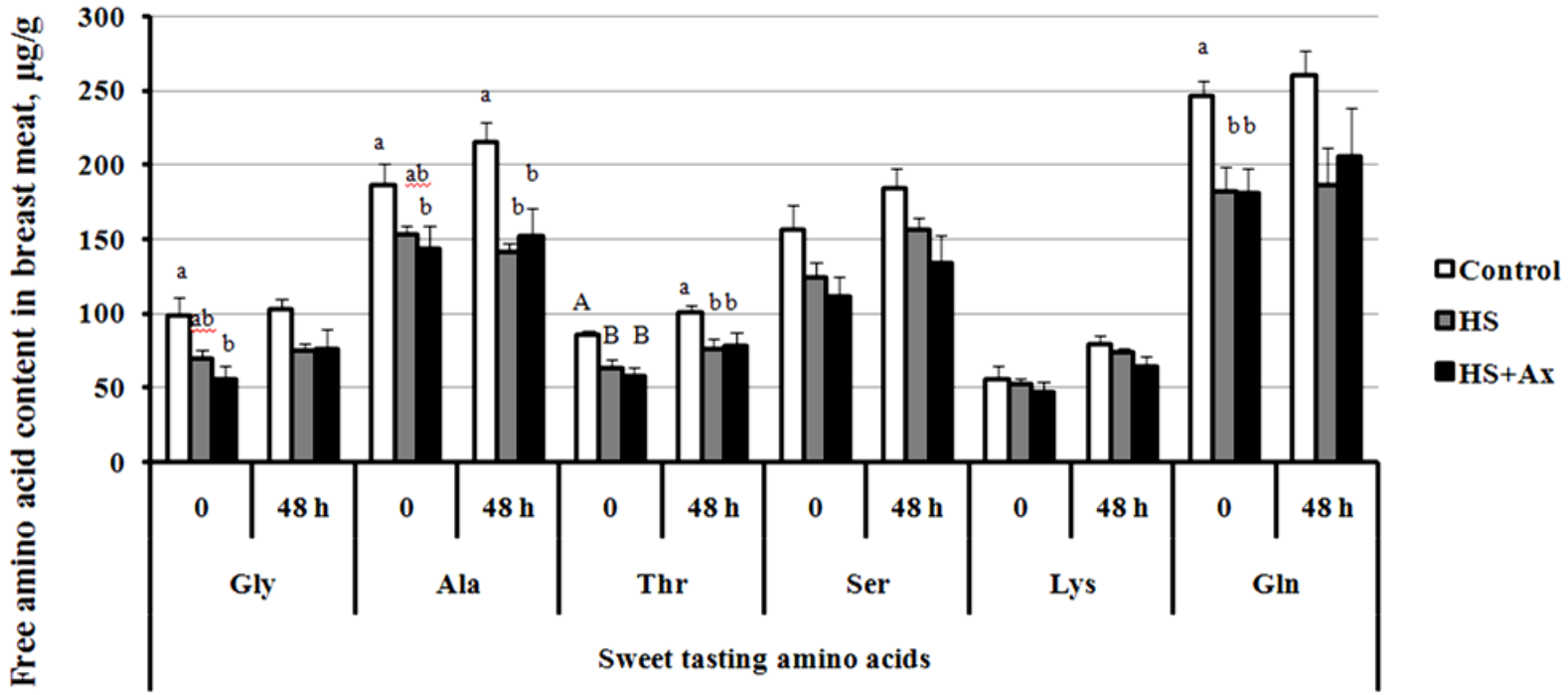


Fig 5-6 Sweet tasting free amino acid content in breast meat from chronic heat stressed chickens during aging (0 – just after slaughter sample and 48 h – 48 h postmortem samples)

[a.b] $P < 0.05$

[A,B] $P < 0.01$

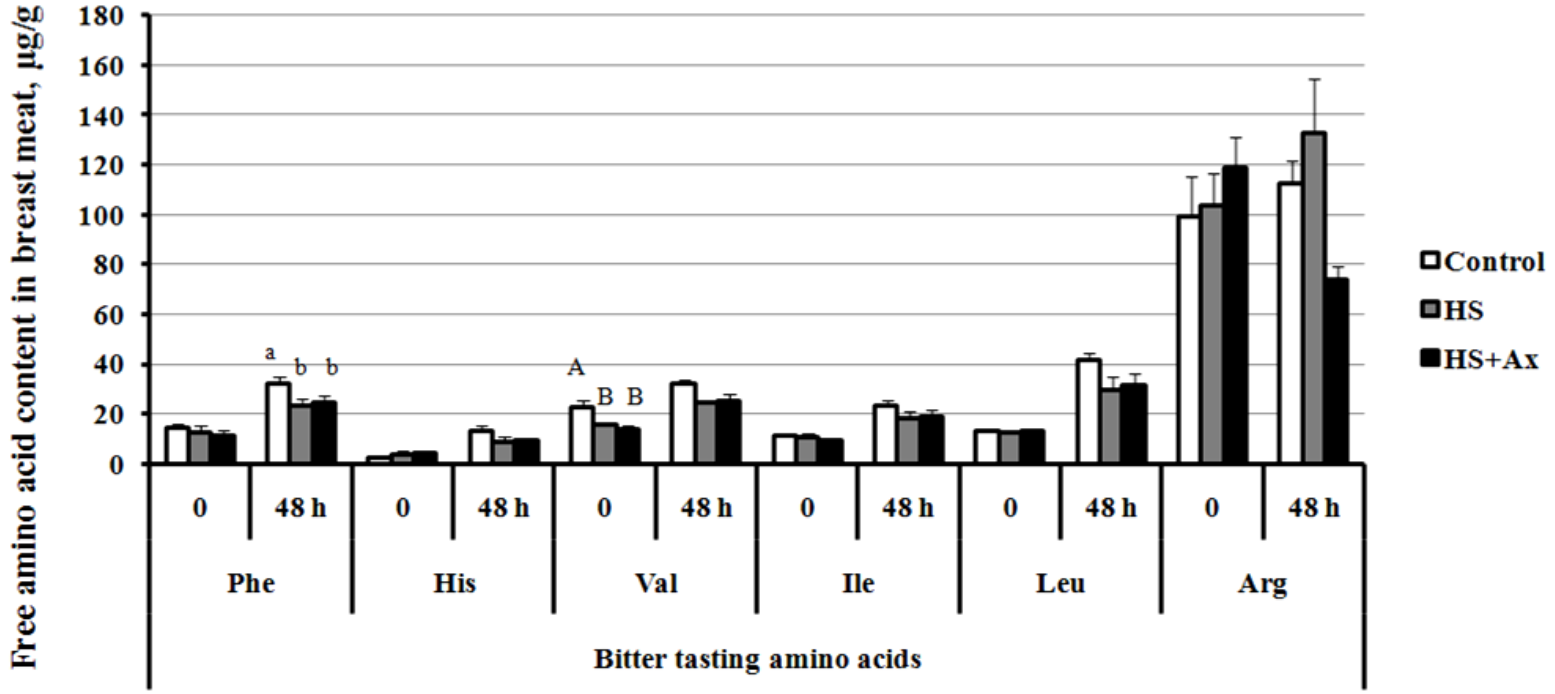


Fig 5-7 Bitter tasting free amino acid content in breast meat from chronic heat stressed chickens during aging (0 – just after slaughter sample and 48 h – 48 h postmortem samples)

[a.b] $P < 0.05$

[A,B] $P < 0.01$

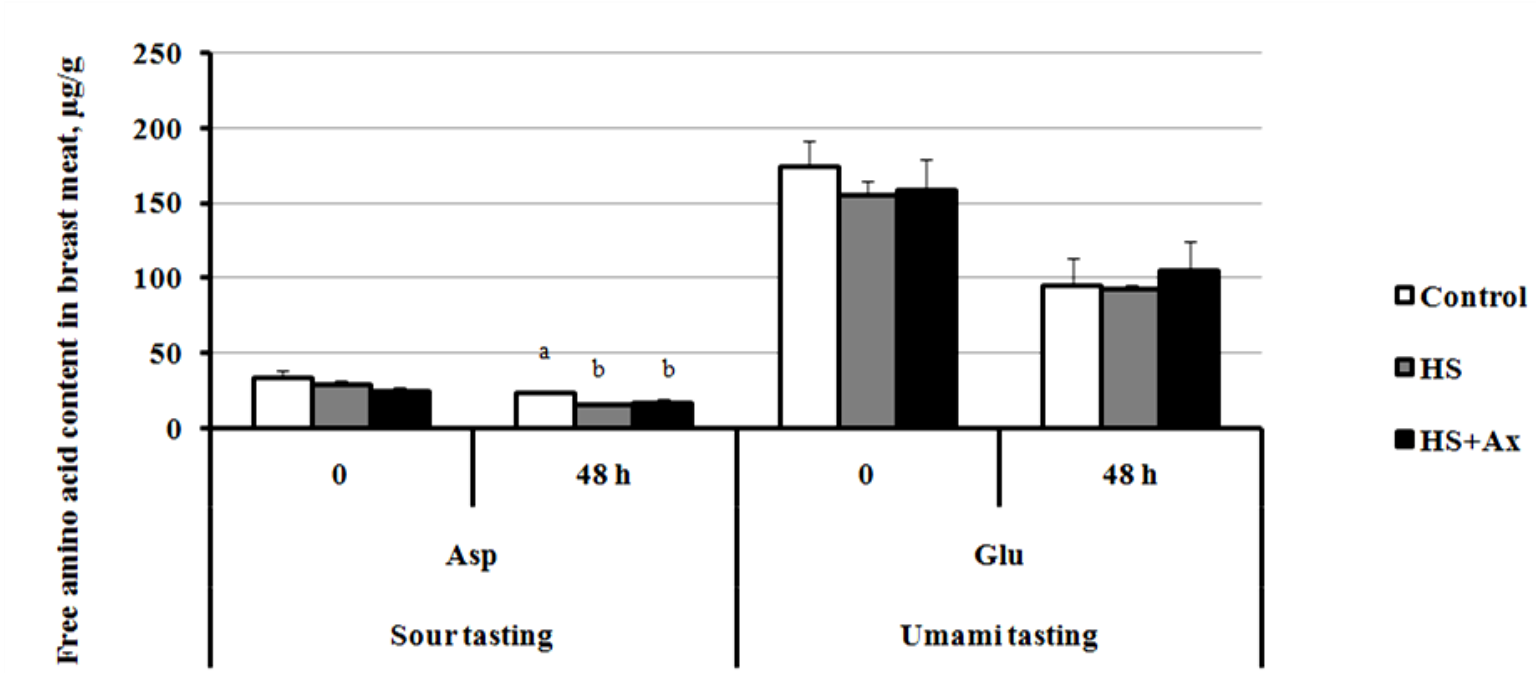


Fig 5-8 Sour and *umami* tasting free amino acid content in breast meat during aging (0 – just after slaughter sample and 48 h – 48 h postmortem samples)

[a.b] $P < 0.05$

Breast meat: Sensory evaluation of breast meat for meat texture in Table 5-14 and Table 5-15. However a paired comparison test of breast meat for texture assessment reveals that no significant difference heat stressed groups comparing to the control group but markedly decrease sensory attributes for tenderness, residual particles in mouth, juiciness, and fibrousness ($P > 0.05$), beside first bite ($P < 0.05$) compared with the control group. In paired difference test, all panelists could detect difference between control group to HS group (Fig 5-17) and to HS+Ax group (Fig 5-19) and in paired preference test nine panelists from 14 chose the control group comparing to HS group (Fig 5-18) and comparing to HS+Ax group (Fig 5-20).

5.4.4 Oxidative stress

The results of oxidative stress indicators of broiler plasma and breast meat under chronic heat exposure illustrated in Table 5-16. Comparing to HS group, HS+Ax group had significantly low MDA content in breast muscle ($P < 0.05$) but no significant difference were observed in plasma and protein carbonyl content in breast muscle between all groups ($P > 0.05$).

Table 5-12 Sensory evaluation of broiler chicken breast meat soup for flavor attributes from control and HS groups

Parameters	Control: HS[1]	F value	Significance
Aroma	-0.57	13.19	**
Umami taste	-0.19	0.28	NS
Sour taste	-0.13	0.2	NS
Bitter taste	0.38	2.29	NS
Sweet taste	-0.31	7.61	*
Chicken-like taste	0.06	0.07	NS
Koku taste	0.13	0.11	NS
Taste intensity	0.31	1.02	NS
After taste	0.25	1.00	NS

[1]Average HS group score of each subject when the contrrol group score is 0.00.

[*] $P < 0.05$ F value > 4.6; [**] $P < 0.01$ F value > 8.86

Table 5-13 Sensory evaluation of broiler chicken breast meat soup for flavor attributes from control and HS+Ax groups

Parameters	Control: HS+Ax[1]	F value	Significance
Aroma	0.00	0.00	NS
Umami taste	-0.06	0.04	NS
Sour taste	0.69	6.89	*
Bitter taste	0.13	0.37	NS
Sweet taste	0.00	0.00	NS
Chicken-like taste	0.19	0.64	NS
Koku taste	0.31	2.22	NS
Taste intensity	0.56	7.56	*
After taste	0.50	6.22	*

[1] Average HS+Ax group score of each subject when the contrrol group score is 0.00.

[*] $P < 0.05$ F value > 4.6

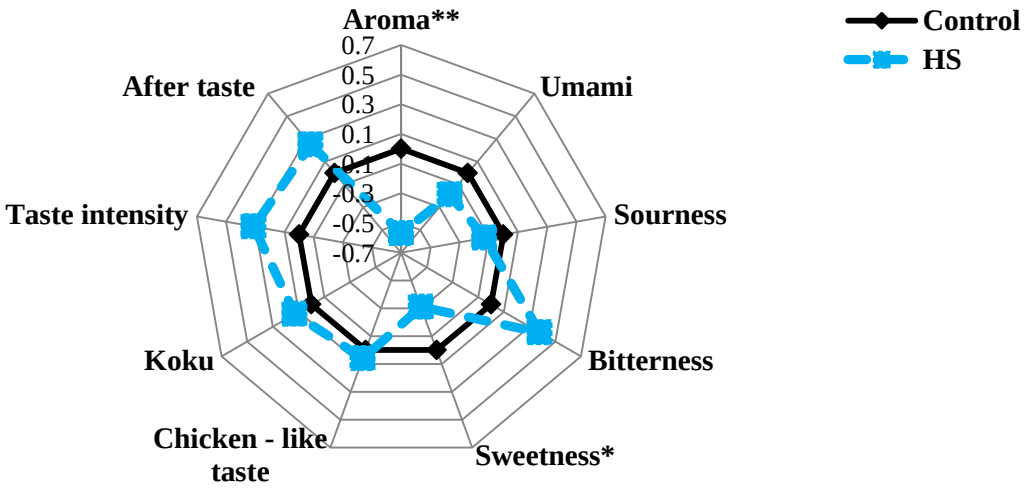


Fig 5-9 Paired comparison test of breast soup from the control and HS+Ax group

** $P < 0.01$

* $P < 0.05$

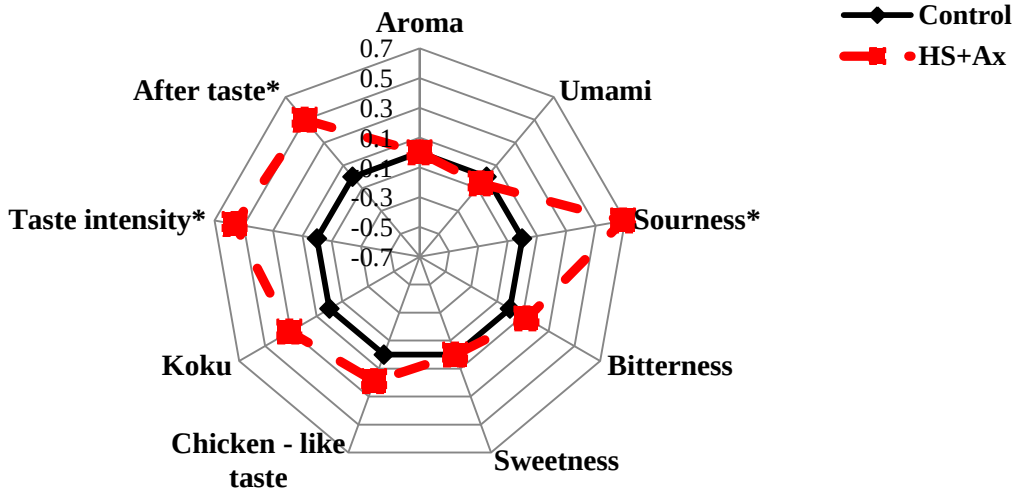


Fig 5-10 Paired comparison test of breast meat soup from the control and HS group

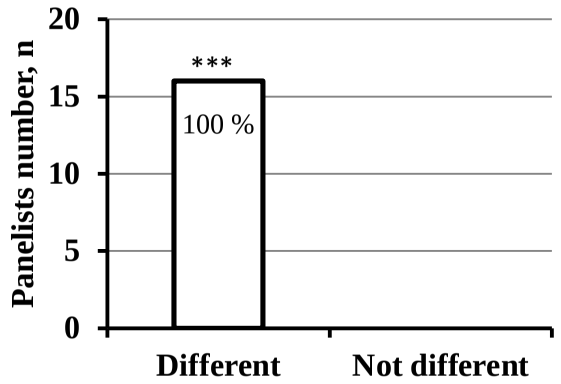
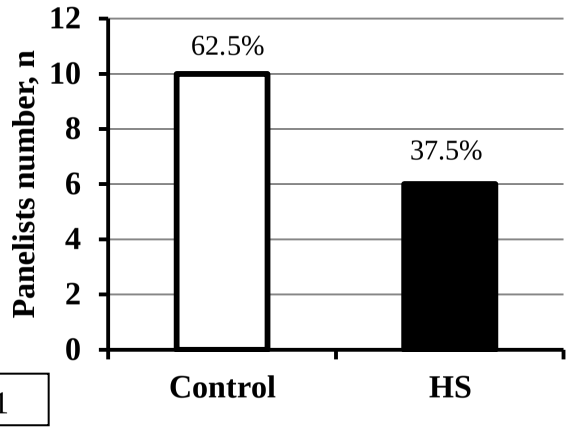


Fig 5-11 Paired difference test of breast meat soup from the control group and HS group



*** $P < 0.001$

Fig 5-12 Paired preference test of breast meat soup from the control group and HS group

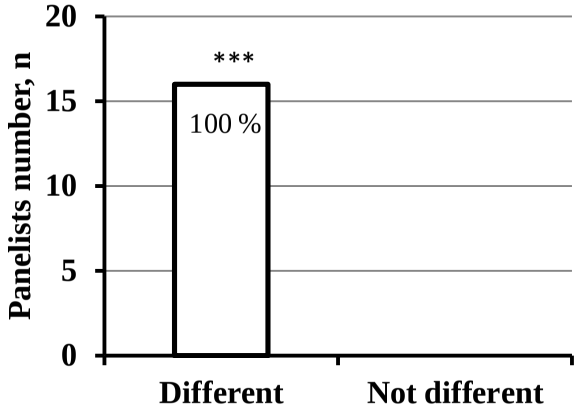


Fig 5-13 Paired difference test of breast meat soup from the control group to HS+Ax group

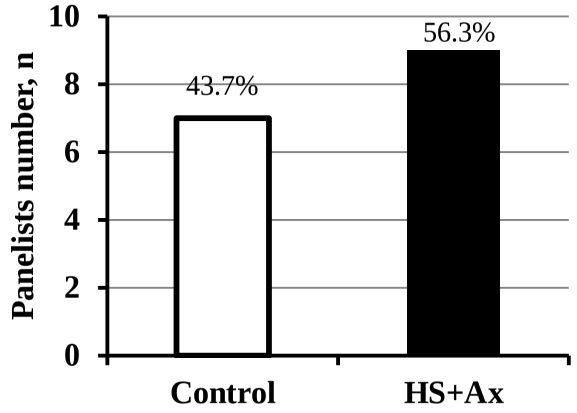


Fig 5-14 Paired preference test of breast meat soup from the control group to HS+Ax group

Table 5-14 Sensory evaluation of the texture of broiler chicken breast meat from control and HS groups

Parameters	Control: HS[1]	F value	Significance
First bite[2]	-1.29	9.08	*
Tenderness[3]	-0.79	2.67	NS
Fibrousness[4]	-0.64	2.27	NS
Juiciness[5]	-0.57	2.56	NS
Overall preferences[6]	-0.79	3.00	NS

[1] Average HS group scores of each subject when the control group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that overall preference is more acceptable.

[*] $P < 0.05$ F value > 4.74

Table 5-15 Sensory evaluation of the texture of broiler chicken breast meat from control and HS+Ax groups

Parameters	Control: HS+Ax[1]	F value	Significance
First bite[2]	-0.93	6.18	*
Tenderness[3]	-0.79	4.65	NS
Fibrousness[4]	-0.5	2.10	NS
Juiciness[5]	-0.07	0.08	NS
Overall preferences[6]	-0.71	3.00	NS

[1] Average HS+Ax group scores of each subject when the control group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that overall preference is more acceptable.

[*] $P < 0.05$ F value > 4.74

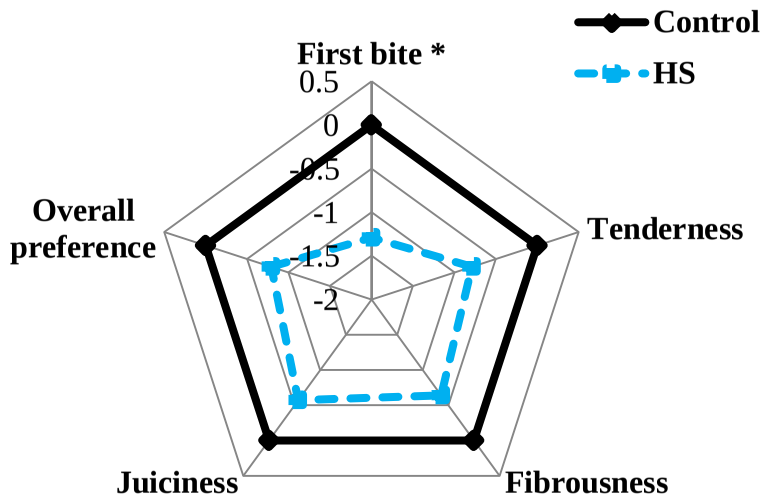


Fig 5-15 Paired comparison test of breast meat from the control and HS group

* $P < 0.05$

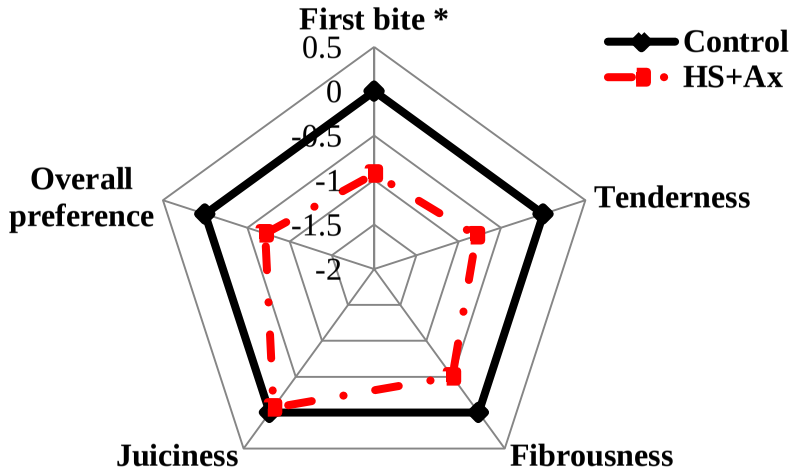


Fig 5-16 Paired comparison test of breast meat from the control and HS+Ax group

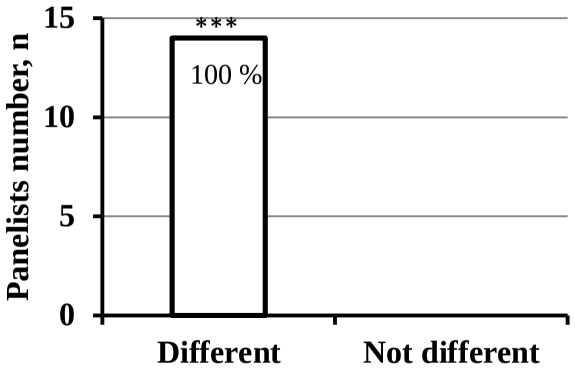


Fig 5-17 Paired difference test of breast meat from control and HS group

*** $P < 0.001$

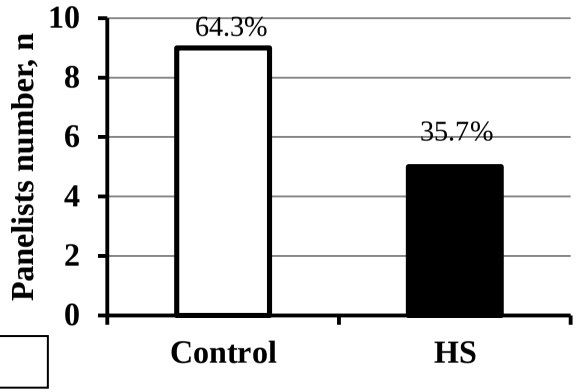


Fig 5-18 Paired preference test of breast meat from control and HS group

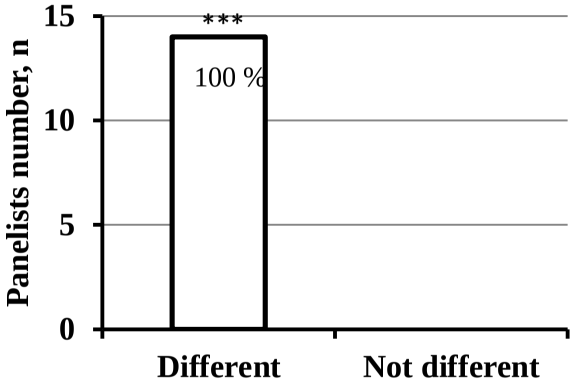


Fig 5-19 Paired difference test of breast meat from control and HS+Ax group

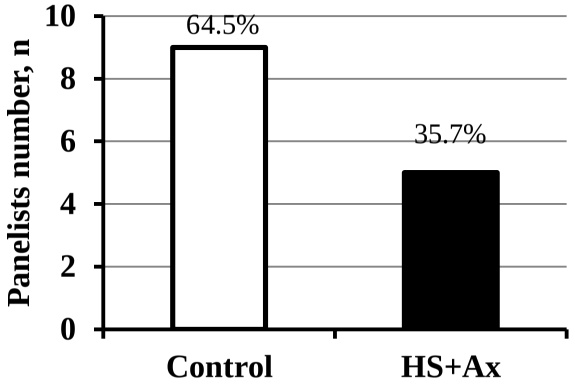


Fig 5-20 Paired preference test of breast meat from control and HS+Ax group

5.5 DISCUSSION

5.5.1 Growth performance

Heat stressed groups were significantly lower daily body weight gain and feed intake comparing to the control. It is already investigated that environmental stress, especially heat stress adversely influenced growth performance of poultry. Heat stress leads to excretion of stress hormones in the organism, and these hormones results in decreased performance as they reduce the feed consumption of animals and affect the metabolism of nutrients adversely (Seigel 1995; Lin *et al.* 2006; Imik *et al.* 2009; Virden and Kidd 2009). In our previous studies, however 20 mg/kg Ax containing diet had no effect on growth performance at normal ambient temperature condition, but 100 mg/kg Ax containing diet increased growth performance of broiler. This time Ax-rich yeast had a significant improvement on feed efficiency under heat stress. Although some researchers reported that antioxidants such as ascorbic acid, vitamin E and vitamin A can improve performance of broiler under chronic heat exposure (Sahin *et al.* 2001; Imik *et al.* 2012a, 2012b) but there was no study about Ax-rich yeast relation to heat stress condition, however Inborr and Lignell (1997) revealed that high concentration of Ax fed to broiler chickens, improved growth performance more rapidly at normal ambient temperature. Present study implies that Ax-rich yeast diet can inhibit the effect of heat exposure on growth performance and it may be beneficial method for productivity of broilers under hot area.

Table 5-16 Effect of Ax-rich yeast on malonedialdehyde content and protein carbonyl content of broiler chicken plasma and breast muscle under chronic heat stress

Parameter	Control	HS	HS+Ax
	In plasma		
MDA, μM	13.54 ± 0.52	12.12 ± 0.25	12.08 ± 0.87
	In breast muscle		
MDA, μM	2.23 ± 0.13[ab]	2.47 ± 0.28[a]	1.70 ± 0.11[b]
Protein carbonyls, nmol/ml	6.9 ± 0.36	6.75 ± 0.71	6.76 ± 0.46

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

5.5.2 Meat quality

Meat texture: One of the important parameters for measuring meat quality is drip loss. Under chronic heat exposure breast meat drip loss increased significantly, but Ax-rich yeast can significantly inhibit this effect as decreasing drip loss. Heat stress is due to oxidative stress. Poly unsaturated fatty acids which located in the membrane structure are easily oxidized and cell membrane is destroyed during the oxidative stress. Due to this process membrane loss their ability to hold water and may contribute to drip loss. Owing to the fact that, Ax is located in the cell membrane and it inhibit lipid peroxidation during heat exposure. This hypothesize was proved in lipid peroxidation analysis. Dietary Ax-rich yeast significantly decreased MDA content in muscle. Another one reason is high carcass temperature caused the denaturation of muscle sarcoplasmic and contractile proteins, which resulted in meat with poor WHC, which was reflected by higher drip loss and cooking loss (Mckee and Sams 1997), but this process is related to meat pH values. In our study no difference was observed in pH value. In this case Ax may inhibit protein denaturation. In addition, Warris and Brown (1987) suggested that pH is important factor in determining drip loss in porcine muscle, but in this study no correlation was detected in broiler meat. This newly demonstrates that Ax-rich yeast can improve the ability of meat to retain water even during ambient heat exposure and it is not only important for meat quality as loss nutrient values with water, it is also a great importance for the economic value of chicken meat production. Heat stress did not influence on SFV and although Ax-rich yeast had a tendency to improve meat tenderness at normal temperature, but under heat exposure it had no effect on it meat tenderness. Some studies reported that breast meat from broilers

suffered heat stress had higher SFV (Lu *et al.* 2007; Gu *et al.* 2008; Zhang *et al.* 2012) and but some studies reported that there was no effect on broiler chicken meat SFV (Lu *et al.* 2007). From these results it is assumed that heat stress effect on SFV change is different that is relating to heat stress type (temperature, humidity, area and chicken population). In addition, water binding capacities of muscles from heat stressed groups were not significantly different from the control group. At normal temperature Ax-rich yeast can improve meat cooking loss, but under heat stress, there had no effect. It may be related to Ax usage for oxidative stress during rearing heat exposure. Until now there have been no data regarding the effect of Ax-rich yeast on poultry meat quality attributes such as meat tenderness, WBC, cooking loss, and drip loss under heat stress condition. Therefore, our study is beneficial fur further study about Ax.

pH: This study did not revealed pH difference between heat stressed group and control groups. Moreover pH value decrease of HS group was higher than other groups. Meat pH value depends on both the glycogen and lactic acid content of muscle tissue and mechanism that converts glycogen into lactic acid (Mckee and Sams 1997; Wang *et al.* 2009). Analysis of metabolites revealed that there was not marked increase in lactic acid in chronic stressed broiler chicken breast meat. Therefore, pH value may not differ in this study.

Meat color: There were no significant differences in L* values between the three groups. Meat pH and lightness are the main indicators of defective meat conditions such as PSE (pale, soft, and exudative) and DFD (dark, firm, and dry) meat. Heat stress accelerates postmortem metabolism and biochemical changes in the muscles which produces faster pH

decline and higher L* values in turkey meat (Mckee and Sams 1997) and broiler meat (Lu *et al.* 2007; Zhang *et al.* 2012). From results, this time heat exposure may was not enough for glycolysis metabolism changes because the sensitivity of chickens to heat stress has been shown to be dependent on the age of birds and strain. In addition, a* values from HS+Ax group 48 h postmortem samples was significantly higher than the control ($P < 0.05$) and HS group ($P < 0.01$) even no significant in just after slaughter samples. In previous study it influences meat redness significantly in meat either from just after slaughter or 48 h postmortem storage, but in this study, increase of redness was not revealed in just after samples. It may be related to utilization of Ax in oxidation processes during the rearing heat exposure. In samples from 48 h postmortem storage the sample from control group and HS group had lower a* values comparing to HS+Ax group and the decrease of a* values in control group from 0 h to 48 h aging was not as lower as HS group: control group – 0.41, HS group – 0.86, and HS+Ax – 0.15 values. It is may related to heme pigment oxidation during the postmortem storage. The myoglobin is responsible for the natural color of meat. This pigment is made of globin, a porphyrin ring, and ferrous ion which are very sensitive to oxidation (Barbut 2002; Mancini and Hunt 2005). Therefore it suggested that during storage disappearance of myoglobin is occurred more efficiently in HS group. However no studies had reported about Ax effect under heat exposure, but these results agreed with some scientists' report about other antioxidants that dietary vitamin E, ascorbic acid and α-lipoic acid have an effect on increase of a* values of breast meat during chronic heat exposure (Imik *et al*. 2012a; Imik *et al.* 2012b) and during post mortem storage. In previous study, we revealed that Ax-rich yeast containing diet can increase meat

color includes redness and yellowness, under chronic heat exposure Ax-rich yeast also influence on meat b* values same effect as normal ambient temperature rearing condition.

Free amino acid content:

Analysis revealed that just after slaughter the content of several free amino acids in plasma and breast muscle samples from the heat stressed groups were significantly lower than the control group. Heat exposure results noticeable decrease of feed intake. Therefore it may influence on free amino acid content of muscle. In addition, the result of free amino acid content in plasma was asserted by report of Temim *et al* (1998) that chronic heat stress depresses protein synthesis in muscle, reduces protein breakdown and decreases the level of most plasma free amino acid content. After 48 h postmortem storage, free amino acid increase in breast meat from Ax-rich yeast diet group was more effective than HS group even not significantly. It means that more postmortem proteolysis may be occurred in Ax group. These results indicated that under chronic heat stress, dietary Ax-rich yeast affects on free amino acid content in meat during postmortem storage with same effect with previous study that Ax-rich yeast can increase the content of free amino acid during the aging of broiler chicken meat at normal ambient temperature condition. From results, we considered that Ax-rich yeast may influence positively on meat flavor from chronic heat stressed broiler chickens during the postmortem storage.

5.5.3 Sensory attributes

Meat soup: The sensory analysis of meat soup from the HS group indicated some attributes had a tendency to decrease and significant difference was shown in some flavor attributes,

aroma and sweetness, compared with those from the control group using Scheffe's test. The oxidation products of fatty acid or amino acids oxidation are aldehydes and ketones which are main contributors of off-flavor attributes, especially unpleasant aromas. Therefore, HS group showed unpleasant aroma comparing to control group. In addition, some attributes, taste intensity, after taste, and sourness, of samples from HS+Ax group increased significantly comparing to the control and above half percent of the assessors preferred the flavor of meat soup from the HS+Ax group. Free sugars, sugar phosphates, nucleotide-bound sugars, free amino acids, peptides, nucleotides, and other nitrogenous components such as thiamine are considered as the main water-soluble flavor precursors (Mottram 1998). During cooking these compounds extracted in water and high amount results intensive flavor. However, total free amino acid content in 48 h postmortem sample of HS+Ax group was lower than control group; other taste active compounds may affect on this flavor attributes.

Breast meat: Meat has many kinds of texture attribute and we chose the main four: tenderness, juiciness, first bite, and fibrousness for the sensory evaluation of breast meat. The results revealed that the heat stressed groups had tendency to decrease meat texture attributes but not significantly, but first bite significantly decreased. Even to date, no other studies have examined the effects of the chronic heat stress on the sensory characteristics of broiler meat, but Sandercock *et al.* (2001) reported that acute heat stress did not affect breast meat eating quality, although overall reductions in taste attributes were observed with age. In paired preference test of breast meat texture attributes of sensory quality, the most assessors preferred the HS+Ax group comparing to the control. From these results, we

can assume that dietary Ax-rich yeast may inhibit unpleasant sensory attributes which are resulted from chronic heat stress.

5.5.4 Oxidative stress

Under chronic heat stress Ax-rich yeast can significantly decrease MDA content which is main indicator of lipid oxidation. Chronic heat exposure increase ROS production in broiler chicken via enhanced mitochondrial respiratory chain activity (Azad *et al.* 2010). Comparing to protein oxidation, lipid oxidation mechanism and antioxidant effect on oxidation process, even under heat stress is well discovered and also it is well known that lipid oxidation process is regulated by dietary antioxidant. Chronic heat stress increase level of lipid oxidation (Azad *et al.* 2010; Imik *et al.* 2012a) and dietary vitamin E had an inhibitory effect on lipid oxidation in breast muscle of broiler (Imik *et al.* 2012a). However dietary Ax-rich yeast decreases lipid oxidation of laying hen skin (An *et al*. 2004) and anti oxidative role in the lipid oxidative process of egg yolk (Yang *et al.* 2006) but Ax-rich yeast influence on oxidative stress in broiler chicken meat is still unclear. Ax is lipid-soluble antioxidant thus it is may effectively influenced in lipid oxidation.

In conclusion, *Phaffia* yeast, which contains a high amount of Ax, is an effective dietary supplement for meat quality improvement as reducing heat stress-related disorders in broiler chickens meat quality.

CHAPTER VI. METABOLOME ANALYSIS OF BROILER CHICKEN MUSCLE FED BY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA* UNDER CHRONIC HEAT STRESS

6.1 ABSTRACT

This study evaluated effect of dietary Ax-rich yeast on broiler chicken breast muscle metabolites change under chronic heat stress. There are totally 120 compounds in control group, 122 compounds in HS group, and 123 compounds in HS+Ax group were detected in analysis of metabolites. The results revealed that comparing to control group, downregulated compounds were 58 and 52 in HS group and in HS+Ax group and upregulated compounds were 11 and 28 in HS group and in HS+Ax group respectively. The final products of the main altering metabolism (glycolitic metabolism) during heat exposure, pyruvate and lactate which are main indicating compounds of heat stress was not altered in between all groups. In TCA cycle, comparing to control group, citric acid in HS group was decreased and in HS+AX group was increased. Malic acid of HS+AX group was increased comparing to HS group. In addition ATP in heat stressed group was downregulated but Ax-rich yeast group upregulated. Finally, one of the main antioxidant compounds in organism, glutathione is upregulated in HS+AX group comparing to others.

6.2 INTRODUCTION

During the heat stress various metabolic changes occurs in organisms (Rhoads *et al.* 2013). Poultry especially, high growth rate, broiler is very susceptible in heat stress. During the heat stress protein synthesis was reduced in the breast muscle (35%) than in the leg muscles (20%) (Temim 1998). Acute heat stress results in a reduction in protein synthesis, a depletion of essential and non-essential plasma free amino acid, an increased plasma uric acid levels, possibly reflecting active protein catabolism and lower N retention (Ostrowski 1981). In chronic heat stress, protein synthesis is depressed in various muscles, reduces protein breakdown and decreases the levels of most plasma free amino acids (Temim *et al*. 2000). Furthermore, during heat exposure, glycolytic metabolism is increased which results high lactic acid formation and decrease of pH value in muscle which is influenced crucially and it deteriorates on meat quality. In addition, heat stress increased lipid retention in broiler (Gereart *et al.* 1996) and pigs (Collin *et al.* 2001) and heat stress effects in chicken are most pronounced in the abdominal fat (Yunianto *et al.* 1997). The metabolism alterations in poultry during heat stress well defined but antioxidant effects on it is not well studied yet. In addition, there is no study about Ax effect on muscle metabolism in chronic heat stress is not revealed. Therefore in this study we purposed to evaluate the effect of Ax-rich yeast on muscle metabolism under chronic heat exposure.

6.3 MATERIALS and METHODS

Instrument settings: **Cationic metabolites (cation mode**)

Equipment

Agilent Capillary Electrophoresis - Time-Of-Flight Mass Spectrometry (CE-TOFMS) system（Agilent Technologies）unit 6

Capillary : Fused silica capillary i.d. 50 μm × 80 cm

Measurement condition

Run buffer : Cation Buffer Solution (p/n : H3301-1001)

Rinse buffer : Cation Buffer Solution (p/n : H3301-1001)

Sample injection : Pressure injection 50 mbar, 10 sec

CE voltage : Positive, 27 kV

MS ionization : ESI Positive

MS capillary voltage : 4,000 V

MS scan range : *m/z* 50-1,000

Sheath liquid : HMT Sheath Liquid (p/n : H3301-1020)

Anionic metabolites (anion mode)

Equipment

Agilent CE-TOFMS system（Agilent Technologies）unit 5

Capillary : Fused silica capillary i.d. 50 μm × 80 cm

Measurement condition

Run buffer : Anion Buffer Solution (p/n : I3302-1023)

Rinse buffer : Anion Buffer Solution (p/n : I3302-1023)

Sample injection : Pressure injection 50 mbar, 25 sec

CE voltage : Positive, 30 kV

MS ionization : ESI Negative

MS capillary voltage : 3,500 V

MS scan range : *m/z* 50-1,000

Sheath liquid : HMT Sheath Liquid (p/n : H3301-1020)

Metabolome analysis was performed in Huamn Metabolome Technologies Inc. Muscles were prepared with same method in Chapter 3 but pooled all muscles with one for each groups.

6.4 RESULTS

Table 1 Effect of dietary Ax-rich yeast on metabolism of broiler breast muscle under chronic heat stress.

Num.	ID	Compound name	Area		
			Control	HS	HS+AX
1	C_0001	Urea	N.D.	N.D.	2.1E-02
2	C_0002	Ethanolamine	N.D.	1.0E-03	8.9E-04
3	C_0012	Acetoacetamide	3.2E-03	2.6E-03	2.4E-03
4	C_0071	Imidazolelactic acid	2.0E-04	1.6E-04	1.7E-04
5	C_0079	N5-Ethylglutamine	2.4E-03	2.2E-03	1.6E-03
6	C_0003	Isobutylamine	N.D.	N.D.	3.8E-03
7	A_0058	UTP	4.2E-03	3.7E-03	4.3E-03
8	A_0017	Dihydroxyacetone phosphate	2.2E-02	1.1E-02	2.9E-02
9	A_0018	Glycerol 3-phosphate	1.6E-02	1.7E-02	2.2E-02
10	A_0027	Phosphocreatine	3.9E-02	1.0E-02	2.7E-02
11	A_0041	Fructose 1,6-diphosphate	3.2E-02	1.0E-02	7.0E-02
12	A_0057	CTP	1.4E-03	6.8E-04	1.0E-03
13	C_0052	Adenine	N.D.	N.D.	1.9E-04
14	C_0115	S-Lactoylglutathione	1.2E-03	8.0E-04	1.9E-03
15	C_0059	Ectoine	1.0E-01	9.8E-02	7.7E-02
16	C_0096	Carnosine	1.2E+00	1.1E+00	1.0E+00
17	A_0060	ATP	2.9E-01	1.7E-01	2.6E-01
18	C_0070	His	4.2E-03	4.4E-03	5.9E-03
19	A_0061	GTP	2.3E-03	1.4E-03	2.1E-03
20	C_0030	5-Aminovaleric acid	3.0E-02	2.7E-02	2.5E-02
21	C_0089	Homoarginine	1.7E-03	2.2E-03	1.5E-03
22	C_0065	Lys	5.0E-02	4.0E-02	4.0E-02
23	C_0078	3-Methylhistidine	3.9E-03	4.1E-03	4.8E-03
24	C_0029	Guanidoacetic acid	4.9E-03	3.6E-03	1.5E-03

Num.	ID	Compounds name	Control	HS	HS+Ax
25	C_0069	4-(β-Acetylaminoethyl)imidazole	5.5E-04	7.8E-04	1.0E-03
26	C_0025	Histamine	1.6E-03	1.3E-03	2.7E-03
27	C_0054	Trigonelline	1.5E-03	1.6E-03	1.2E-03
28	C_0014	Cadaverine	N.D.	5.3E-05	N.D.
29	C_0006	Putrescine	2.8E-03	1.7E-03	1.8E-03
30	C_0040	XC0016	4.6E-02	3.8E-02	3.7E-02
31	C_0094	β-Ala-Lys	2.3E-02	2.2E-02	1.7E-02
32	C_0113	Glutathione (GSSG)_divalent	3.4E-03	2.0E-03	3.9E-03
33	C_0074	2-Aminoadipic acid	8.9E-04	9.5E-04	7.9E-04
34	C_0081	Arg	8.1E-02	8.1E-02	9.1E-02
35	C_0086	N6-Acetyllysine	1.2E-03	1.0E-03	1.3E-03
36	A_0028	Pantothenic acid	4.6E-03	4.6E-03	5.7E-03
37	C_0106	Glycerophosphocholine	1.1E-02	1.2E-02	2.5E-02
38	A_0024	Citric acid	5.1E-03	3.8E-03	5.9E-03
39	C_0103	Uridine	1.0E-03	7.9E-04	1.1E-03
40	C_0016	GABA	3.4E-03	3.4E-03	3.5E-03
41	C_0111	Ophthalmic acid	1.6E-03	1.2E-03	7.5E-04
42	C_0043	3-Guanidinopropionic acid	2.6E-02	2.0E-02	1.6E-02
43	C_0099	N2-Succinylornithine	1.4E-03	1.3E-03	1.5E-03
44	C_0073	N6-Methyllysine	1.1E-03	1.4E-03	1.4E-03
45	C_0045	Leu	5.2E-02	4.6E-02	4.5E-02
46	C_0005	Trimethylamine N-oxide	3.2E-04	4.3E-04	2.1E-04
47	A_0063	NAD+	2.3E-02	2.2E-02	1.6E-02
48	C_0102	Cytidine	5.0E-04	3.1E-04	3.7E-04
49	C_0049	Ornithine	2.1E-03	1.4E-03	1.1E-03
50	C_0098	Butyrylcarnitine	1.1E-03	8.8E-04	1.2E-03
51	A_0020	Ascorbic acid	9.0E-04	N.D.	N.D.
52	C_0023	Hypotaurine	1.5E-02	7.8E-03	6.9E-03
53	C_0021	Ser	1.9E-01	1.4E-01	1.4E-01
54	A_0011	Malic acid	2.0E-02	1.4E-02	1.9E-02
55	C_0075	Carnitine	5.8E-02	6.1E-02	4.4E-02
56	C_0057	1-Methyl-4-imidazoleacetic acid	2.3E-03	1.5E-03	1.7E-03
57	C_0061	4-Guanidinobutyric acid	2.8E-03	2.1E-03	2.0E-03
58	A_0031	XA0033	2.3E-03	2.5E-03	4.5E-03
59	C_0042	Hydroxyproline	2.7E-02	2.4E-02	2.9E-02
60	A_0009	Succinic acid	1.4E-02	1.4E-02	1.2E-02
61	C_0017	N,N-Dimethylglycine	1.7E-01	1.2E-01	1.2E-01
62	C_0085	Phosphorylcholine	9.1E-03	1.0E-02	1.8E-02
63	C_0092	Trp	1.1E-02	8.5E-03	8.4E-03
64	C_0064	Gln	1.9E-01	1.4E-01	1.5E-01
65	C_0083	Theobromine	5.5E-03	4.3E-03	5.0E-03
66	C_0100	Anserine	1.1E+00	1.1E+00	1.2E+00
67	C_0082	Citrulline	1.1E-03	8.6E-04	9.3E-04
68	C_0038	Taurine	4.2E-02	3.2E-02	2.8E-02
69	C_0091	O-Acetylcarnitine	4.0E-02	3.9E-02	3.8E-02
70	C_0020	Choline	4.8E-03	5.1E-03	8.5E-03
71	C_0037	Nicotinamide	2.3E-02	2.4E-02	3.8E-02
72	C_0077	Phe	3.3E-02	3.0E-02	3.2E-02
73	C_0018	N-Methylalanine	1.5E-03	1.1E-03	1.3E-03
74	C_0048	Creatine	1.9E-01	1.9E-01	1.9E-01
75	C_0088	N-Acetyllysine	3.4E-04	4.0E-04	4.9E-04
76	C_0090	Spermine	5.9E-04	1.1E-03	1.5E-03
77	C_0032	Betaine	5.8E-01	3.7E-01	3.7E-01
78	C_0116	S-Adenosylhomocysteine	5.0E-04	3.0E-04	2.2E-04
79	C_0053	Hypoxanthine	2.7E-03	3.1E-03	2.8E-03
80	C_0063	Spermidine	9.5E-03	6.7E-03	6.1E-03
81	C_0010	Glycerol	1.7E-01	3.3E-01	3.2E-01
82	C_0050	Asp	4.2E-02	2.9E-02	3.4E-02
83	C_0022	Diethanolamine	N.D.	4.4E-04	5.4E-04
84	C_0062	γ-Butyrobetaine	1.5E-02	2.0E-02	2.4E-02
85	C_0060	Stachydrine	N.D.	N.D.	4.4E-04
86	A_0005	3-Hydroxybutyric acid	3.2E-03	2.6E-03	2.7E-03
87	C_0047	Asn	5.1E-02	3.6E-02	2.8E-02
88	A_0012	Ethanolamine phosphate	1.5E-03	1.0E-03	1.3E-03

Num.	ID	Compounds name	Control	HS	HS+Ax
89	A_0004	Lactic acid	3.0E+00	3.3E+00	3.3E+00
90	C_0066	Glu	1.4E-01	1.3E-01	1.4E-01
91	A_0015	Uric acid	1.2E-03	8.7E-04	1.2E-03
92	C_0011	Azetidine 2-carboxylic acid	1.3E-03	1.1E-03	7.4E-04
93	C_0019	3-Aminoisobutyric acid	1.3E-03	1.2E-03	1.4E-03
94	C_0009	Ala	3.5E-01	2.8E-01	2.8E-01
95	C_0027	Creatinine	9.1E-03	1.1E-02	1.1E-02
96	C_0114	Glutathione (GSH)	3.4E-02	3.2E-02	4.8E-02
97	C_0110	Guanosine	1.8E-04	1.5E-04	1.5E-04
98	A_0010	5-Oxoproline	6.7E-04	4.6E-04	1.0E-03
99	A_0032	Glucose 1-phosphate	1.8E-02	1.5E-02	1.0E-02
100	C_0034	Thr	1.2E-01	8.7E-02	8.2E-02
101	C_0076	Methionine sulfoxide	6.8E-04	5.5E-04	6.0E-04
102	A_0030	Ribulose 5-phosphate	7.4E-04	7.9E-04	1.0E-03
103	C_0105	Dyphylline	4.7E-03	5.3E-03	5.5E-03
104	C_0051	S-Methylcysteine	4.2E-04	N.D.	N.D.
105	A_0033	Glucose 6-phosphate	2.8E-01	2.7E-01	1.3E-01
106	A_0062	ADP-ribose	2.8E-04	3.6E-04	3.8E-04
107	A_0034	Fructose 6-phosphate	7.1E-02	6.3E-02	3.4E-02
108	C_0112	5'-Deoxy-5'-methylthioadenosine	1.1E-04	5.3E-05	8.7E-05
109	C_0109	Saccharopine	N.D.	2.0E-04	2.5E-04
110	C_0108	Inosine	5.2E-03	5.8E-03	6.4E-03
111	C_0084	Tyr	3.0E-02	2.5E-02	2.5E-02
112	C_0004	Gly	1.6E-01	1.2E-01	1.0E-01
113	C_0007	Sarcosine	4.4E-03	3.0E-03	3.0E-03
114	C_0041	Pipecolic acid	1.8E-03	9.6E-04	9.0E-04
115	C_0104	Isovalerylcarnitine	5.3E-04	5.5E-04	4.4E-04
116	C_0015	2-Aminobutyric acid	4.1E-03	3.5E-03	3.3E-03
117	A_0029	Ribose 5-phosphate	N.D.	1.9E-04	N.D.
118	C_0028	Pro	3.1E-01	1.6E-01	1.4E-01
119	C_0067	Met	7.4E-03	6.5E-03	6.6E-03
120	C_0117	S-Adenosylmethionine	1.1E-03	9.1E-04	8.8E-04
121	A_0044	IMP	4.2E-02	9.7E-02	6.1E-02
122	C_0107	Adenosine	1.3E-04	1.1E-04	4.7E-05
123	C_0044	Ile	4.0E-02	2.7E-02	2.8E-02
124	C_0031	Val	5.5E-02	4.0E-02	3.8E-02
125	C_0093	XC0061	2.3E-03	2.1E-03	1.0E-03
126	C_0008	β-Ala	1.8E-01	1.6E-01	1.1E-01
127	A_0053	ADP	2.0E-03	2.0E-03	1.9E-03
128	C_0097	Isobutyrylcarnitine	3.8E-04	3.5E-04	N.D.
129	C_0087	Gly-Leu	6.0E-04	N.D.	N.D.

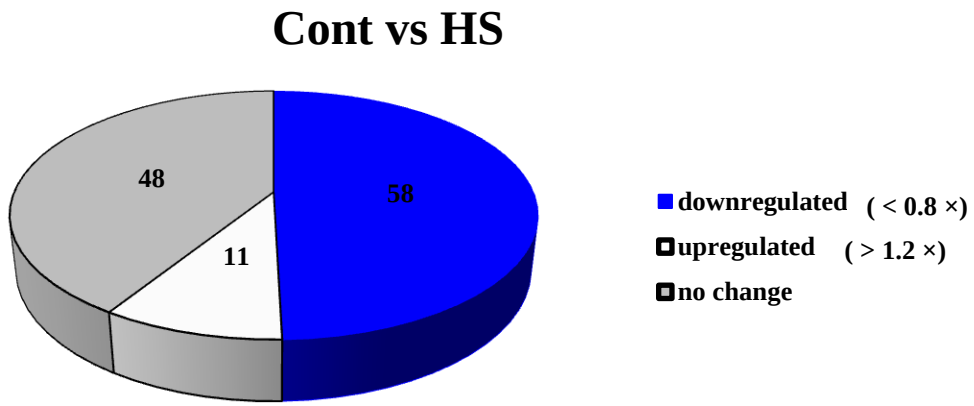


Fig 6-1 Chronic heat stress changed metabolites in broiler breast muscle

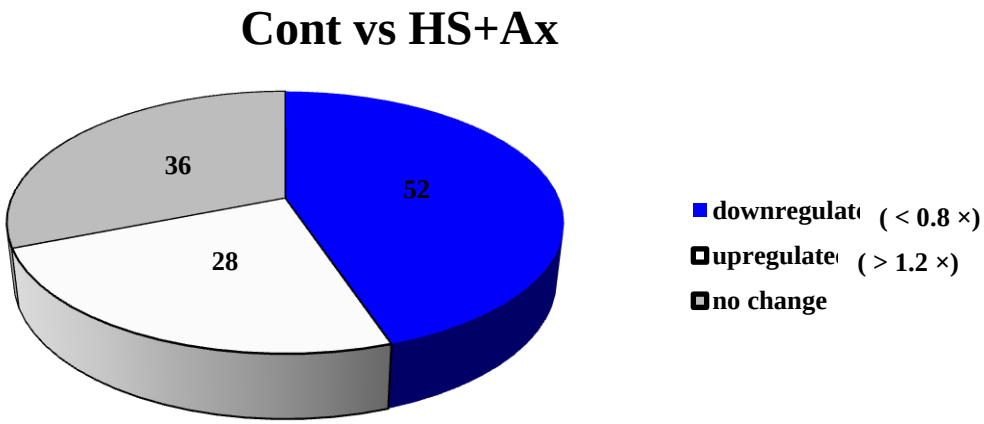


Fig 6-2 Ax-rich yeast chronic changed metabolites in breast muscle under heat stress

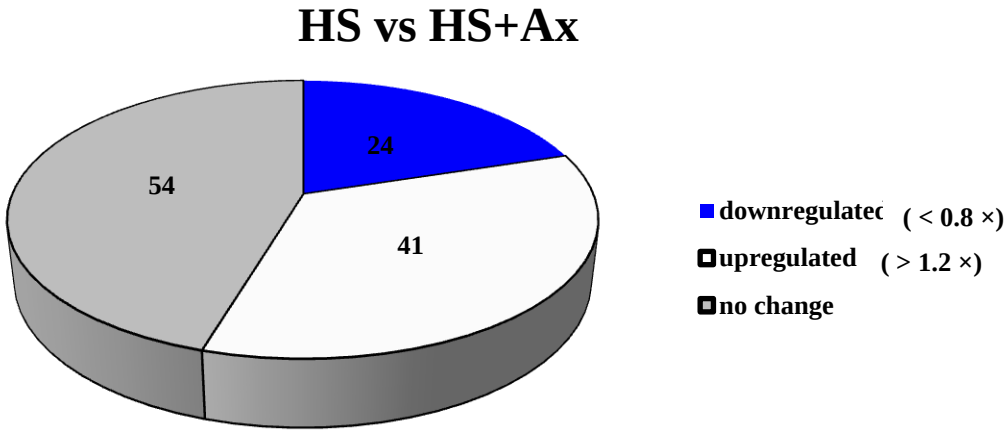


Fig 6-3 Ax-rich yeast chronic changed metabolites in breast muscle under heat stress

Table 6-2 Chronic heat stress upregulated metabolites in breast muscle of broiler (Ratio of metabolites in HS and Control)

Num.	Compound name	HS/Control
1	Homoarginine	1.28
2	4-(β-Acetylaminoethyl)imidazole	1.43
3	N^6-Methyllysine	1.27
4	Trimethylamine *N*-oxide	1.34
5	*N*-Acetyllysine	1.17
6	Spermine	1.85
7	Hypoxanthine	1.16
8	Glycerol	1.96
9	γ-Butyrobetaine	1.32
10	ADP-ribose	1.29
11	IMP	2.29

Table 6-3 Chronic heat stress downregulated metabolites in breast muscle of broiler (Ratio of metabolites in HS and Control)

Num.	Compound name	HS/Control
1	Acetoacetamide	0.83
2	Imidazolelactic acid	0.80
3	Dihydroxyacetone phosphate	0.51
4	Phosphocreatine	0.27
5	Fructose 1,6-diphosphate	0.33
6	CTP	0.49
7	*S*-Lactoylglutathione	0.68
8	ATP	0.60
9	GTP	0.61
10	Lys	0.79
11	Guanidoacetic acid	0.73
12	Histamine	0.84
13	Putrescine	0.60
14	XC0016	0.84
15	Glutathione (GSSG)_divalent	0.60
16	Citric acid	0.76
17	Uridine	0.77
18	Ophthalmic acid	0.75
19	3-Guanidinopropionic acid	0.79
20	Cytidine	0.62
21	Ornithine	0.64
22	Butyrylcarnitine	0.82
23	Hypotaurine	0.53
24	Ser	0.75
25	Malic acid	0.67
26	1-Methyl-4-imidazoleacetic acid	0.68
27	4-Guanidinobutyric acid	0.74
28	*N*,*N*-Dimethylglycine	0.73
29	Trp	0.79
30	Gln	0.74
31	Theobromine	0.78
32	Citrulline	0.79
33	Taurine	0.77
34	*N*-Methylalanine	0.72
35	Betaine	0.63
36	*S*-Adenosylhomocysteine	0.60
37	Spermidine	0.71
38	Asp	0.69
39	3-Hydroxybutyric acid	0.82
40	Asn	0.70
41	Ethanolamine phosphate	0.64
42	Uric acid	0.74
43	Azetidine 2-carboxylic acid	0.80
44	Ala	0.80
45	Guanosine	0.83
46	5-Oxoproline	0.69
47	Glucose 1-phosphate	0.83
48	Thr	0.73
49	Methionine sulfoxide	0.81
50	5'-Deoxy-5'-methylthioadenosine	0.49
51	Tyr	0.84
52	Gly	0.72
53	Sarcosine	0.69
54	Pipecolic acid	0.55
55	2-Aminobutyric acid	0.85
56	Pro	0.50
57	Ile	0.66
58	Val	0.72

Table 6-4 Ax-rich yeast upregulated metabolites in breast muscle of broiler under chronic heat stress comparing to control

(Ratio of metabolites in HS+Ax and Control)

Num.	Compounds name	HS+Ax/Control
1	Dihydroxyacetone phosphate	1.29
2	Glycerol 3-phosphate	1.43
3	Fructose 1,6-diphosphate	2.21
4	*S*-Lactoylglutathione	1.60
5	His	1.40
6	3-Methylhistidine	1.23
7	4-(β-Acetylaminoethyl)imidazole	1.90
8	Histamine	1.67
9	Pantothenic acid	1.24
10	Glycerophosphocholine	2.21
11	Citric acid	1.17
12	N^6-Methyllysine	1.27
13	XA0033	1.96
14	Phosphorylcholine	1.99
15	Choline	1.77
16	Nicotinamide	1.67
17	*N*-Acetyllysine	1.41
18	Spermine	2.55
19	Glycerol	1.91
20	γ-Butyrobetaine	1.56
21	Creatinine	1.17
22	Glutathione (GSH)	1.38
23	5-Oxoproline	1.49
24	Ribulose 5-phosphate	1.39
25	Dyphylline	1.18
26	ADP-ribose	1.39
27	Inosine	1.25
28	IMP	1.45

Table 6-5 Ax-rich yeast downregulated metabolites in breast muscle of broiler under chronic heat stress comparing to control

(Ratio of metabolites in HS+Ax and Control)

Num.	Compound name	HS+Ax/Control
1	Acetoacetamide	0.75
2	N^5-Ethylglutamine	0.68
3	Phosphocreatine	0.71
4	CTP	0.71
5	Ectoine	0.75
6	5-Aminovaleric acid	0.83
7	Lys	0.80
8	Guanidoacetic acid	0.32
9	Trigonelline	0.78
10	Putrescine	0.62
11	XC0016	0.81
12	β-Ala-Lys	0.77
13	Ophthalmic acid	0.47
14	3-Guanidinopropionic acid	0.61
15	Trimethylamine *N*-oxide	0.66
16	NAD^+	0.70
17	Cytidine	0.75
18	Ornithine	0.53
19	Hypotaurine	0.46
20	Ser	0.71
21	Carnitine	0.77
22	1-Methyl-4-imidazoleacetic acid	0.74
23	4-Guanidinobutyric acid	0.70
24	*N*,*N*-Dimethylglycine	0.73
25	Trp	0.79
26	Gln	0.79
27	Taurine	0.67
28	Betaine	0.64
29	*S*-Adenosylhomocysteine	0.45
30	Spermidine	0.65
31	Asp	0.81
32	Asn	0.55
33	Azetidine 2-carboxylic acid	0.55
34	Ala	0.80
35	Glucose 1-phosphate	0.57
36	Thr	0.70
37	Glucose 6-phosphate	0.47
38	Fructose 6-phosphate	0.47
39	5'-Deoxy-5'-methylthioadenosine	0.81
40	Tyr	0.84
41	Gly	0.64
42	Sarcosine	0.68
43	Pipecolic acid	0.51
44	Isovalerylcarnitine	0.83
45	2-Aminobutyric acid	0.80
46	Pro	0.47
47	*S*-Adenosylmethionine	0.83
48	Adenosine	0.36
49	Ile	0.69
50	Val	0.70
51	XC0061	0.45
52	β-Ala	0.60

6.5 DISCUSSION

The results revealed that comparing to control group, HS group had downregulated compounds were 58 and 52 in HS group and in HS+Ax group respectively, and upregulated compounds were 11 and 28 in HS group and in HS+Ax group respectively. Ax-rich yeast upregulated 41 compounds than HS group. It means heat stress group generally decrease metabolism in muscle and Ax-rich yeast may alleviate this effect on some metabolites. The main altering metabolism during heat exposure is glycolitic metabolism. Glycolysis' final products, especially lactic acid which is main indicating compound of heat stress were not altered in between all groups in chronic heat stress. However, fructose-1-6-phosphate and dihydroxyacetone phosphate was down-regulated in HS group and upregulated in HS+Ax group. However some study reported that heat stress increased glycoytic metabolism and resulted increase of lactic acid and decrease of ultimate pH (Zhang *et al.* 2012). In addition heat stress increases glucose use by skeletal muscle, and because skeletal muscle is responsible for the majority of glucose disposal in changes in its fuel efficiency can have large impacts on whole-body nutrient flux (DeFronzo *et al.* 1992). It suggested that heat temperature that exposed in this time may was not enough for the glycolysis alteration. Furthermore, in TCA cycle, comparing to control group, citric acid in HS group was decreased and in HS+AX group was increased. Malic acid of HS+AX group was increased comparing to HS group. During the heat stress, pyruvate dehydrogenase kinase 4 (PDK4) in skeletal muscle is increased in pig (Won *et al.* 2012). PDK4 inhibits pyruvate dehydrogenase (PDH) complex that control flux of glucose carbons through the TCA cycle

and is responsible for the irreversible conversion of pyruvate to acetyl-CoA. However in the absence of hypoxia, heat stress reduces acetyl-CoA flux through the TCA cycle (Rhoads *et al.* 2013). Thus, it is implied that Ax-rich yeast may influence on PDK4 enzyme and increases TCA circle flux. In addition ATP in heat stressed group was downregulated comparing to the control but Ax-rich yeast group upregulated it than HS group and also phosphicreatine in HS group was lowered comparing to control, but it was upregulated in Ax group comparing to HS group. Lister *et al.* 1967 reported that heat stressed muscles had high G6P, low ATP, and low phosphocreatine. Results clarified that Ax-rich yeast may inhibit heat stress effects on these metabolites. Finally, one of the main antioxidant compounds in organism, glutathione is upregulated in HS+AX group comparing to others. Ax can enhance nuclear factor E2 related factor 2 (NRF2)-mediated endogenous antioxidant defense system (Yang *et al.* 2011). Activation of NRF2 pathway improves endogenous antioxidant defense by increasing the expression of antioxidant enzymes, such as glutathione peroxidase, glutathione S-transferase and hemeoxygenase. Moreover, NRF2 enhances antioxidant capacity of glutathione. Therefore, glutathione may be upregulated in this study.

In conclusion, chronic heat stress influences adversely on some metabolites, but Ax-rich yeast has an ability to alleviate heat stress effects.

CHAPTER VII. EFFECT OF DIETARY ASTAXANTHIN RICH YEAST, *PHAFFIA RHODOZYMA*, ON MEAT QUALITY AND OXIDATIVE STRESS OF BROILER CHICKENS UNDER ACUTE HEAT STRESS

7.1 ABSTRACT

We evaluated effects of dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, on broiler chicken meat quality under acute heat stress. Fourteen-day-old female Ross broilers were divided into two groups: control group: Ax-free diet and Ax group: 20 mg/kg Ax diet for 28 days at normal ambient temperature for pre-feeding. After feeding trial, at forty-two-day old, removed feed and chickens were exposed in two ambient temperatures: 24°C (Control group) and 34°C (HS group and HS+Ax group) for two hours before slaughter. After heat exposure, blood samples were taken and chickens were slaughtered, and then body organ weights measured and meat quality, sensory attributes were analyzed. Under acute heat stress, drip loss increased significantly in HS group ($P < 0.05$) but HS+Ax group was no difference were observed comparing to control ($P > 0.05$). However acute heat stress did not influence on breast meat color from either just after slaughter or 48 h post-mortem ($P > 0.05$), Ax-rich yeast can effectively increase meat b* values in just after slaughter and 48 h post-mortem ($P < 0.05$) comparing to other groups. In sensory evaluation of meat texture, no significant differences were observed. The main indicating parameter of protein oxidation, protein carbonyl content was tend to increase in HS group, but HS+Ax group significantly degreased the protein carbonyl content from HS group ($P <$

0.05) under acute heat exposure, but malondialdehyde content was not differed significantly between all groups ($P > 0.05$).

In conclusion, Ax-rich yeast may inhibit heat exposure during crating the broiler as improving meat quality but not so effective as in chronic heat stress.

7.2 INTRODUCTION

Most countries, especially developed countries, poultry production is in controlled environment housing. Under supervision of the farmer, the houses provide everything birds need to maintain their welfare and performance, including protection from the weather. Environmental conditions during transport and holding of livestock have been shown to affect live shrink and subsequent meat quality (Petracci *et al*. 2001). Seasonal heat exposure has been reported to cause undesirable changes in meat characteristics in broiler production (Northcutt *et al.* 1994). Acute heat stress is experienced by broiler chickens under normal production conditions and during procedures such as transportation and preslaughter holding (Warris *et al.* 1993). During acute heat exposure, there are many metabolic changes, increase of creatine kinase activity, change cell membrane integrity in breast muscle glycolytic metabolism, and increase the osmotic effect of the membrane, are occurred in broiler muscle (Sandercock *et al.* 2001). The alteration in muscle membrane permeability and changes in metabolism in broiler is leading to alteration in meat characteristics such as increased drip loss and decrease of flavor of meat (Sandercock *et al.* 2001). As said in previous chapter heat stress results oxidative stress and especially, acute

heat exposure stimulates mitochondrial superoxide production in broiler skeletal muscle (Mujahid *et al.* 2006). This superoxide becomes the initiator of oxidation process of meat and due to poor meat quality. Once during heat stress, antioxidant system balance is lost, therefore dietary antioxidant is beneficial method to regulating oxidation process in meat. Unfortunately, there are numerous studies about effect of acute heat stress on meat quality but no study has been conducted to Ax-rich yeast regulation, even other antioxidant regulation of meat quality.

Therefore in this study we purposed to evaluate Ax-rich yeast, *Phaffia rhodozyma*, on meat quality and oxidative stress under acute heat exposure.

7.3 MATERIALS AND METHODS

7.3.1 Birds and housing

One-day-old female Ross strain broilers were purchased from a commercial hatchery (Ohnuma Co. Ltd., Niigata, Japan). From 1 to 14 days, the broilers were housed and kept warm in a brooder and fed a commercial starter diet based on corn and soybean meal (crude protein (CP), 22%; metabolizable energy (ME), 3.10 kcal/g). At 14 days of age, they were separated into two cages and started feeding on the experimental diets. Feed and water were provided *ad libitum*. The ambient temperature was gradually decreased from 36 to 24°C over the period from 1 to 14 days during which lighting was given for 15 h from 04:00 to 19:00 each day. All procedures were performed using the 'Management manual' for Ross

broiler chickens and all experimental protocols were approved by the Niigata University Animal Care Committee.

7.3.2 Diets and experimental design

Fourteen-day-old broilers were separated into two parts: Part 1: broilers fed an Ax-free diet; Part 2: broilers fed a diet containing 20 mg/kg Ax. The formulation and nutritional values of the experimental diets are shown in Table 7-1. All nutrition levels fulfilled the requirements of the National Research Council (1994). At forty-two day of age, two hours prior to slaughter, feed was removed, and chickens were allotted into two environmental conditions: normal condition – control group (24°C) and high ambient temperature condition – HS group and HS+Ax group (34°C) for two hours before slaughter. Environmental condition report is shown in Table 7-2.

7.3.3 Measurements and analysis

Sampling, percentage of body organs calculation, meat quality analysis, sensory analysis, and oxidative stress analysis were performed same methods which are described in Chapter 3, blood plasma analysis and oxidative stress parameters were made in same methods which are described in Chapter 5.

7.4 RESULTS

7.4.1 Body yields

Table 7-3 shows the organ's yields of the broiler chickens. Comparing to the control HS group had a significantly smaller liver ($P < 0.05$), but HS+Ax group had no difference was observed ($P > 0.05$).

7.4.2 Meat quality

Meat texture: The results of the meat SFV measurement are shown in Table 7-4 and water holding abilities are shown in Table 7-5. Acute heat stress increased drip loss significantly comparing to the control ($P < 0.05$) but the Ax-rich yeast significantly decreased drip loss: those from the HS+Ax group was not significant from the control group ($P > 0.05$). However, the meat cooking loss, WBCs, and SFV were not significant different in either heat stress or Ax-rich yeast groups comparing to the control ($P > 0.05$).

Meat color: The color changes in the broiler breast meat are shown in Table 7-6 and thigh muscles are illustrated in Table 7-7. The HS group was higher L* values comparing to the control in just after slaughter sample and 48 h postmortem samples but not significantly ($P > 0.05$). In further, there were not any significant differences in a* values between all groups ($P > 0.05$). For just after slaughter samples and 48 hours post mortem, significant increases in b* values ($P < 0.01$) were seen in samples from the HS+Ax group compared with the control and HS groups. For the thigh muscle color alteration, there were no statistically significant differences were observed between all groups in L* values and a*

Table 7-1 Composition and nutritional content of the experimental diets.

Ingredients (%)	Control		Ax[1]	
	14–21d	21–42d	14–21d	21–42d
Soybean meal	50.68	44.13	50.68	44.13
Corn starch	37.82	46.76	37.82	46.76
Soybean oil	7.24	5.46	7.24	5.46
$CaCO_3$	1.28	1.34	1.28	1.34
$CaHPO_4$	1.55	1.10	1.55	1.10
NaCl	0.47	0.35	0.47	0.35
Vitamin and mineral premix[2]	0.50	0.50	0.50	0.50
Aquasta[3]	0.00	0.00	0.20	0.20
Agar	0.21	0.21	0.01	0.01
Methionine	0.26	0.155	0.26	0.155
Calculated analysis				
CP (%)	23.00	20.00	23.00	20.00
ME (kcal/g)	3.20	3.20	3.20	3.20

[1] Ax: 20 mg/kg astaxanthin contains diet for pre-feeding for 28 days before heat exposure.

[2] Vitamin and mineral premix provided the following per kg of diet: vitamin A 300000 IU, vitamin D 40000 IU, vitamin E 2 g, vitamin K_3*$3H_2O$ 0.37 g, vitamin B_1 0.36 g, vitamin B_2 0.72 g, vitamin B_6 0.7 g, vitamin B_{12} 2 mg, pantothenic acid 2.17 g, nicotinic acid 6.94 g, biotin 0.03 g, folic acid 110 mg, choline 299.81 g, $MnSO_4$ 32.98 g, $FeSO_4$ 43.52 g, $CuSO_4$ 4.02 g, $ZnSO_4$ 19.75 g, $Ca(IO_3)_2$ 0.11 g, and MgO 198.95 g.

[3]Aquasta®: Red yeast, *Phaffia Rhodozyma*, providing astaxanthin at 10000 mg/kg (Aska Pharmaceutical Co. Ltd, Tokyo, Japan).

Table 7-2 Environment control protocol of heat exposure for two hours before slaughter.

Groups	Ambient t°, C
Control	24.5 ± 0.3
HS	33.5 ± 0.4
HS + Ax	33.5 ± 0.4

Table 7-3 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma,* on body organ weights of broiler chickens under acute heat stress.

Body organs (% of whole body weight)	Control	HS	HS+Ax
Breast muscle	15.51 ± 0.3	15.94 ± 0.42	16.04 ± 0.64
Liver	2.12 ± 0.04 [a]	1.84 ± 0.07 [b]	2.07 ± 0.11 [ab]
Abdominal fat	1.18 ± 0.08	1.33 ± 0.12	1.42 ± 0.19

Values are mean ± SE.

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Table 7-4 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on the breast meat SFV of broiler chickens under acute heat stress.

Parameters	Control	HS	HS+Ax
Shear force value$_{48}$, kg	2.93 ± 0.20	2.91 ± 0.30	2.81 ± 0.26

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

Table 7-5 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on the breast meat water holding ability of broiler chickens under acute heat stress.

Parameters	Control	HS	HS+Ax
Drip loss$_{48}$, %	0.29 ± 0.04^{a}	0.41 ± 0.04^{b}	0.35 ± 0.03^{ab}
Cooking loss$_{48}$, %	11.34 ± 0.38	10.96 ± 0.35	11.09 ± 0.45
Water binding capacity, %	86.00 ± 2.30	89.09 ± 1.27	90.41 ± 1.58
Water binding capacity$_{48}$, %	78.85 ± 0.84	81.19 ± 1.67	80.95 ± 1.06

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

Table 7-6 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat color of broiler chickens under acute heat stress, initially and at 48 h post mortem.

Parameters	Control	HS	HS+Ax
L* value	41.06 ± 0.38	42.30 ± 0.49	41.16 ± 0.42
L* value$_{48}$	49.95 ± 0.47	50.71 ± 0.51	49.19 ± 0.59
a* value	3.50 ± 0.25	3.57 ± 0.13	3.57 ± 0.17
a* value$_{48}$	3.09 ± 0.25	2.94 ± 0.17	3.66 ± 0.28
b* value	1.08 ± 0.20^{B}	1.04 ± 0.15^{B}	4.10 ± 0.38^{A}
b* value$_{48}$	4.37 ± 0.34^{B}	3.97 ± 0.36^{B}	7.00 ± 0.53^{A}

$^{A, B}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

$_{48}$ 48 h postmortem meat sample.

Values are mean ± SE.

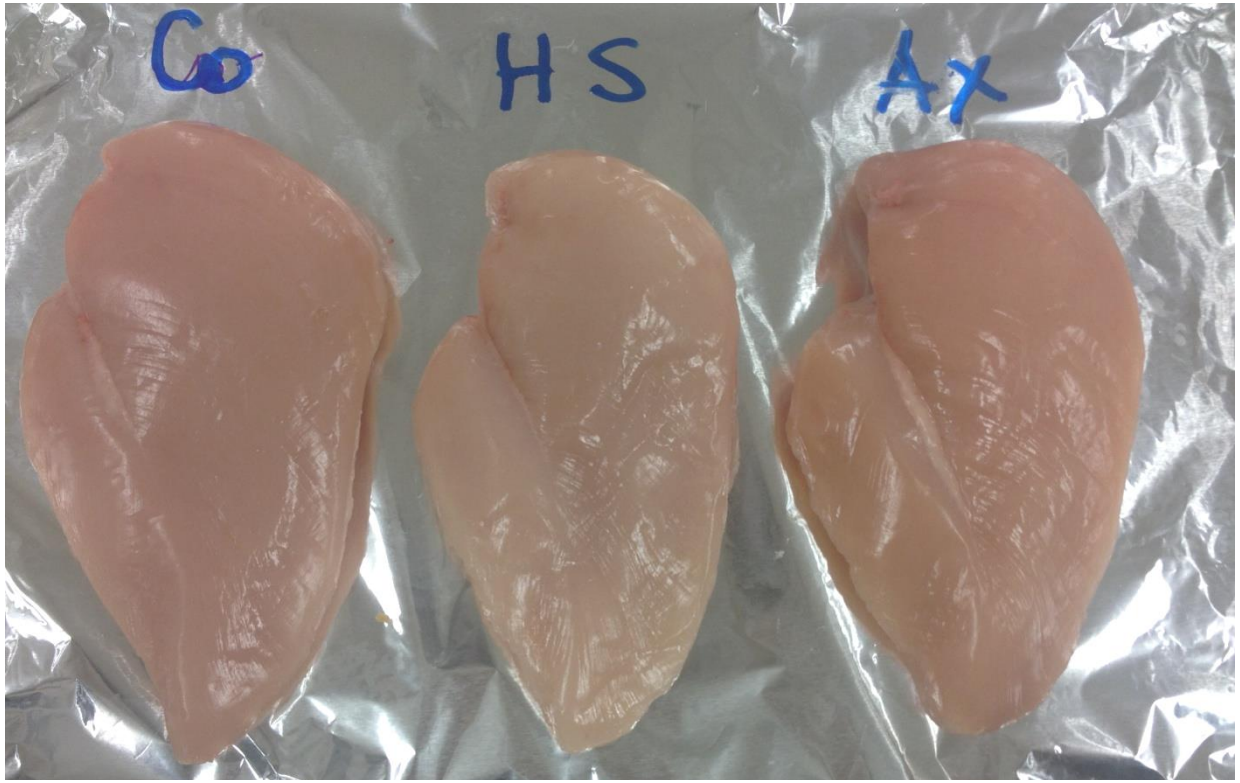


Fig 7-1 Effect of Ax-rich yeast, *Phaffia rhodozyma*, on breast meat color of broiler chickens under chronic stress, just after slaughter samples. From the left side Control group, HS group and HS+Ax group.

values ($P > 0.05$), but b* values of thigh muscles from HS+Ax group was significantly higher than control and HS groups ($P < 0.05$) (Table 7-7).

pH: This study did not revealed influence of heat stress on meat initial pH, no significant differences were observed between all groups ($P > 0.05$) (Table 7-8). However, acute heat stress significantly decrease ultimate pH values comparing to the control ($P < 0.05$), but Ax containing diet significantly inhibit heat stress effect on ultimate pH values. In addition, decrease of pH value from 0 h to 48 h in HS group was higher than others, but not significantly.

Free amino acid content: The free amino acid contents in plasma are shown in Table 7-9 and breast meat that from just after slaughter samples are shown in Table 7-10. Analysis revealed that in blood plasma the contents of Tyr, Met, Gln, Glu, Leu, Asp ($P < 0.05$), Val, and Ile ($P < 0.01$) from HS group were significantly lower than control group and HS+Ax group had also significant differences were observed in same free amino acids except Leu and Met content comparing to the control. Furthermore, Thr, Glu, and Val content in just after slaughter breast muscle samples from HS and HS+Ax groups were significantly lower than the control ($P < 0.05$).

7.4.3 Sensory attributes

The results from sensory evaluation of breast meat of broiler chickens are shown in Table 7-11. A paired comparison test of breast meat for texture attributes assessment reveals there were no significant differences between HS group and HS+Ax group. In addition to paired preference test, panelists chose HS and HS+Ax group in 50% and 50%.

7.4.4 Oxidative stress

Table 7-12 shows the effect of Ax-rich yeast on oxidative stress on broiler plasma and breast muscle under acute heat stress. Ax-rich yeast significantly decreased protein carbonyl content in breast muscle comparing to HS group ($P < 0.05$) under acute heat exposure. No significant differences were observed in MDA content either in plasma or breast muscle ($P > 0.05$).

Table 7-7 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on thigh muscle color of the broiler chickens under acute heat stress, just after slaughter samples.

Parameters	Control	HS	HS+Ax
L* value	45.43 ± 0.44	45.79 ± 0.38	42.53 ± 0.79
a* value	4.79 ± 0.29	4.17 ± 0.40	5.02 ± 0.34
b* value	1.65 ± 0.30^{B}	1.56 ± 0.35^{B}	3.98 ± 0.62^{A}

$^{A, B}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

Table 7-8 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on breast meat pH values of the broiler chickens under acute heat stress, initially and 48 h postmortem.

Parameter	Aging, h	Control	HS	HS+Ax
pH	0	6.51 ± 0.17	6.52 ± 0.08	6.47 ± 0.10
	48	5.66 ± 0.02[a]	5.55 ± 0.04[b]	5.60 ± 0.04[ab]
	Decrease	0.84 ± 0.17	0.99 ± 0.14	0.88 ± 0.14

Values are mean ± SE.

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

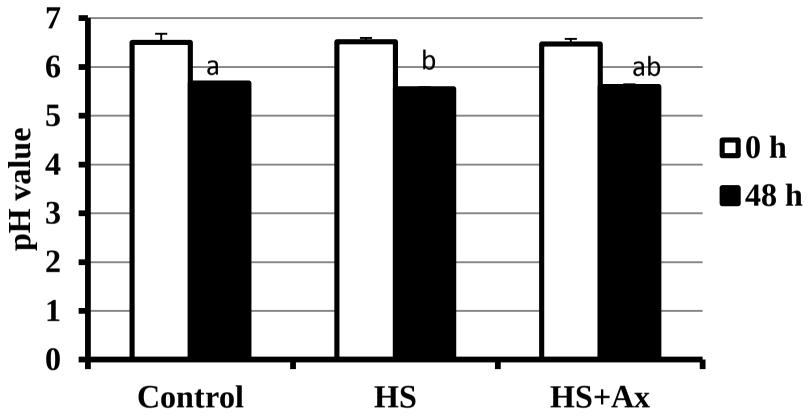


Fig 7-2 pH values of breast meat from just after slaughter and 48 h postmortem

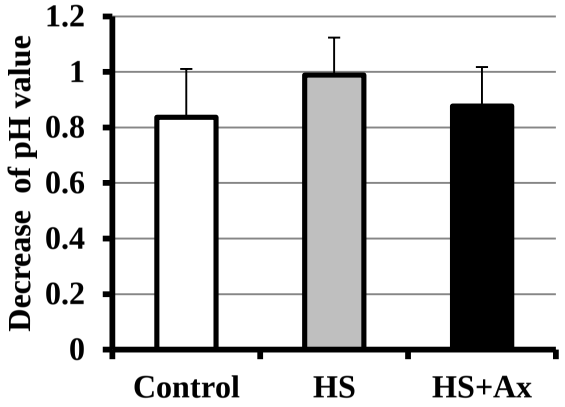


Fig 7-3 pH value decrease of breast meat during aging (from 0 h until 48 h)

Table 7-9 Effect of dietary Ax-rich yeast, *Phaffia rhodozyma*, on free amino acid content of blood plasma.

Free amino acid content, mmol/l	Groups		
	Control	HS	HS+Ax
Gly	0.66 ± 0.02^{A}	0.45 ± 0.02^{B}	0.45 ± 0.03^{B}
Ala	0.94 ± 0.06^{A}	0.55 ± 0.04^{B}	0.61 ± 0.1^{B}
Thr	0.53 ± 0.02	0.43 ± 0.007	0.44 ± 0.07
Ser	0.8 ± 0.05	0.6 ± 0.05	0.7 ± 0.08
Tyr	0.19 ± 0.01^{a}	0.14 ± 0.007^{b}	0.14 ± 0.01^{b}
Lys	0.3 ± 0.03	0.26 ± 0.03	0.30 ± 0.04
Gln	0.93 ± 0.04^{a}	0.73 ± 0.02^{b}	0.70 ± 0.08^{b}
Phe	0.12 ± 0.001	0.10 ± 0.004	0.12 ± 0.01
Arg	0.45 ± 0.02	0.37 ± 0.06	0.37 ± 0.04
Ile	0.15 ± 0.005^{A}	0.09 ± 0.006^{B}	0.10 ± 0.01^{B}
Val	0.25 ± 0.01^{a}	0.18 ± 0.01^{B}	0.19 ± 0.02^{b}
Leu	0.21 ± 0.006^{a}	0.17 ± 0.008^{b}	0.18 ± 0.01^{ab}
Met	0.057 ± 0.004^{a}	0.041 ± 0.002^{b}	0.047 ± 0.004^{ab}
His	0.073 ± 0.007	0.042 ± 0.003	0.043 ± 0.004
Asp	0.067 ± 0.003^{a}	0.051 ± 0.004^{b}	0.052 ± 0.006^{b}
Glu	0.22 ± 0.007^{a}	0.17 ± 0.004^{b}	0.16 ± 0.02^{B}
Total free EAA, mmol/l	1.71 ± 0.07^{A}	1.32 ± 0.03^{B}	1.43 ± 0.03^{B}
Total free NEAA, mmol/l	4.27 ± 0.17^{a}	3.14 ± 0.17^{B}	3.22 ± 0.3^{b}

$^{a, b}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

$^{A, B}$ Means in a row within an effect with no common superscript differ significantly ($P < 0.01$).

Values are mean ± SE.

Table 7-10 Effect of dietary astaxanthin-rich yeast, *Phaffia rhodozyma*, on the free amino acid content of broiler chicken breast meat.

Amino acid content, μg/g	Groups		
	Control	HS	HS+Ax
Gly	98.6 ± 11.7	104.1 ± 10.7	73.3 ± 11.2
Ala	186.7 ± 13.6	179.8 ± 37.0	143.7 ± 5.5
Thr	86.1 ± 1.8 [A]	71.8 ± 4.3 [B]	60.1 ± 9.6 [B]
Ser	156.2 ± 16.7	147.4 ± 20.5	143.0 ± 9.5
Lys	55.3 ± 9.4	52.1 ± 12.3	47.6 ± 7.2
Glu	246.7 ± 9.3 [a]	178.9 ± 19.4 [b]	160.5 ± 17.6 [B]
Phe	14.4 ± 1.4	13.6 ± 1.4	18.3 ± 3.0
Arg	98.9 ± 16.4	81.8 ± 1.8	80.3 ± 10.0
Ile	11.1 ± 0.9	12.4 ± 3.0	9.6 ± 1.2
Val	23.0 ± 2.7 [A]	19.2 ± 1.5 [B]	17.6 ± 1.9 [B]
Leu	13.3 ± 0.6	17.2 ± 1.8	16.8 ± 1.5
His	2.6 ± 0.4	3.2 ± 0.4	3.0 ± 0.3
Asp	33.7 ± 4.7	36.5 ± 3.8	34.7 ± 3.3
Glu	174.4 ± 17.0	145.5 ± 17.2	157.3 ± 8.8
Total free EAA, mg/g	0.20 ± 0.003	0.19 ± 0.022	0.17 ± 0.007
Total free NEAA, mg/g	1.07 ± 0.05	0.96 ± 0.09	0.86 ± 0.01

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

Table 7-11 Sensory evaluation of the texture of broiler chicken breast meat.

Parameters	HS: HS+Ax[1]	F value	Significance
First bite[2]	0.36	0.69	NS
Tenderness[3]	0.21	0.79	NS
Fibrousness[4]	-0.21	0.47	NS
Juiciness[5]	0.0	0.0	NS
Residual particles[6]	-0.07	0.07	NS

[1] Average HS+Ax group score of each subject when the HS group score is 0.00.

[2] > 0 means that the force required to compress the sample is lower.

[3] > 0 means that the force required to bite through the sample to rupture it is lower.

[4] > 0 means that degree of fibrousness is smaller.

[5] > 0 means that amount of moisture in the meat is higher.

[6] > 0 means that amount of loose particles left in the mouth after swallowing is less.

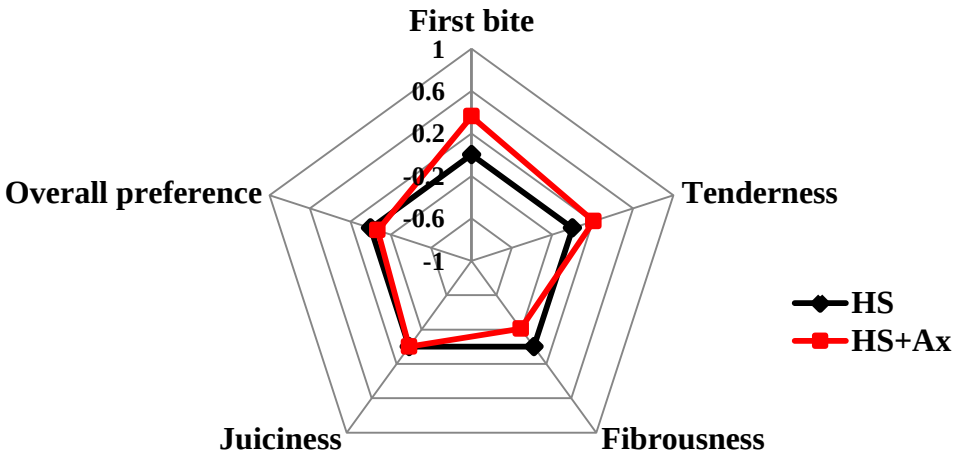


Fig 7-4 Paired comparison test of breast meat from the HS group and HS+Ax group.

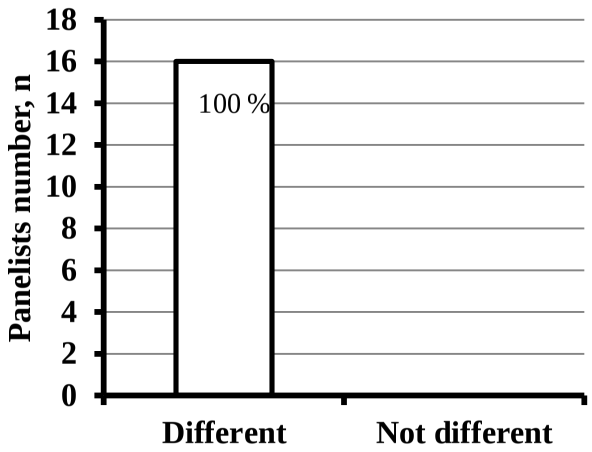


Fig 7-5 Difference test of breast meat soup from the HS group to HS+Ax group.

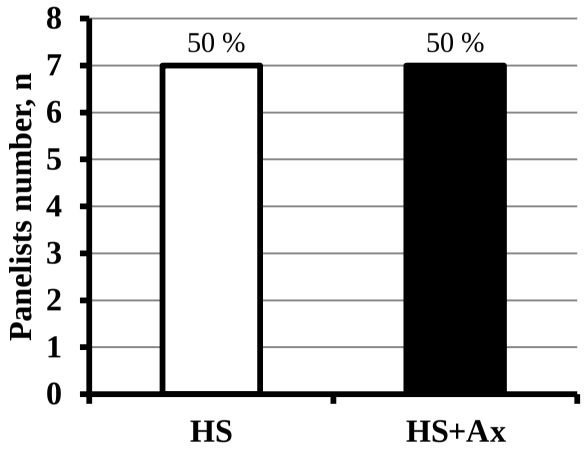


Fig 7-6 Preference test of breast meat soup from the HS group to HS+Ax group.

Table 7-12 Effect of Ax-rich yeast on malondialdehyde content and protein carbonyl content of broiler chicken plasma and breast muscle under acute heat stress.

Parameter	HS	HS+Ax
	In plasma	
MDA, μM	23.18 ± 1.46	28.05 ± 1.27
	In breast muscle	
MDA, μM	4.16 ± 0.82	4.02 ± 0.68
Protein carbonyls, nmol/ml	8.08 ± 0.37[a]	6.76 ± 0.46[b]

[a, b] Means in a row within an effect with no common superscript differ significantly ($P < 0.05$).

Values are mean ± SE.

7.5 DISCUSSION

7.5.1 Body yields

No significant differences were observed in breast meat and abdominal fat yields between all groups, but liver amount of HS group was significantly lower than control group ($P < 0.05$). Some studies reported that heat stress decreased body organ yields especially, liver, gizzard, and heart under chronic heat stress, a result of the reduction of in feed intake thereby providing less nutrients for the proper development of these organs (Shim *et al.* 2006; Rosa *et al.* 2007; Salabi *et al*. 2011). This study selected heat exposure for two hours. However, there was no report about short term heat exposure effect on body organ yields, sudden and harmful heat exposure may affect negatively on liver function.

7.5.2 Meat quality

Meat texture: Acute heat stress did not influence on SFV and Ax-rich yeast had no effect on it meat tenderness. This result agreed with some studies reported that acute heat stress does not influence meat SFV (Santos *et al*. 2008; Zhang *et al.* 2012). Santos *et al*. 2008 reported that acute heat stress did not influence myofibrillar protein fragmentation index which is main indicator of meat myofobrillar protein proteolysis. Therefore, acute heat stress has no effect on meat SFV. One of the important parameters for measuring meat quality is drip loss. Sudden short term heat exposure increase breast meat drip loss significantly, but Ax-rich yeast can significantly inhibit this effect as decreasing drip loss. This result affirmed with reports that drip loss from breast muscles increases in chickens subjected to acute heat stress (Sandercock *et al.* 2001; Wang *et al.* 2009). As mentioned

before, heat stress is due to oxidative stress includes lipid and protein oxidation which is main reason of water loss. Ax may have significant effect that inhibits oxidation processes also in acute heat exposure. This newly demonstrates that Ax-rich yeast can improve the ability of meat to retain water even under acute heat exposure. In addition, water binding capacities of muscles from heat stressed groups were not significantly different from the control group.

pH: However, acute heat stress had no significant effect on initial pH of muscle but decrease of ultimate pH value was observed. Ax-rich yeast can inhibit heat stress influence on ultimate pH value. A rapid formation of lactic acid results the rapid decrease of pH which is main reason of alteration of meat quality including decrease WHC and increase lightness. (Mckee and Sams 1997; Wang *et al.* 2009). The heat stressed birds have high R-value (McKee and Sams, 1997), which represents the ratio of inosine:adenosine-containing compounds in the muscle, and the rate of the ATP hydrolysis determines the rate of post-mortem glycolysis (Bendall 1973). The rapid increase in the R-value and a high rate of hydrolysis of ATP thus fast post-mortem energy production leading to a rapid pH decrease.

Meat color: Acute heat stress did not influence on meat lightness. This result did not agreed with some studies which initially and 24 h postmortem meat lightness significantly increased in three days heat exposure at 38°C (Mckee and Sams 1997). In our study birds were exposed to increasing temperature for two hours of 34°C. It means that heat exposure was may not enough for alteration of meat lightness. Furthermore, Ax-rich yeast had a same effect on meat yellowness with previous studies that increase of meat yellowness

either in normal ambient temperature or in chronic heat exposure. However, Ax-rich yeast had a significant effect on meat redness increase either in normal condition or in chronic heat exposure condition of rearing broiler chicken but interestingly, under acute heat exposure Ax-rich yeast cannot influence on meat redness. During the acute heat exposure, we removed feed, but it is difficult to explain that tissue accumulated Ax was disappeared only two hours heat exposure. Exact reason is not understandable.

Free amino acid content: Almost all free amino acid content in plasma from heat stressed groups is significantly lower than control group. This result agreed with report from Ostrowski (1981) that short term exposure to high temperature results in reduction in protein synthesis, a depletion of essential and non-essential plasma free amino acids. Ax-rich yeast inhibited the reduction of two free amino acids (Leu and Met), but generally had no effect on total free amino acid content in plasma. Comparing to plasma, a few free amino acid content decreased in breast muscle.

7.5.3 Sensory attributes

Sensory analysis of breast meat for meat texture attributes between HS and HS+Ax groups revealed that no significant differences were observed in all attributes. This result affirmed report of Sandercock *et al*, (2001) that exposure to acute heat stress did not affect breast meat eating quality. To date, no other studies have reported about dietary Ax-rich yeast effect on meat eating quality under acute heat stress even other antioxidants. In paired preference test of breast meat texture attributes of sensory quality, sensory evaluation

assessors divided 50:50% HS and HS+Ax group. From these results, we can assume that dietary Ax-rich yeast has no effect on meat eating quality under acute heat stress condition.

7.5.4 Oxidative stress

Our study revealed that Ax-rich yeast has significant effect on protein oxidation under acute heat stress. However, the occurrence of protein oxidation in food system has been largely unexplored but it is believed to proceed *via* a free radical chain reaction similar to lipid oxidation (Lund *et al.* 2011). Acute heat stress is result of sudden and short term high heat exposure. It increases mitochondrial ROS production via increased substrate oxidation, which is linked to mitochondrial electron transport chain, and down regulation of the avian form of mitochondrial uncoupling protein, resulting in higher damage to mitochondrial proteins and lipids (Mujahid *et al.* 2007). Even the antioxidant regulation on protein oxidation is not well discovered, but recent studies reported that dietary vitamin E can inhibit protein oxidation in turkey meat (Mercier *et al.* 1998), beef (Rowe *et al.* 2004), and pork (Ventanas *et al.* 2006) during post-mortem storage. Unfortunately there is no study about dietary Ax-rich yeast in relation to protein oxidation of broiler meat under heat exposure, even in normal ambient temperature, but some researchers revealed that the carotenoid canthaxanthin has a positive effect on protein oxidation in rainbow trout during post-mortem storage (Baron *et al.* 2009). As mentioned previously, Ax is the powerful antioxidant that is 100 times stronger than vitamin E for singlet oxygen quenching (Miki 1991). Therefore in this study, Ax may suppress ROS production and could inhibit protein oxidation under acute heat exposure. The protein oxidation in muscle food results the poor

meat quality includes discoloration, loss of water retain capability and decrease of tenderness so on. Thus protein oxidation inhibition ability of Ax-rich yeast is very important and beneficial property for broiler meat quality.

In conclusion, *Phaffia* yeast, which contains a high amount of astaxanthin, has a possibility to improve meat quality but not eating quality and the improvement rate is lower than those in chronic heat exposure.

8. GENERAL DISCUSSION

The general purpose of this study was to evaluate effect of dietary Ax-rich yeast on meat quality of the broiler chicken.

The effect of two concentrations (10 mg/kg and 20 mg/kg Ax contain) of dietary supplementation with Ax-rich yeast, *Phaffia rhodozyma*, *during long period feeding* on broiler chicken meat quality was evaluated in Chapter 3.

In Chapter 4 we evaluated the effect of high concentration (50 mg/kg and 100 mg/kg Ax contain) Ax-rich yeast on meat quality and performance of broiler during short period of feeding.

From Chapter 3 and Chapter 4, we assumed that broiler chicken growth performance effectively improved by high concentration of dietary Ax-rich yeast even in short period, and meat quality can be influenced by low concentration of dietary Ax-rich yeast but feeding is for a long period. The broiler growth performance may be affected by Ax source not from Ax directly, but meat quality improvement is only possibility to conduct dietary Ax, especially due to its strong antioxidant property. The newly demonstration about Ax-rich yeast was its ability to improve meat water retain property during cooking. Cooking loss is affected from protein denaturation under the heat. The oxidized protein is easy to denaturated. Therefore, Ax-rich yeast may decrease meat cooking loss as inhibiting protein denaturation by prevent protein from oxidation process. The breast muscle is the driest part of chicken, thus it is beneficial for make a meat more juicy and high consumer acceptance.

In addition, cooking loss is great economic importance to the catering industry except meat quality.

The Ax's main studies for chicken are particularly focused on skin, egg yolk, and meat pigmentation. However these studies mainly discovered increase of meat redness according to dietary Ax feeding. In our study obviously increase of meat redness was observed but additionally, meat yellowness increased as well as its redness. Although meat redness is related to Ax concentration in the diet because of, its greater accumulation in broiler muscles and its antioxidant property. Ax may inhibit heme pigments oxidation in meat during storage. Interesting point of this study is intensive increase of meat yellowness. However, Ax's red color affects meat redness directly, but reason of yellowness increase is unclear. Maybe it is influenced by other carotenoids found in *Phaffia* yeast or it may be related from reductive degradation products of Ax, crustaxanthin and idoxanthin, which are yellower compounds, but the exact reason is uncertain. This is may become interesting topic for further study.

The most important part of this study is the first findings that Ax-rich yeast can increase the content of free amino acid content during the postmortem storage of broiler chicken meat. It may be related to Ax's powerful antioxidant property can influence on proteolytic system of postmortem storage as inhibiting oxidation processes (enzymes oxidation of proteolytic system or amino acid oxidation) in muscle. It suggests that *Phaffia* yeast can influence meat aging as resulting improvement of meat taste. Furthermore, except improvement of meat flavor attributes, Ax-rich yeast also improve meat tenderness. It was affirmed by result of

sensory evaluation and analysis of breast meat SFV, because of the intensive proteolysis in muscle during storage results tender meat.

Chapter 5 discussed about effect of Ax-rich yeast on meat quality, growth performance and oxidative stress under chronic heat exposure. In the next study (Chapter 6) muscle metabolites changes during chronic heat stress and Ax-rich yeast influence on it was described. In final phase of this study (Chapter 7) Ax-rich yeast influence on meat quality under acute heat stress was researched.

Chapter 5, 6, 7 assumed that even it is already investigated that environmental stress, especially heat stress adversely influenced growth performance of poultry, but Ax-rich yeast diet can alleviate the effect of heat exposure on growth performance and it may be beneficial method for production of broilers in hot area. As similar effect with normal condition, Ax can increase water holding ability under heat exposure (either acute or chronic). It released in drip loss parameter which is seriously influenced by lipid and protein oxidation that are intensified in heat stress. Lipid oxidation harmfully destroys cell membrane and results loss of fluid inside the cell but oxidized proteins loss their structure and cannot hold water which is located between them. Ax-rich yeast alleviated oxidation process during heat stress and this is may influence water retain ability.

High ability to hold water is not only important for meat quality as nutrient value loss or deterioration of eating quality; it is also a great importance for the economic value of chicken products such as weight loss during the trade. The hypothesis about Ax-rich yeast effect on oxidation process was confirmed in analysis of oxidative stress parameters, MDA

content and protein carbonyl content. Ax-rich yeast inhibited lipid oxidation intensively under chronic heat stress and protein oxidation under acute heat stress. It considered that sudden and critical high heat exposure influences more effectively on protein in muscle food.

As same with normal rearing ambient temperature, meat redness and yellowness can be influenced by dietary Ax-rich yeast even under heat exposure but intensity was lower. It means that Ax may be utilized more under high ambient temperature. It was clarified by influence on meat tenderness. Ax-rich yeast has no increasing effect on meat tenderness under heat exposure, even in this time heat stress did not affect on meat texture. The heat stress effect on meat tenderness may be related to heat stress type (temperature, humidity, area and chicken population).

However some studies revealed that heat stress decreases free amino acid content in plasma and muscle, but unfortunately dietary antioxidant effect on it has been not reported yet. Our study demonstrated that acute and chronic heat stress both decrease free amino acid content in plasma and muscle and dietary Ax-rich yeast has no effect on it. Nevertheless, during the postmortem storage, free amino acid content in Ax-rich yeast diet meat has a tendency to increase more effectively than in the not supplemented broiler's meat. It means postmortem proteolysis may occur more intensively in meat from Ax fed broiler.

Sensory evaluation also confirmed that oxidation process in heat stressed meat as showing unpleasant aroma. The oxidation products of fatty acid or amino acids oxidation are aldehydes and ketones which are main contributors of off-flavor attributes, especially

unpleasant aromas. Whereas, Ax-rich yeast disappears negative effect of heat stress on aroma and can increase meat soup intensity and after taste. During cooking these compounds extracted in water and high amount results intensive taste. From these results, we can assume that dietary Ax-rich yeast may alleviate unpleasant sensory attributes which are resulted from chronic heat stress and increase taste-active compounds.

Metabolome analysis of breast muscle under chronic stress resulted that no differences in glycolysis final products which is main reason of poor meat quality. It means heat exposure may was not enough for glycolysis metabolism changes because the sensitivity of chickens to heat stress has been shown to be dependent on the age of birds. Even so, some heat stress influencing compounds such as phosphocreatine and ATP negatively regulated (but on creatine had no effect) in heat stress, but Ax-rich yeast has positive regulation on these compounds. In addition Ax-rich yeast may prevent natural antioxidant enzymatic system, because of it increase glutathione in muscle. Thus Ax-rich yeast may affect positively on muscle metabolism under chronic heat stress.

In general, there are some studies related to dietary carotenoids for color improvement of poultry shank, egg yolk and skin because of their brilliant color, but unfortunately, no study has related to other meat eating qualities. Therefore, this study is in advance that is reflecting dietary Ax-rich yeast improvement on various meat eating qualities such as meat texture, water retain ability, and sensory attributes in normal rearing condition and also reflecting acute and chronic heat exposures. Furthermore this study is including the new finding that the increase of free amino acid content in meat of broiler chickens can be

regulated by dietary Ax-rich yeast, during the postmortem storage. Finally, we hypothesized that even Ax is lipid-soluble antioxidant but it inhibits protein oxidation in broiler meat and this property is may related to its spanning location in the cell membrane that is different from other carotenoids and other antioxidants.

In conclusion, *Phaffia* yeast that contains high amount of astaxanthin is an effective dietary supplementation for the improvement of meat quality of broiler chicken at either normal ambient temperature or high ambient temperature and it is beneficial for broiler production even under hot climate area**.**

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LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 7

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

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