CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Agrobiology, Food and Natural Resources

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MUSCLE FIBRE DEVELOPMENT IN BROILER CHICKEN UNDER FEED RESTRICTION

Ph.D. Thesis

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Prague 2014

"With them the seed of wisdom I did sow,
And with my own hands laboured it to grow
And this was all the harvest that I reaped
"I came like water, and like wind I go."

Omar Khayyam

DECLARATION

This thesis details the results of original investigations undertaken on Muscle fibre development in broiler chicken under feed restriction. Except where reference is made to the work of others, the study described in this thesis is my own original work and I am the author of this thesis. This thesis has not been submitted previously for the purpose of obtaining any other degree or diploma in any other university. Data including growth and carcass composition were measured at the International Poultry Testing Station Ústrašice. Nutrient content of the diets were calculated at Institute of Animal Science Prague-Uhříněves. Muscle fibre characteristics were assessed at the university laboratory.

In Prague, date,.....

Signature,……Ahmad Teimouri…….....

ACKNOWLEDGEMENTS

Thank you **Prof. Ing. Eva Tůmová, CSc.,** my supervisor, mentor and major professor for your keen insight, guidance, vision, encouragement, trust, patience, perseverance, thoroughness, commitment, assistance, challenging, queries, advices, thoughtful comments, support, conception and design of the experiments. Thank you for pushing me to give to the study my all!

Thanks to Faculty of Agrobiology, Food and Natural Resources Department of Animal Husbandry for their valuable help and kindness.

Thanks to the International Poultry Testing Station Ústrašice for al1 their cooperation in the conduction of the experiment. Thank you for keeping such a watchful eye over my feathered friends.

Grateful thanks to my colleagues especially **Ing. Lukáš Zita, Ph.D.,** and **Ing. Zuzana Bízková, Ph.D.,** who as coworker assisted me at the farm, in the laboratory and during data preparation. More importantly, however, their friendship and support always helped me get through the rough periods when things would not work out just right. I am particularly appreciative of **Ing. Vladimíra Charvátová, Ph.D.,** for the time she spent proof reading some of my chapters and of course for friendship and helping in my experiment. I also acknowledge with thanks the helps of persons who lent me a hand when I needed it. Their memory will remain in my mind forever.

Most of all, thanks to my parents who have provided me with relentless financial supports and love as well as continuous and unwavering encouragement in all of my educational endeavours. They never doubted my ability to succeed. My father has provided continued long distance support and love. He has visited me several times in Prague. My father's persistence, eagerness and rapturous provided me with the support to complete my thesis. Lastly, special thanks to my brother for his visits and encouragement, and sisters for their motivation.

THANKS TO EVERY ONE

DEDICATION

Dedication is derived from a research project. Project of Ministry of Education of the Czech Republic No. MSM 6046070901.

TABLE OF CONTENTS

1. Introduction

Broiler chickens are the chickens raised for meat. Generally, meat is believed to have a significant role in maintaining the health and nutrition of the people. Chicken has long been recognized as an excellent source of high quality protein globally, which is nutritious, healthy and safe. Over the past 5 decades, the consumption for chicken meat has grown at an exceptional rate all over the world due to population growth, consumer preference for non red meat or healthy meat and for convenient value-added or processed chicken products which reduce meal preparation time. A major increase in the per capita consumption of chicken meat began in the late 1970s as consumers became increasingly concerned about the level of cholesterol in their diets. Breast fillet is perceived by the consumer as having a lower level of cholesterol than trimmed steak or pork chop. Not only that, chicken is lower in all saturated fats than salmon and leaner than sirloin steak, pot roast, beef tenderloin and pork chops. It is noteworthy that about half of the world's population consumes chicken nowadays. Chicken meat also has the most unique advantage of not being discriminated against due to religious or cultural beliefs. Broiler chicken industry is able to rapidly respond these high demands because of low production costs. Chicken has fast growth rate, short life span and low feed conversion ratio. Moreover, the growth of this industry has been, and continues to be, very large and very dynamic and many changes in production techniques have accompanied its worldwide expansion. It seems there is no limit to this expansion. For instance, small chicken farms have been replaced to some extent by larger highly specialized types of poultry companies. Similarly, broiler chicken industry has been typified with unprecedented growth in terms of production.

Chicken meat representing about 85.6% of the total poultry meat output that this percentage has been rather constant during the last 40 years. The forecast for world broiler chicken meat production for 2013 was 83.5 million tons. However, this production in 2013 (April) is revised upward by 1.1 million tons to 84.61 million tons with the majority of growth provided by the United States, Thailand and Russia. World total broiler chicken meat consumption was 83.25 in 2013 (April). Over 6 billion broiler chickens are slaughtered for meat in the EU-27 each year, producing around 9.4 million tones of chicken meat, with an average per capita consumption of 17.4 kg/year. Total broiler chicken meat consumption was 9.2 million tons in 2013 (April) for the EU-27.

The broiler chicken industry is changing. Today's industry is the multiform world industry in which chicken producers have products with characteristics according to customer demand which may differ per region. So, the broiler chicken industry is a consumer driven market. Nonetheless, everywhere the focus of the industry is on bottom line performance. Live performance of broiler chickens has been improved tremendously since the early 1950s mainly due to selection for rapid early growth, improvements of nutrition, rearing environment so that it takes only 35 days to reach finishing body weight of about 2 kg. Therefore, these modern broiler chicken strains are characterized by very high growth rate and low feed conversion ratio. Unfortunately, this increase in growth rate is associated with high body fat deposition, high mortality and high incidence of metabolic diseases and skeletal disorders. These negative aspects are major concern for the farmer and processor, because they can bring about important economic losses. These situations most commonly occurs with broiler chickens that consume feed *ad libitum*. It is generally assumed that the faster chickens reach market weight, sooner, the better the feed conversion. While this is usually true, there may be some potential for modifying the growth pattern of the chicken in favor of an even greater reduction in maintenance requirement. Feed restriction maybe used for this purpose. Feed restriction programs are strategies that can be used to alter feeding management in order to decrease some extent feed intake and therefore modify to the growth rate, alleviating the occurrence of metabolic disorders, improving feed efficiency and decreasing the meat cost.

The broiler chicken industry no longer has the single objective of achieving an acceptable economic performance. Increasingly, they are having extra objectives such as maintaining and improving meat quality and quantity and so on. For example, a growing interest in obtaining meat products with specific quality traits has been observed lately among broiler chicken producers. The continuous demand for these aims in broiler chicken production of today and tomorrow calls for undergoing considerable scrutiny and commitment to transparency in muscle growth and development and subsequent meat quality and quantity. Since muscle fibres are the major component of muscle, a better understanding of their characteristics are very important for both quality and quantity characteristics of muscle as meat. They have great effect on meat quality and quantity and the effect of different factors such as chicken's age, sex, genetics strain and plan of nutrition (e.g., *ad libitum* or feed restriction) on muscle fibre characteristics, growth and carcass composition would influence broiler chicken producer profit and consumer health and nutrition.

2. Literature Review

2.1. Feed restriction in broiler chickens

The commercial broiler chicken is the product of selection over many generations for rapid growth and enhanced muscle mass (Richards *et al.*, 2003). Genetic selection and improved nutrition and chicken management have increased the growth rate of broiler chickens by over 300 percent from 25 g per day to 100 g per day in the past 50 years (Knowles *et al.*, 2008). The most dramatic increase in growth occurs in the first four weeks post hatch (Zubair and Leeson, 1996a). Selection for rapid growth and enhanced muscle mass has been accompanied by an increase in voluntary feed intake, resulting in chickens that do not adequately regulate feed intake to achieve energy balance (Richards *et al.*, 2003). Therefore, chickens consume energy at two or three times greater than their maintenance needs and so high body fat deposition often accompanies this early rapid growth phase (Zubair and Leeson, 1996a; Tůmová *et al.*, 2002; Lippens, *et al.*, 2009). This fact is of economical concern because fat represents an undesirable and uneconomical product (Urdaneta-Rincon and Leeson, 2002). It is well documented in human studies that high dietary fat intake has related to the incidence of cardiovascular diseases and cancer (Zubair and Leeson, 1996a). To produce a leaner chicken and reduce the unfavorable effects of fat on human health, there is interest in the broiler chicken industry to reduce fat deposition in broiler chicken carcasses (Urdaneta-Rincon and Leeson, 2002).

Rapid growth rate and high muscle mass have increased chicken oxygen needs more than oxygen supplies by heart and lungs and high oxygen requirements of tissue result in metabolic disorders. Also, high growth rate cause an abnormal cartilage mass in long bones which is compressed by high body weight as the chicken walks, causing painful lameness and skeletal disorders. So, rapid growth rate and high body weights are correlated with a significant increase in metabolic and skeletal disorders threaten the viability of the industry (Julian, 1998; Lippens, 2003; Leeson, 2007). Thus, excessive fat accumulation and an increased incidence of skeletal and metabolic diseases often accompany early rapid growth phase in broiler chickens (Sahraei and Mohammadi Hadloo, 2012). For these reasons, fast growth rate of broiler chickens has been blamed for welfare concerns and then the broiler chicken industry has attempted to find the solutions to these concerns. These situations most commonly observed in broiler chickens that consume feed *ad libitum* (Pasternak and Shalev, 1983; Urdaneta-Rincon and Leeson, 2002). Research has shown that a practical solution for these concerns is to slow down the early growth

by feed restriction methods (Baghbanzadeh and Decuypere, 2008; Waldroup, 2011; Svihus *et al*., 2013). Slower growth rate at certain times may be important for optimizing skeletal development. In addition, slowing rate of growth of a chicken during the second to third week of age might alter growth of fat cell which accounts for the most growth of fat tissue. In general, the objective of feed restriction is to reduce nutrient intake for a specified period and determine growth performance during the subsequent realimentation period, which broiler chickens are provided adequate nutrients. In this manner, compensatory gain to a final body weight similar to that of *ad libitum* broiler chickens is possible (Santoso, 2003; Dilger *et al*., 2006; Sahraei *et al*., 2012). A period of feed restriction may, therefore, be beneficial to the broiler chicken industry, not only in combating metabolic and skeletal disorders associated with fast growth rate, but also in placing limits on fat cell size or hyperplasia. For example, the use of such strategies has been shown to reduce incidence of skeletal disorders (Robinson *et al*., 1992; Camacho *et al*., 2004; Wijtten *et al*., 2010), ascetic mortality (Julian *et al*., 2005; Baghbanzadeh and Decuypere, 2008; Singh *et al*., 2011), and sudden death syndrome (Gonzales *et al*., 1998; Julian *et al*., 2005) in fast growing broiler chickens. In addition, feed restriction has been demonstrated potential for reducing feed conversion ratio (Jones and Farrell, 1992; Zubair and Leeson, 1994a) and abdominal fat (Plavnik and Hurwitz, 1985; Leeson and Summers, 2005; De Silva and Kalubowila, 2012). Generally, feed restriction induces compensatory growth, improves feed efficiency, reduces deposition of fat and reduces metabolic and skeletal disorders in the broiler chickens. Also, feed restriction strategies are proven to be effective in increasing the growth performance and carcass parameters of broiler chickens (Zubair and Leeson, 1996a; De Silva and Kalubowila, 2012; Sahraei *et al*., 2012).

The growth curve of a broiler chicken follows a sigmoid or S shaped pattern when weight of the chicken is plotted against time; growth rates change throughout development from an accelerating phase post hatch to a maximum growth rate, followed by a decelerating phase (Knížetová *et al*., 1983; Knížetová *et al*., 1995; Lippens, 2003). It suggested that broiler chickens having a concave-shaped growth curve need less feed than those exhibiting a convex-shaped growth curve. The more concave growth curve the better save maintenance energy requirements. It has been demonstrated that feed restriction in second week post-hatch may produce a concave-shaped (growing slowly initially and faster later) type of growth curve in broiler chickens,

resulting in complete compensatory growth (Plavnik and Hurwitz, 1985; Yu and Robinson, 1992; Velleman *et al*., 2010).

Some of the important hormones involved in growth and development are insulin, growth hormone (GH), Insulin-like growth factor 1 (IGF-1), thyroid hormones, glucocorticoids, and the sex steroids (Hossner, 2005). In poultry, the two main hormones required for the full expression of growth are growth hormones (GH) and triiodothyronine (T3) (Decuypere and Buyse, 2005; Scanes, 2011). There is strong evidence that the effects of GH and thyroid hormones are mediated by hepatic production of insulin-like growth factor-1 (IGF-1). GH and T3 are associated with protein synthesis and energy production and can increase the metabolism rate and the need of oxygen and heat production of broiler chickens (Li *et al.*, 2011). It has been shown that feed restriction reduces serum T3 concentrations (Spaulding *et al*., 1976; Navidshad *et al*., 2006). Previous research in broiler chickens showed that feed restriction may modify the plasma levels of T3 and GH to modulate the energy metabolism and growth (Mcmurtry *et al*., 1988; Zhan *et al*., 2007). Gonzales *et al*. (1998) found that during the period of feed restriction, plasma T3 and IGF-1 concentrations decreased whereas plasma T4 and GH concentrations increased. Likewise, studies by Giachetto *et al*. (2003). Decuypere and Buyse (2005) and Li *et al*. (2007) indicated that feed restriction reduces growth in chicken accompanied by a significant fall in circulating IGF-1 and a rise in plasma GH which are restored to the normal levels by refeeding.

As mentioned earlier, rapid early growth of modern broiler chickens has resulted in increased occurrence of metabolic diseases and skeletal disorder (Robinson *et al*., 1992; Dozier *et al*., 2003; Leeson and Summers, 2005). The three main metabolic and skeletal disorders affecting today's broiler chickens are ascites, sudden death syndrome and tibial dyschondroplasia (Angel, 2007). These metabolic diseases and skeletal disorder are most specifically related to rapid growth (likely because of high metabolic rate and a requirement for very rapid long bone growth) as it is common in broiler chickens (Julian, 1998, 2005; Leeson and Summers, 2005). Control over metabolic diseases and skeletal disorder has led to the industry recommendation of early feed restriction in order to slowing fast growth (Bölükbasi *et al*., 2004; Leeson and Summers, 2005; Butzen *et al*., 2013). The logic is that short term feed restriction applied early in life modify growth curve and this would allow the chicken to restore balance between supply and demand organs (Velleman *et al*., 2010).

When considering feed restriction for slowing early growth, methods can be subdivided in quantitative and qualitative restrictions (Lippens, 2003). Methods of feed restriction vary between countries and even chicken producers. Previous studies has examined different strategies of feed restriction including physical feed restriction, feeding time restriction (e.g., skip-a-day programs, intermittent feeding), lighting program, and diet dilution, the use of low protein or low energy diets, the use of appetite suppressants (calcium propionate) and feed form. Currently, Ali *et al*. (2011) indicated that skip-a-day, diet dilution and intermittent feeding methods during the day may be feasible methods for broiler chickens under heat stress in summer without adverse effect on performance. While each method of feed restriction highlights a different set of pros and cons, a solution that fits both economic and welfare concerns has not yet been found. So, the selection of a suitable feed restriction method is very difficult. Nevertheless, the use of lighting programs, feeding time restriction, mash feed and adding whole wheat to ration are easier than other methods. According to Butzen *et al*. (2013), early restriction programs either by physical feed restriction or feeding time restriction can be used as a method for controlling growth rate in broiler chickens without any damage to performance and meat quality. Generally, the choice of a proper feed restriction technique depends upon simple logistics of prevailing feeding and management systems.

A quantitative feed restriction means that a limited amount of a well balanced diet, with normal nutrient density, is offered to the broiler chickens (Lippens, 2003). A simple quantitative feed restriction (physical feed restriction) provides a calculated quantity of feed per broiler chicken and is one of the most commonly used methods (Zhan *et al.*, 2007). Theoretically, this is the simplest form of nutrient restriction, yet a number of practical problems exist. For example, chickens eat very little feed on a daily basis at this age, and so, daily allocation must be precise, with accurate weighing and feed distribution. Quantitative feed restrictions include physical feed restriction, feeding time restriction (e.g., skip-a-day programs) and lighting program. Quantitative feed restriction has been observed to reduce mortality and culling (Fontana *et al.*, 1992; Robinson *et al.*, 1992), improve feed conversion ratio (Plavnik and Hurwitz, 1988; Fontana *et al.*, 1992; Deaton, 1995; Lee and Leeson, 2001) and allow a complete recovery of body weight if the degree of restriction was not too severe and slaughter ages were extended beyond 6 weeks (Plavnik and Hurwitz, 1988; Deaton, 1995). Khantaprab *et al.* (1997) restricted broiler chickens to 80% or 60% *ad libitum* intake during the age 1 to 50 days and indicated that restriction of feed intake

significantly reduced body weight gain. Lippens *et al.* (2000) demonstrated that broiler chicken restricted to 90% or 80% *ad libitum* from day 4 to day 7 had significantly lower body weight than control group. They also reported that treatment 90% *ad libitum* had no significant effect on body weight. Tůmová *et al.* (2002) indicated an accelerated growth rate on the previously restricted chickens at the age of 21 days resulting in a similar daily weight gain with full-fed cockerel, and from the age of 35 days daily weight gain of the previously restricted chickens was higher at about 15% than in full-fed broiler chickens. Urdaneta-Rincon and Leeson (2002) found that broiler chicken restricted from 5 to 42 day by giving 95, 90, or 85% of *ad libitum* intake had lower body weight at the slaughter age than *ad libitum* group. Saleh *et al.* (2004, 2005) showed that feed restriction at 6.27 kJ/kg×$W^{0.67}$ from 7 to 14 day significantly reduced body weight at the end of experiment. Özkan *et al.* (2006) stated that broiler chickens restricted to 50% normal growth from 5 to 11 days had a greater growth rate from 12 to 39 days of age than the control group. Jang *et al.* (2009) gave broiler chicken to 85% or 70% of *ad libitum* intake from day 8 to day 14 and reported that body weight gain was not affected by feed restriction at the end of experiment. Lippens *et al.* (2009) restricted broiler chickens to 80% of *ad libitum* intake from day 4 to day 7 and reported that growth of the *ad libitum* broiler chickens were not significantly different from that of their restricted counterparts at 42 days of age. Wijtten *et al.* (2010), restricted broiler chickens to 85% of *ad libitum* intake from day 4 to day 14 of age and thereafter gradually diminishing this restriction to 95% of *ad libitum* intake at 21 days of age. The results of study showed that body weight gain was similar for all groups from 14 to 36 d of age. Li *et al.* (2011) restricted broiler chickens to 90%, 80%, 70% of *ad libitum* intake from day 5 to day 12, day 5 to day 15, day 5 to day 19, day 7 to day 14, from day 7 to day 17, day 7 to day 21, day 10 to day 17, day 10 to day 20, from day 10 to day 24 and indicated that average daily gain feed restricted chickens was lower than *ad libitum* chickens except feed restricted chicken to 70% of *ad libitum* day 10 to day 20 during 24-35 days. They also reported that feed restricted chickens to 90% of *ad libitum* from day 5 to day 12 had highest daily weight gain and feed restricted chickens to 70% from day 10 to day 24 had lowest daily weight gain during 36-42 days.

Feed restriction including limiting the time of daily access to feed removal of feed for up to 8 h a day or skip-a-day feeding, allowing chickens to feed only once/h and feeding once every other day can be used as an alternative (Azis, 2012). Feeding time restriction is a feed restriction schedule which chicks have daily free access to feed for 4 and 8 hours per day in specific time. It

is an alternative to lower intensity of early feed restriction (Zhan *et al*., 2007; Onbaşilar *et al*., 2009; Onwurah and Okejim, 2012) and it is less stressful (Susbilla *et al*., 2003). Mohebodini *et al*. (2009) indicated that chickens had free access to feed during four periods of 2 h (06:00-08:00, 12:00-14:00, 18:00-20:00, and 24:00-02:00) from 7-21 days of age had lower weight gain than control, but had no difference on weight gain than control during realimentation period from 22-42 days of age. Svihus *et al*. (2010) and Svihus *et al*. (2013) restricted time access to feed broiler chickens from day 7, with 4 1-h feeding times/day and one 2-h feeding times/day from day 14. It was concluded that broiler chickens fast adapt to intermittent feeding without reduction in final body weight and with improvements in feed efficiency.

Skip-a-day feeding programs providing limited allotments are widely used in broiler breeder growth restriction programs. However, information on the influence of skip-a-day feeding to restrict rapid early growth of broiler chickens is relatively sparse (Dozier *et al.*, 2002). During the period of skip-a-day restriction, the broiler chickens are fed on alternate days. Skip-a-day feed restriction programs during starting period can reduce the incidence of ascites syndrome without compromising body weight gain or feed conversion (Arce *et al.*, 1992; Ballay *et al.*, 1992). Oyedeji and Atteh (2005a) concluded that skip-a-day feeding for 3 weeks starting at day-old would improve carcass quality and reduce sudden death syndrome which is often associated with chickens that are on *ad libitum* feeding. Dozier *et al.* (2002, 2003), Lien *et al.* (2008), Saffar and Khajali (2010) found that skip-a-day feed removal significantly reduced growth without adverse effect on performance of broiler chickens. Benyi *et al*. (2009) suggested that for efficient broiler production in the tropics, Ross 308 could be used under a skip-a-day feeding program for 14 days during the starter or grower period.

As a type of feed restriction, lighting programs may be used. Lighting is the simplest method among feed restriction methods. Continuous lighting (23L:1D) provides for maximum growth; however, additional mortality in the chickens due to metabolic disorders and leg abnormalities, plus loss of feed efficiency with increased physical activity, has led to the use of other lighting schedules. Therefore, continuous exposure to light (23-24 h) has detrimental effects on growth and production. Intermittent lighting and restricted lighting schedules have been used for slowing growth rate of broiler chickens (Lewis and Morris, 1998; Brickett *et al*., 2007; Sahraei, 2012). The original research by Classen (1988, 1990, 1992) demonstrated that using extended daily dark periods (6 h L:18 h D) between 3 and 14 d of age reduced the problems

associated with rapid growth. By increasing day length after 14 d of age, broiler chickens grew faster and were generally able to attain equal market weight (by 42 or greater days of age) to those chickens maintained on a constant 23 h light. Classen and Riddell (1989) noticed that broiler chicks exposed to 6 hours light:18 hours dark (6L:18D) from three to 21 days of age consumed significantly less feed compared to chickens exposed to continuous lighting (23L:1D). Reducing the light period to six hours in a twenty-four-hour day had no effect on overall feed conversion ratio and body weight. Lighting programs providing short day lengths prior to 3 weeks of age, have been observed to decrease rapid early growth, leg abnormalities, ascites, sudden death syndrome, abdominal fat, improve feed conversion ratio and enhance immune function. Restricted lighting programs enhance broiler production through improvements in body weight, feed conversion ratio and health. Similarly, it reduces abdominal fat (Olanrewaju *et al.*, 2006). Ohtani and Leeson (2000) found that broiler chickens grown under intermittent lighting (1 h L: 2 h D) had significantly higher body weight gain than chickens under continuous lighting during the period of 3 to 6 weeks of age. Ingram *et al*. (2000) determined the effect of light restriction (12L:12D) from day 3 to day 42 on broiler chickens performance. The results of this experiment showed that light restriction significantly decreased body weight but significantly improved feed conversion.

Qualitative feed restrictions include diet dilution by addition of an inert ingredient to a complete diet (Leeson *et al.*, 1991), chemical methods and deficiencies in certain nutrients or low energy and/or low protein diets. Polin and Wolford (1973) reported that chickens can overeat as much as 34.1% to compensate for diets diluted with oat hulls. Leeson *et al.* (1991) and Jones and Farrell (1992) used 50% to 65% diet dilution with rice hull in order to retard early growth. This technique appeared to be successful, and even though these chickens consumed more feed, adjustment was insufficient to normalize nutrient intake, and so growth rate was reduced. Chickens showed complete growth compensatory at the end of the experiment. These results are in agreement with those of Zubair and Leeson (1994a), who showed no difference in body weight at either 42 or 49 days when chickens were fed a 50% oat-hull diluted diet for six days. In another trail, Leeson *et al.* (1992) offered broiler chickens a conventional finisher diet diluted up to 50% with a 50:50 mixture of sand: oat hulls from 35 to 49 days of age, and showed no significant difference in body weight at 49 days or breast weight at 42 or 49 days of age. Cabel and Waldroup (1990) indicated that diluting the starter diet with sand from 5 to 11 days of age

moderately restricted growth, which was completely recovered by 49 days of age. Madrigal *et al.* (2002) observed that feeding a diet containing 60% rice bran from 7 to 21 days of age had no adverse effect on body weight in the end of experiment. Teimouri *et al.* (2005) showed that diet dilution to 20% with wood charcoal from day 8 to day 14 had no significant effect on performance of broiler chickens. Diet dilution can be achieved by combining the whole wheat with commercial diets (Taylor and Jones, 2004).

The use of chemicals during the early period of growth may also depress the feed intake of broiler chicken. Fancher and Jensen (1988) first suggested restriction of feed intake by chemical means as an alternative for diet dilution. These authors examined glycolic acid to restrict feed intake in broiler chickens. According to Pinchasov and Jensen (1989), the inhibitory mechanism of glycolic acid acts through the brain serotonergic.

Sodium is an essential nutrient known to influence several aspects of normal animal growth. A sodium-deficient diet also is known to reduce the appetite of the broiler chickens (Plavnik and Hurwitz, 1990; Meluzzi *et al.*, 1998; Lippens *et al.*, 2003). Sodium deficiency leads to reduced growth and feed consumption and impairs feed conversion (Vieira *et al.*, 2003). An alternative for sodium-deficiency is the use of low protein or low energy diets (Plavnik and Hurwitz, 1990).

A chicken whose growth has been slowed by feed restriction may exhibit an enhanced rate of growth when realimented. If this exceeds the maximal rate of gain when adequate nutrition has been provided, the chicken is said to have undergone compensatory or catch up growth (Zubair and Leeson, 1996a). This faster rate of growth relative to age termed compensatory growth (Bohman, 1955) or catch-up growth (Prader *et al.*, 1963). As mentioned in Yu and Robinson (1992), catch-up growth is a more precise term because the word compensatory suggests excessive growth of a body part in compensation for the loss of part of its function. Hence, depending on the authors and their preference, these terms are used interchangeably. Compensatory growth has been shown to occur in most farm animals, even the broiler chickens, which has a very short grow-out cycle. This phenomenon has long been recognized as having the potential to have profound effects on the rate of growth and body composition of most animals. Two theories have been proposed to explain how compensatory growth is regulated (Zubair and Leeson, 1996a). First, compensatory growth mechanisms may involve a set-point or reference for body size appropriate for each age and that the control is regulated by the central nervous system

(Wilson and Osbourn, 1960; Mosier, 1986). The second theory relates to so called peripheral control which suggest that tissues *per se*, control body size through cell number or by the total content of DNA (Winick and Noble, 1966; Pitts, 1986). McMurtry *et al.* (1988) stated that changes in the weight gain composition, higher efficiency of energy utilization, and reduction in maintenance requirements, or a combination of these factors, contribute to the phenomenon of compensatory growth. The key mechanisms of compensatory growth are decreased maintenance requirement. The reduction in maintenance costs would then allow for comparatively more energy for growth upon realimentation, thus contributing to the compensatory growth responses (Ryan, 1990; Rowan *et al.*, 1996; Benschop, 2000). Feed restriction will reduce the maintenance requirements by reducing the energy loss (total heat production) and the basal metabolic rate and the specific dynamic action of feed (Zubair and Leeson, 1994b). The energy and nutrients which support compensatory growth may come from the reduction of maintenance requirement include four components: basal metabolic rate (BMR), specific dynamic action (the transient increase in metabolic rate that accompanies digestion of a meal), energy for activity, energy for maintenance of body temperature (Yu and Robinson, 1992; Leeson and Summers, 2001). In the other word, when refed, the concomitant compensatory growth is characterized by increased intakes and improved efficiency of energy and protein utilization due to an accelerated tissue metabolism (Buyse *et al.*, 1996), a reduced maintenance requirement and an activated endocrine status. Numerous hormones are directly or indirectly involved in the metabolic responses to feed restriction and the subsequent period of refeeding. There are e.g., fast increases in plasma concentrations of insulin (Yambayamba *et al.*, 1996), triiodothyronine (T3, Nir *et al.*, 1996; Buyse *et al.*, 2000), growth hormone and insulin-like growth factor-I (IGF-I, Kühn *et al.*, 1996; Buyse *et al.*, 2000) during the refeeding and compensatory growth phase. Indeed, these hormones are all known to be regulated by diet and to promote protein accretion and growth rate (Grizard *et al.*, 1999).

Whether market broiler chickens achieve total compensatory growth following an early feed restriction is open to question. In general, a significant compensatory growth following early feed restriction has been indicated by numerous studies in broiler chickens (Dozier *et al*., 2003; Mahmood *et al.*, 2005; Khajali *et al.*, 2007). However, other authors showed that feed restriction did not result in significant compensatory growth (Li *et al.*, 2007; Lippens *et al*., 2009; Wijtten *et al*., 2008, 2010). Others reported that complete growth compensation was not attained by the

chickens subjected to early feed restriction (Urdaneta-Rincon and Leeson, 2002; Saleh *et al.*, 2005; Chopde *et al.*, 2008). To understand the differences in the results, it is necessary to examine the phenomenon of compensatory growth and some of the factors that influence response of broiler chickens to a short term feed restriction and refeeding. The inconsistency between these findings could be attributed to several factors. It is well documented that these factors include not only the nature, timing, duration and severity of the feed restriction and length and nutrient composition of realimentation period but also genetic factors, such as strain and sex (Zubair and Leeson, 1996a; Lawrence and Fowler., 2002; Andersen *et al*., 2005).

Time of imposing a feed restriction program is important because the later that chickens are feed-restricted the less the opportunity to achieve desirable productive performance. Restricting feed intake of broiler chickens in the final stages (5-8 weeks) of production allows little or no time for compensatory growth to occur. Benyi and Habi (1998) feed-restricted chickens from 4 to 8 weeks of age and showed that feed-restricted chickens were not able to achieve normal final body weight at 56 d. Sahraei and Shariatmadari (2007) compared the effects of diet dilution with sand and wheat bran (in levels, 0, 7, 14, 21 or 28%) on broiler chicken performance during finisher periods from 35 to 45 days of age. The results of study showed that live weight (at 45 days of age), body weight gain only in 28% levels were less than control chickens. Some researchers suggest that the most favorable time to apply a feed restriction program is during the second week, rather than later (Robinson *et al*., 1992). Plavnik and Hurwitz (1988) suggested that feed restriction programs may start at 6 days of age, and continue no longer than 7 days in order to allow chickens to attain growth compensation by 49 days of age. Feed restriction programs beginning at an earlier age rather than later seem to be more beneficial to achieving the objectives on the performance response of broiler chickens. As it mentioned earlier, it has been demonstrated that feed restriction in second week post-hatch may produce a concave-shaped (growing slowly initially and faster later) type of growth curve in broiler chickens, resulting compensatory growth (Plavnik and Hurwitz, 1985; Yu and Robinson, 1992; Velleman *et al*., 2010).

It is generally recognized that with an extended period of feed restriction it is more difficult for broiler chickens to achieve complete growth compensation and so attain normal market body weight. Urdaneta-Rincon and Leeson (2002) evaluated the effect of quantitative feed restriction on broiler chicken from 5 to 42 days. Results from experiment indicated that

restricted chickens had significantly lower weight gain at 49 days than *ad libitum* broiler chickens. In order to obtain complete catch up in growth, McMurtry *et al.* (1988) recommended restricting broiler chicken males for no more than seven days and females no longer than five days.

The length of time allowed for refeeding may influence compensatory growth. Most studies that have reported complete growth compensation either used milder undernutrition programs or growth periods were to extend to at least 56 days of age (Plavnik and Hurwitz, 1985, 1988, 1991; Plavnik *et al*., 1986) but may not compensate fully if slaughtered at 42 days of age (Su *et al*., 1999; Whitehead, 2002). It is not clear at this point whether growth compensation observed in more prolonged growth studies is simply due to early plateau in growth of the *ad libitum* chickens. As the severity of the restriction, as well as its duration, may also influence the length of the refeeding period, it seems that the shorter the duration of feed restriction, the easier it is for the chicken to recover from a growth deficit.

Male and female broiler chickens differ in growth rate and body fat content (Leenstra, 1986; Rehfeldt *et al.*, 1997). Generally, male broiler chickens have a greater growth rate and leaner body composition than do female broiler chickens (Rehfeldt *et al.*, 1997). It seems reasonable to assume that the nutritional requirements of the male broiler chicken may differ from that of the female, since the male grows at a faster rate (Proudfoot, 1973). As a result of the difference in body weight gain, female broiler chickens are frequently housed separately from males to facilitate slaughtering at different ages. This housing method would facilitate feeding the sexes different diets if warranted (Proudfoot and Hulan, 1978). Male and female also may respond differently to compensatory growth. For example, male broiler chickens have been shown to have a greater ability to exhibit compensatory growth than females. This is likely due to the higher innate rate of growth of male broiler chicken and their lower deposition of body fat. Plavnik and Hurwitz (1985, 1990, 1991) obtained complete compensatory growth with male broiler chickens, but they were unable to demonstrate catch up growth in female broiler chickens. Lippens *et al.* (2000), Dagaas and Bustria (2001), Tůmová *et al.* (2002) and Novele *et al.* (2009) reported that cockerels have a greater ability to establish compensatory growth than pullets. However, Deaton (1995) found no significant difference in growth rate between either male or female chicken after feed restriction. Plavnik and Hurwitz (1988) recommended that different responses of male and female broiler chickens to compensatory growth can be exploited by

restricting mixed-sex broiler chickens at seven days or earlier. In addition, using sex separate system can be useful in order to obtain higher compensatory growth.

Most studies of feed restriction in broiler chickens have been concerned with feed efficiency and body fat. Feed restriction in early stage is beneficial for improving the feed efficiency. Urdaneta-Rincon and Leeson (2002) indicated that broiler chicken restricted to 90% *ad libitum* from day 5 to day 30 had a significant improvement on feed conversion ratio than *ad libitum* ones at 42 days of age. Also, Pinherio *et al.* (2004), Yagoub and Babiker (2008) Onbaşilar *et al*. (2009) and Rezaei and Hajati (2010) demonstrated that feed efficiency improved in restricted broiler chicken compared with the *ad libitum* ones. Saleh *et al.* (2004, 2005) reported that feed restriction at 6.27 kJ/kg×$W^{0.67}$ from 7 to 14 day significantly improved feed conversion ratio but body weight was compromised. On the other hand, Lippens *et al.* (2000), Urdaneta-Rincon and Leeson (2002), Camacho *et al.* (2004), Zhan *et al.* (2007) and Chopde *et al*. (2008) showed that there is no significant different overall feed conversion ratio between restricted and full-fed broiler chickens. The superior feed conversion ratio *ad libitum* chickens over the feed restricted broiler chickens during realimentation has been previously cited in other trials (Urdenta-Rincon and Leeson, 2002; Jang *et al.*, 2009; Khetani *et al.*, 2009, Jalal and Zakaria, 2012).

The effect of feed restriction programs on carcass composition has been studied. McGovern (1999) mentioned that quantitative feed restriction reduced breast muscles weights. Lippens *et al.* (2000) noted that broiler chickens restricted to 80% *ad libitum* from day 4 to day 11 had significantly lower carcass yield and thigh percentage than *ad libitum* group. Dozier *et al.* (2002) indicated that subjecting broiler chickens to 4 days of feed removal had no adverse effect on carcass yield. Nielsen *et al*. (2003) showed that feed restriction could decrease fat content and increase protein deposition in carcass, thus resulting in the improved carcass composition. Lippens *et al.* (2003) demonstrated that qualitative feed restriction either a low energy diet or low NaCl from day 4 for 4 days had no significant effect on carcass and breast meat yield of broiler chickens. Saleh *et al.* (2005) restricted broiler chickens to an energy intake 6.3 kJ/kg×$W^{0.67}$ from 7 to 14 d and showed that dressing percentage was significantly reduced. They also found no significant difference in breast yield between feed restricted chickens and *ad libitum* chickens. Khajali *et al.* (2007) reported that skip-a-day feeding at 9, 11 and 13 days of age had no effect on breast yield. Chopde *et al*. (2008) restricted broiler chickens to 50% or 30% of full fed chickens

from day 11 to day 16. The results of study showed that feed restriction had no significant effect on dressing percentages, edible carcass yield, and thigh yield. However, drumstick yields and breast meat of full fed chickens were higher than that of feed restricted chickens. In the study by Novele *et al.* (2008), in which broiler chickens were restricted to 50% or 75% of the *ad libitum* intake at different period of feed restriction during starter period, the breast muscles and leg muscles were not significantly different than *ad libitum* ones. Mohebodini *et al*. (2009) determined the effect of quantitative feed restriction on carcass composition of broiler chickens. The results of study indicated that subjecting broiler chickens to quantitative feed restriction from day 7 to day 14 significantly reduced carcass weight and the weights of thigh and breast. Wijtten *et al*. (2010) indicated that carcass yield and breast yield are consistently compromised with quantitative feed restriction in young broiler chicken. Chen *et al.* (2012) found that feed restricted broiler chickens had significantly lower eviscerated carcass ratio. However, they showed that there was no significant difference in leg muscle ratio and breast muscle ratio between control and feed-restricted groups. Jalal and Zakaria (2012) evaluated the effect of early feed restriction (50%, 65% or 80% *ad libitum* feed intake) from day 8 to day 14 on carcass characteristics in broiler chickens. Results of this study demonstrated that feed restriction had no significant effect on carcass yield and dressing percentage of broiler chickens.

Reducing fat deposition in chicken meat is an industry aim. According to Griffin (1996), not only broiler chickens grow up to 4 times more quickly than layer strains selected for table egg production but they also accumulate much more fat, particularly in the abdominal fat depot. The first results with abdominal fat reduction by early feed restriction were published by Plavnik and Hurwitz (1985). Some other authors reviewed similar results (Meluzzi *et al*., 1998; Choct *et al*., 2005; Oyedeji and Atteh, 2005b). Quantitative feed restriction had a meaningful reduction in abdominal fat in broiler chicken (Khadem *et al*., 2006; Chen *et al*., 2012; Jalal and Zakaria, 2012). Likewise, abdominal fat deposition was not significantly affected by quantitative feed restriction (Camacho *et al.*, 2004; Mohebodini *et al*., 2009; Jalal and Zakaria, 2012). Zhan *et al.* (2007) found that a feed restriction for 4h per day from 1 to 21 day of age significantly increased abdominal fat yield in Acorned female broiler chicken. Also, Wijtten *et al*. (2010) indicated that abdominal fat yield is consistently increased with quantitative feed restriction in young broiler chicken. As mentioned, results obtained with feed restriction programs intended to diminish the carcass fat content in broiler chicken have been contradicted. This contradiction may be caused

by nature, severity, timing, and duration of under-nutrition, condition of realimentation, strain and sex of broiler chicken, experimental circumstances and all factors which may affect the broiler chicken's response.

Feed restriction programs have shown the potential to reduce the incidence of ascites, sudden death syndrome (SDS) and skeletal disorders. As metabolic disorders are prevalent during the first weeks of life, reducing initial growth and thus, the metabolic load and oxygen requirements in this crucial phase, is a good way of avoiding metabolic disorders (Buys *et al*., 1998). In the study, Urdenta-Rincon and Leeson (2002), in which male broiler chickens were restricted to 85% of the *ad libitum* level from 5 to 42 days, the prevalence of ascite and sudden death syndrome did not significantly reduce. Ingram *et al.* (2000), Lippens *et al.* (2000), Lee and Leeson (2001), Lippens *et al.* (2002), Lippens *et al.* (2003), Dozier *et al.* (2002, 2003) and Lien *et al.* (2008) showed that mortality was not influenced by feed restriction programs. Bowes *et al*. (1988) restricted broiler chickens to 75% of *ad libitum* intake from 5 to 39 days of age. Results of the study confirmed the hypothesis that the incidence of SDS is related to growth rate and suggest that SDS mortality may be reduced by growing broiler chickens at a slower rate. For example, Acar *et al.* (2001), Tůmová *et al.* (2002), Urdenta-Rincon and Leeson (2002) and Özkan *et al.* (2006) indicated that feed restriction reduced the total mortality percentage by 45%, 53%, 60%, 28%, respectively. McGonern *et al.* (1999) and Saleh *et al.* (2004, 2005) revealed that feed restriction reduced mortality by around 60%, 25%, respectively. This could provide the greatest economic encouragement for implementing early feed restriction by allowing for more broiler chickens to be marketed from a flock.

2.2. Muscle fibres in broiler chickens

Skeletal muscle is the largest tissue mass, comprising approximately 40-50% of body weight in most animal species and contributes to the regulation of metabolic homeostasis (Iñarrea *et al*., 1990; Scheuermann, 2003; Li *et al*., 2007). Skeletal muscle comprised of muscle fibres, connective tissue, intramuscular fat, blood vessels and nerves. The major components of skeletal muscles are muscle fibres. Muscle fibres are multinucleate and membrane-bound cells. In addition, muscle fibres are highly specialized cells acting as the structural units of skeletal muscle tissue (Hedrick *et al.*, 1994; Chang, 2007; Choi and Kim, 2009). It is absolutely clear that

biophysical, histological and biochemical characteristics of muscle fibres play a key role of meat quality and quantity. These characteristics of muscle fibres can be conveniently defined under three distinct but overlapping categories: fibre number, fibre size and fibre type. So, understanding and investigating these characteristics are one of the most important practical to poultry and meat scientists (Rehfeldt *et al.*, 2000; Kuttappan *et al*., 2013; Verdiglione and Cassandro, 2013).

Because muscle fibres occupy 75-90% of the muscle volume, muscle fibre characteristics are main determinant factor of muscle mass as well as meat quality. Muscle fibre characteristics are represented by their total number of fibres (TNF), cross-sectional area of muscle fibre (CSA), and length of muscle fibre and muscle fibre type (Choi and Kim, 2009; Lee *et al*., 2010). Considering the anatomy of the breast muscles, the measurement of muscle fibre density (MFD; muscle fibre number in a given cross-sectional area of the muscle) has a practical advantage over TNF, because it does not require the measurement of total muscle cross-section area (Scheuermann *et al*., 2003). It is well known that muscle fibre number, size, and fibre-type composition are closely related to each other (Ryu *et al.*, 2004; Chang, 2007). Average fibre size in musculus *pectoralis major*, musculus *biceps femoris*, musculus *extensor hallucis longus* and musculus *gastrocnemius* muscle in broiler chicken are 60, 51.6, 59.8, 60.45 μm (Papinaho *et al.*, 1996; Geyikoğlu *et al.*, 2005). In general, the fibre diameter varies from 10 to 100 μm but is dependent on such factors as health, species, breed, sex, age and plane of nutrition (Choi and Kim, 2009). Their lengths can vary from several millimeters to more than 30 cm. As the muscle fibres are enormous cells, spanning up to 100 μm in length, multiple nuclei are required to provide the synthesis machinery needed to increase muscle protein synthesis that must accompany cell growth. It is interesting to note that not all muscle fibres are equivalent. Muscles are rarely composed of only one population of myofibres. Muscle fibres have different types. Distribution of different muscle fibre types creates a mosaic pattern for healthy muscle.

Muscle fibres are commonly classified based for their ATPase activity. A modification of Brooke and Kaiser method for demonstration of myosin ATPase activity after preincubation at differing pH were used in order to classification muscle fibres types (Dubowitz and Brooke, 1973; Geyikoğlu *et al*., 2005). One of the unique features of chicken muscle is its numerous fibre types and their distinct functional characteristics and compositions, which contribute to a variety of functional capabilities. Chicken skeletal muscle can be divided into five distinct muscle fibre

types. These fibre type variations differ according to their molecular, metabolic, structural, and contractile properties as well as biochemical and biophysical characteristics, such as fibre size, colour, glycogen and lipid content (McKee, 2003; Choi and Kim, 2009; Lee *et al*., 2010). There is the type I, smallest slow-contracting-oxidative, high lipid content, many mitochondria and myoglobin "red"fibres, type IIA intermediate fast-contracting oxidative-glycolytic "red" fibres and IIB largest, fast-contracting glycolytic, few mitochondria "white "fibres, and a type IIIA and IIIB which are slow, tonic "intermediate"fibres (McKee, 2003; Taylor, 2004). Recently, muscle fibre type composition of skeletal muscle has attracted great attention both in human health and animal production because of its close association with insulin sensitivity in mammals and its significance for lean-mass deposition and meat quality (Li *et al*., 2007). Fibre type composition can vary markedly in different muscle types, depending on function. Therefore, the differences in muscle function will determine the types of fibres present (Taylor, 2004). Moreover, there are many factors that contribute to fibre type variation, such as sex, age, breed, hormones and physical activity. So, a better understanding of such muscle fibre characteristics is important for the study of overall muscle characteristics and subsequent meat quality. It is noteworthy that, in broiler chicken the *pectoralis* muscle is composed of only type IIB muscle fibre (Iwamoto *et al*., 2003; MacRae *et al*., 2006, 2007; Roy *et al*., 2006). It has been proposed that because chickens do not have the ability for long-term flight, the breast muscles do not require slow, oxidative type I fibres but rely on fast, glycolytic type IIB fibres, which generate a high power output over a short period for short-term intense flight or flapping activities (Suzuki, 1978). However, some authors reported that the breast muscle of chickens may include several percent of the red or intermediate fibres (Elminowska-Wenda, 2007; Jaturasitha *et al*., 2008ab; Zhao *et al*., 2012). These apparent differences in percentage occurrence of fibres in *pectoralis* muscle might be due to two main reasons. Firstly, the differences between strains, whether they are broiler or layer chickens, are related to their genetics. Secondly, the differences could be due to the different locations of the tissue samples, especially how close they are to the deep side (close to the sternum) of the *pectoralis* muscle (Sabbagh, 1990). The musculus *biceps femoris* is composed of type I, IIA and IIB (Papinaho *et al*., 1996; Dahmane Gošnak *et al*., 2010). Type IIIA and IIIB fibres are not found in mammals but are found in muscles such as the *plantaris* and *anterior latissimus dorsi* of the avian species (McKee, 2003; Geyikoğlu *et al*., 2005). Postnatal the apparent number of muscle fibres of each muscle does not change, while a transformation of type

IIA into IIB fibres during the first few months after birth and an enlargement of muscle fibre size up to 10-fold during the first two years of age can be observed (Wegner *et al.*, 2000).

The performance of muscle in adult animal largely depends on muscle fibre number and type and therefore on fibre size. Similarly, in chicken, selection for overall growth has been shown to induce greater muscle weight at the same age by increasing the fibre size, length and number (Burke and Henry, 1997; Scheuermann *et al.,* 2004; Berri *et al.,* 2007). It is well known that the muscle fibre number in chickens is established before hatching (Smith, 1963). So, any increase in muscle weight post-hatching depends on the increase in length and diameter of the muscle fibres (Knížetová *et al*., 1972; Burke and Henry, 1997; Chen *et al.*, 2007). Growth of muscle fibre is considered to be controlled by an increase in diameter and an elongation due to the addition of newly formed sarcomeres to the ends. Scheuermann *et al.* (2004) evaluated breast muscle development in chicken genotypes and reported that broiler chickens have higher total apparent myofibre number in the breast muscles than Leghorn-type chickens, and high breast yield of broiler chicken strains may be due to increased total apparent myofibre number. They suggested that increased muscle fibre number may also participate to improve breast meat yield even though it confirmed that fibre hypertrophy is an essential factor for an increase in muscle volume. Moreover, interestingly, males exhibited muscle fibre CSA about 16% smaller than females, whereas their *pectoralis major* muscle weight was less than 4% lower. This suggests a greater muscle fibre number in male broiler chickens. Surprisingly, the relationship between muscle mass and CSA is highly controversial. This could be due to the fact that muscle mass is mainly influenced by TNF, a highly variable trait. Further, according to Rehfeldt *et al*. (2000), among the muscle fibre characteristics, the total number of muscle fibres is an important factor affecting muscle mass and meat quality. Most studies report that glycolytic fibres exhibit the largest CSA, suggesting that, for a given TNF, an increase in the proportion of glycolytic fibres must lead to an increase in muscle weight. It is well known that postnatal muscle growth is mainly realized by an increase muscle fibre size and a change in muscle fibre type towards glycolytics type (Chen *et al.*, 2007). Indeed, research shows that selection for growth rate and breast meat yield has led to a shift from type I towards more type IIB muscle fibres which has a major impact on post mortem energy metabolism and thus, on meat quality (Lippens, 2003). Thus, in the chicken muscle, the fibre number is fixed at hatch and with growth and development the size and type changes (Taylor, 2004).

Histochemical characteristics are primarily the result of genetic and environmental factors. Selection for increased breast meat in chicken has no effect on fibre type and fibre diameter or meat quality, whereas in turkeys improvement of growth and breast meat yield is based on an increase of the fibre size with impaired meat quality (Berri, 2000; Iwamoto *et al.*, 2003). Voutila (2009) showed that muscle fibre area in the chicken breast muscles was significantly smaller than in the turkey breast muscles. Fast growing chickens have larger diameter fibre than slow growing lines. This increase is also associated with an increase in the number of giant fibres, which typically have cross-section area three to five times larger than normal, although this may also result from severe contraction (Dransfield and Sosnicki, 1999). Mizuno and Hikami (1971) also reported that differences in muscle volume between the laying type and the meat type in chickens mainly resulted from differences in fibre number. Differences in muscle fibre characteristics have been found between breeds. Burke and Henry (1997) indicated that muscle fibre numbers in *semimembranosus* muscle of broiler chicken were significantly more than muscle fibre numbers in the same muscle of Bantam chicken. Rahaman *et al*. (2010) evaluated meat characteristics of Cobb 500 and Ross broiler chicken strains in terms of histomorphometry of muscle fibres. Results of the study showed that thicker muscle fibre in breast and thinner muscle fibre in thigh were found in Ross strain. As mentioned in An *et al*. (2013a), commercial crossbred chickens had significantly higher muscle fibre diameter and lower muscle fibre density than Chinese native chickens. In essence, genetically programmed increases in chicken muscle mass must be due to a larger number of muscle fibres, larger muscle fibres, or a combination of these two factors.

Intact males mostly exhibit larger muscle fibre than females or male castrates. Contradictory results have been reported concerning the determination of the number of muscle fibres by gender. Differences in fibre number and size are primarily under the control of sex hormones, and differences in fibre number between males and females can arise by hormonal action if differences in androgen hormones are sufficiently high during periods of prenatal fibre formation (Rehfeldt *et al.*, 2004; Choi and Kim, 2009). Chiang *et al.* (1995) found that sex of chickens had no influence on either the proportion of muscle fibre types or areas. Dransfield and Sosnicki (1999) reported that fast growing male chickens had *pectoralis* muscle fibres three to five times wider than slower growing chickens and an increase in the number of giant fibre. Scheuermann *et al.* (2004) indicated that male broiler chickens had higher muscle fibre density in

pectoralis muscle than female broiler chickens from day 7 to day 21. They also reported that males had a faster increase in the cross-sectional area of muscle fibres (i.e., a higher rate of hypertrophy) compared to females. An *et al*. (2013a) showed that cock had significantly higher muscle fibre diameter and lower muscle fibre density when compared to hen. In addition, testosterone treatment in later postnatal periods can stimulate muscle hypertrophy in a direct or indirect manner. Additionally, differences in fibre number and size have been related to differences in physical activity between the sexes (Rehfeldt *et al.*, 2004).

The size and number of muscle fibre are factors that influence muscle mass and meat quality (Hossner, 2005; Lee *et al*., 2010; An *et al*., 2013a). During postnatal development, when the number of muscle fibres is high, fibre generally grows more slowly. Conversely, fibre grows more rapidly when the number of fibre is low in poultry (Choi and Kim, 2009). Thus, fibre number is negatively correlated with fibre area, whereas both fibre number and area are positively correlated with muscle mass in broiler chicken (Gille and Salomon, 1998; Rehfeldt *et al.*, 2004). Papinaho *et al.* (1996) found that there was a significant correlation between final meat quality and biochemical and histological properties of breast muscle such as fibre cross-sectional area, pH and so on. The higher breast muscle fibre size of broiler chickens, the higher growth of breast muscle weight and the higher values for the meat: bones ratio (Marcu *et al*., 2013). Furthermore, muscle fibre size is an important factor in determining meat tenderness. As broiler chickens age, the cross sectional area of muscle fibre increases in size. For example, in broiler chicken muscle with a larger fibre size exhibits tougher meat than muscles of smaller fibre size (Chen *et al.*, 2007). Bünger *et al.* (2009) and Choi and Kim (2009) demonstrated that animals with greater numbers of muscle fibres of moderate size produced a higher quantity and quality of meat. In contrast, Berri *et al.* (2007) reported that increased fibre size was associated with higher pH and darker meat. The authors suggest the meat from broiler chickens with larger fibre would therefore be better adapted to further processing compared to broiler chickens with smaller fibre. Likewise, Duclos *et al.* (2007) showed that breast muscles with the largest fibres exhibited the highest pH, lower drip loss, darker lightness value and greater tenderness after cooking than breast muscle with smallest fibre. For the majority, giant fibres are considered to arise from hypercontraction of individual fibres. Muscles consisting of higher proportion of IIB fibres have more giant fibre (Chiang *et al.*, 1995). Miraglia *et al.* (2006) found that Ross and Kabir hybrid have giant fibre. They also showed that the percentage of giant fibres in the

musculus *pectoralis major* muscle is higher ($P < 0.001$) in the Ross, while there are no significant differences in the musculus *ileotibialis lateralis* and musculus *semimembranosus* muscles. The greater fibre diameter observed in the Ross chickens, in spite of the younger age of the chickens at slaughter, can be easily explained by the faster growth speed typical of this hybrid. The presence of more giant fibres in the muscles of chickens selected for fast meat production could be considered as one of the side effects of genetic selection. As far as the giant fibre percentage is concerned, the most significant difference ($P < 0.001$) was found in the musculus *pectoralis major* muscle that, interestingly, is the muscle that genetic selection mainly aims to increase because of its commercial value (Miraglia *et al.*, 2006). Additionally, type of muscle fibre can affect the meat quality by influencing the post mortem changes during conversion of muscle into meat. As we expressed chicken breast muscle are approximately entirely type IIB fibre (glycolytic), capable of short burst of activity for the fight or flight response. According to Taylor (2004), chicken *pectoralis* muscle is about 95% type IIB, very white, not juicy, and of bland flavor. Chicken legs muscles are rich in type I fibres, have more flavour, are juicier, and are tenderer. Generally, muscles with a high content of type I are positively correlated with juiciness and flavour, and those with a high content of type IIB are negatively correlated with shear force and tenderness (Taylor, 2004). As mentioned in Barbut *et al.* (2008), metabolism of the breast muscle could contribute to pale, soft and exudative (PSE) chicken meat. Le Bihan-Duval (2003) showed that fast-growing chicken had more IMF in breast meat, which was usually associated with higher tenderness. However, Fanatico *et al.* (2007) found that breast meat from slow-growing chicken was tendered than meat from fast-growing chickens. Likewise, Chen *et al.* (2007) mentioned that higher breast meat shear force was found in broiler chicken compared to the crosses and Leghorns. Zhao *et al*. (2007) demonstrated that selection for breast muscle IMF leads to desirable changes in meat quality, carcass, sexual maturity, and egg production traits. An *et al*. (2013a) reported that native chicken has good meat quality due to its lower shear force value than commercial crossbred chicken at market time.

2.3. Muscle fibres and feed restriction in broiler chickens

Growth of muscle fibres is considered to be controlled by three factors: (1) enlargement by increase in diameter, mainly due to the accumulation of muscle fibrils; (2) elongation due to the addition of newly formed sarcomeres to the ends; (3) increase from proliferation of satellite cells (Gille and Salomon, 1998; Rehfeldt *et al.*, 2004; Chen *et al.*, 2007). Satellite cells are mononuclear myogenic stem cells located between the basal lamina and plasmalemma of the skeletal muscle fibre (Allouh, 2007). Satellite cells are critical to postnatal growth of muscle because they are the progenitors of all nuclei added to muscle fibres for postnatal skeletal muscle growth (Carpenter *et al.*, 2000; Halevy *et al.*, 2001; Allouh and Rosser, 2010).

In the recent years, the growth and development of skeletal muscle has long been of interest to animal scientists. Not only will a better understanding of this process lead to improved strategies to increase the efficiency of lean tissue deposition in domestic animals, but it also has human health implications (Reecy *et al.*, 2003; Li *et al.*, 2007). In addition, because of the emphasis on meat quality and quantity in broiler chickens, the poultry industry has become focused on muscle morphology and fibre type characteristics (Li *et al.*, 2007).

Considering that postnatal muscle growth and development is due to muscle fibre area, diameter and length growth and development, many studies have attempted to find out factors can influence on muscle fibre development. It has been shown that species, genotype, sex, age and plane of nutrition (e.g., *ad libitum* or feed restriction), hormones and physical activity are some of the main factors affecting muscle fibre development (Rehfeldt *et al.*, 2004; Velleman *et al.*, 2010; Verdiglione and Cassandro, 2013). It well established that strain, sex and nutrition have major influence on growth in broiler chickens (Bilgili *et al.*, 2006; Berhe and Gous, 2008; Brewer *et al.*, 2012ab). The change in the chicken market toward processing has been strongly related to the improvement in chicken growth and carcass weight, with a significant increase quality and yield of major carcass part (breast without bone, thighs and drumsticks). There are many different factors that can affect on meat quality and quantity. Genotype, age, sex and nutrition have the greatest impact among them (Shahin and Abdelazeem, 2005; Nikolova and Pavlovski, 2009; Fernandes *et al.*, 2013).

Plane of nutrition (e.g., *ad libitum* or feed restriction) promotes healthy, balanced growth with good skeletal and muscle development. It is well known that feed restriction during postnatal growth reduces growth including skeletal muscle growth. In particular, it both in

quantity and in quality has been reported to lead to decreases in muscle fibre size (area or diameter) (Rehfeldt *et al*., 2004). It seems that feed restriction exclusively affect fibre size by means of reduced nuclear and protein accumulation. Studies by Moss (1968), Timson *et al.* (1983), Roy *et al*. (2006) and Marcu *et al.* (2013) indicated that qualitative feed restriction gave similar results (i.e., reducing muscle fibre size) in the chickens. According to Li *et al*. (2007), skip-a-day feed restriction has been shown to delay postnatal *gastrocnemius* muscle growth in short term in broiler chickens, but may induce an accelerated muscle fibre hypertrophy in the long term. However, some reports found that immediate posthatch feed restriction regimen is not appropriate for morphological development of the musculus *pectoralis major* muscle of broiler chickens (Mozdziak *et al*., 2002a; Velleman *et al*., 2010). Consequently, the plane of nutrition (e.g., *ad libitum* or feed restriction) after hatch has a considerable impact on growth and development of chicken muscle fibre. If chickens achieve a fast growth over the first 5-7 days, it then becomes more possible to slow growth during the second week if desire, as means of improving muscle fibre development.

Generally, since each muscle has different fibre type and role in body in comparison with another muscle, it seems that effect of feed restriction on muscle fibre might be dependent on the muscle type. In galliform hatchlings, the leg muscles are larger and more developed than the breast muscles (Ricklefs, 1983), and are assumed to less sensitive to early feed restriction. The different responses of musculus *pectoralis superficial and* musculus *biceps femoris* muscles to feed deprivation have been found previously (Warriss *et al*., 1988). In addition, as suggested by Tesseraud *et al*. (1996) and Yaman *et al*. (2000), the white *pectoralis* muscle responds markedly to nutritional alteration and the red *gastrocnemius* is less sensitive. Therefore, based on the aforementioned studies, it is plausible to assume that thigh muscle fibre and breast muscle fibre respond differently to feed restriction. Consequently, not surprising, the relationship between feed restriction and muscle fibre is fallible, especially due to differences between commercial strains, between sexes, between implementing methods of feed restriction (i.e., the type, timing, duration and severity of the feed restriction), between types of muscle and alterations in age at slaughter. Nevertheless, limited information is available concerning the effects of strain, gender and feeding group (*ad libitum* or quantitative feed restriction) and their possible interactions as a means of improving growth and carcass composition as well as modification of muscle fibres development in broiler chickens.

3. Hypotheses and Objectives

3.1. Hypotheses

The literature review suggests that quantitative feed restriction during early life is able to reduce growth rate. It also shows that tempering growth rate can greatly improved daily weight gain and carcass composition (decrease fat weight and increase meat weight) in feed-restricted broiler chickens compared to *ad libitum* broiler chickens. Presumably, feed restriction may affect selected characteristics of muscle fibres development. Also, there may be interaction with selected effects such as strain, sex and feeding group (*ad libitum* or quantitative feed restriction) affecting selected traits of muscle fibre development.

3.2. Objectives

The objective of the study was to evaluate level and significance of the effect of strain, sex and feeding group (*ad libitum* or quantitative feed restriction) on growth, carcass composition and mainly on development of muscle fibres in broiler chickens. It is important to note that the major objective of this study was to evaluate the selected effects of strain, sex and feeding group (*ad libitum* or quantitative feed restriction) on selected characteristics of muscle fibres development (muscle fibre number density, area, diameters, perimeter, length, width and circularity) in broiler chickens during growth period. Moreover, the effect of strain, sex, feeding group (*ad libitum* or quantitative feed restriction) on selected traits of growth (daily weight gain) and (carcass composition, carcass weight, breast muscles weight, thigh muscles weight, abdominal fat weight) was evaluated for minor objective as an additional effect to explain larger proportion of biological variability as well as reliability of results.

4. Materials and Methods

In order to verify our hypotheses an experiment with a completely randomized design with a 3×2×2 factorial arrangement was done. Three feeding groups (*ad libitum*, 80% *ad libitum* and 65% *ad libitum*), two strains (Ross 308 and Cobb 500) and both sexes were the main factors. In the experiment, 4,860 (one day old) sexed broiler chicks were used for 5 weeks. Ross 308 and Cobb 500 broiler chicks were obtained from International Poultry Testing Station Ústrašice hatchery, sexed. Ross and Cobb strains are mainly used in chicken production worldwide. Chickens were randomly split into 12 groups according to restriction intensity, strain and sex with three replications in each group. In total, the chicks were assigned to 36 experimental pens, with 135 birds per pen. These numbers of replications and birds per pen were chosen to support statistical validity. Each pen (7.2 m^2) equipped with two tube feeder and one automatic nipple drinking system, had pine wood shaving litter over concrete floor. Stocking density was 18.6 chicks per m^2. The broiler chicks were raised under standard commercial conditions in an environmentally controlled windowless poultry house at International Poultry Testing Station Ústrašice. Scheme of the experiment is given in Table 1. As mentioned earlier in the literature review, the optimum timing for feed restriction was found to be during the second week. So, feed was provided for consumption *ad libitum* for the first 7 days of age. Then, during the feed restriction period (from day 8 to day 14), chicks in two feed-restricted groups received 80% (R1) and/or 65% (R2) of the amount of feed voluntarily consumed each day by *ad libitum* chickens. This amount was calculated by averaging the daily feed intake for all of *ad libitum* chickens and then using 80% and 65% of this as the feed allocation for group R1 and group R2 for following day, respectively. This methodology was repeated on a daily basis for 7-day period of feed restriction. After the end of the feed restriction, the chickens consumed feed *ad libitum* until 35 days of age. Chickens were fed these diets in a pellet form. Diets were made in the International Testing of Poultry, s.p., VSK Lysá nad Labem. Composition of the diets is shown in Table 2. Nutrient component of the diets: dry matter, crude protein, crude fibre, ether extract and ash were analysed at Institute of Animal Science Prague-Uhříněves. Environmental conditions were kept according to methodology stated (Table 3). The following lighting program was adopted: 23 h of light in the first 14 days (i.e., 23L:1D from day 1 to day 14), followed by 19 h of light per day until day 28 (i.e., 19L:5D from day 15 to day 28), and 23 h of light per day until the end of the growing period (i.e., 23L:1D from day 29 to day 35). Air temperature in poultry house was

maintained at 33°C for the first week and then reduced step-by-step by some 3°C each week to a final temperature of 21°C at 35 days. A brooder guard was used around chickens for each pen for the first week to confine chicks to the "Chick Comfort Zone". Water was freely available to all chickens during the entire experiment. Fifty chickens from each pen were weighed individually each week with a precision manual poultry scale with large weight range up to 30 kg and accuracy 1 gram (www.veit.cz) to determine average body weight. Daily weight gain for each chicken for each interval was calculated as average daily change in average body weight between two consecutive average body weight measurements for each chicken.

Prior to actual processing, 8 chickens were chosen in approximately similar live weight from each group at 14, 21, 28 and 35 days of age. The chickens were wing-banded and slaughtered. First the chickens were electrically stunned for 5 seconds. Approximately 10 seconds after being stunned, a deep manual throat cut was made to sever the carotid artery and jugular vein with a minimum 120 seconds bleeding time. After bleeding was completed chickens were scalded with hot water (54°C) for 2 minutes in a dunking scalder. Later than defeathering, the chickens were eviscerated manually. Abdominal fat (fat surrounding the cloaca) was removed and weighed with precision scale with weighing range of 600 grams and high accuracy (0.01 gram). Carcasses (considered as the chickens without viscera, head, feather, and shanks) were weighed with precision scale with weighing range of 6000 grams and high accuracy (0.1 gram). Next carcasses were cut up into various portions and then assayed. First the wings were removed by a cut through the shoulder joint at the proximal end of the humerus. Then the thigh portion (i.e., whole leg) was obtained by cutting through the joint between the femur and the ilium bone of the pelvic girdle. Next both thighs skin was separated and thigh muscles (i.e., whole leg muscles) were dissected and weighed. Then the breast muscles on both right and left side of carcass were excised and weighed.

After carcass analyses, musculus *pectoralis major* and musculus *biceps femoris* were dissected from each sample right away. Samples of both breast and thigh muscle were bagged in labeled plastic bags, transferred directly to the university laboratory. The samples were frozen by immersion 10 second in isopentane cooled with liquid nitrogen bath and used for frozen sectioning followed by morphological analysis. Muscle samples were placed in cryovials and stored in laboratory freezer at -80°C in air tight containers until sectioning. Frozen muscles were transferred to a cryostat (-21°C) and placed on mounting chucks. Ten micron thick cryosections

from the midbelly were serially cut on a cryostat at -21°C. One or more sections were adhered to a coverslip. Sections were processed for hematoxylin and eosin (H&E) staining for histology. In order to preparing sections for processing, the procedure for staining was followed: the coverslips were placed in water for 1 minute. The sections were stained by placing the coverslips in hematoxylin stain for 4 minutes. The coverslips were transferred from hematoxylin stain to tap water for 20 seconds. The sections were then counterstained in eosin for 3 minutes. Finally, the coverslips were rinsed in distilled water for 1 minute, dehydrated in alcohol 96% for 3 minutes. The sections mounted, dehydrated, and cover slipped using DPX *(*Fluka*;* Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany).

Sections stained with hematoxylin and eosin and for muscle fibre morphology were magnified to 200× under a light microscope (www.arsenal.cz) and their images were taken through microscope using a digital camera (Olympus, Model C5060 wide zoom, Japan). All fibres in each hematoxylin-stained section were counted, and the cross-sectional areas (μm^2), fibre densities, diameters (µm), perimeters (µm), lengths (µm), widths (µm) and circularities ($4\pi \times$ area / $(\text{perimeter})^2$) of all fibres were measured using Nis-Elements AR 2.30 imaging system (www.lim.cz).

Differences in daily weight gain was analysed by analysis of variance and 3-way ANOVA for the effect of feed restriction, strain, sex and their interactions using procedure of the SAS (SAS Institute Inc., Cary, NC, 2002) and the following statistical model [1]:

$$Y_{ijk} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + e_{ijk} \quad [1]$$

Where Y_{ijk} is the value of the response (dependent) variable estimated at i, j, and k factor levels, µ: the overall mean, A_i: the effect of strain (i= 1: Ross 308; i=2: Cobb 500); B_j: the effect of sex (j= 1: cockerel; j= 2: pullet); C_k: the effect of feed restriction (k= 1: *ad libitum*; k=2: 80% of *ad libitum* ; k= 3: 65% of *ad libitum*); (AB)ij: the interaction of strain and sex; (AC)ik: the interaction of strain and feed restriction; (BC)jk: the interaction of sex and feed restriction; $(ABC)_{ijk}$: the interaction of strain, sex and feed restriction; e_{ijkl}: the residual error.

Differences in carcass composition and breast muscle fibre characteristics and thigh muscle fibre characteristics were analysed by 4-way ANOVA for the effect of feed restriction, strain, sex, age and their interactions using the following statistical model [2]:

$$Y_{ijkl}= \mu + A_i + B_j + C_k + D_l + (AB)_{ij} + (AC)_{ik} + (AD)_{il} + (BC)_{jk} + (BD)_{jl} + (CD)_{kl} + (ABC)_{ijk} + (ABD)_{ijl} + (ACD)_{ikl} + (BCD)_{jkl} + (ABCD)_{ijkl} + e_{ijkl} \quad [2]$$

Where Y_{ijkl} is the value of the response (dependent) variable estimated at i, j, k, and l factor levels, μ: overall mean, A_i: the effect of strain (i= 1: Ross 308; i=2: Cobb 500); B_j: the effect of sex (j= 1: cockerel; j= 2: pullet); C_k: the effect of feed restriction (k= 1: *ad libitum*; k=2: 80% of *ad libitum* ; k= 3: 65% of *ad libitum*); D_l: the effect of age (l= 1: 14; l=2: 21; l=3: 28; l=4: 35 days); $(ABCD)_{ijkl}$: the interaction of strain, sex, feed restriction and age; e_{ijkl}: the residual error. Least Squares Means were reported as significantly different at the $P \leq 0.05$ level. Standard Error of the Mean (SEM) was reported for the factors.

Table 1. Scheme of the experiment

Group	Number of chickens	Characteristics
1	405 (3×135)	*ad libitum*, Ross males
2	405 (3×135)	R_1 80% *ad libitum*, Ross males
3	405 (3×135)	R_2 65% *ad libitum*, Ross males
4	405 (3×135)	*ad libitum*, Ross females
5	405 (3×135)	R_1 80% *ad libitum*, Ross females
6	405 (3×135)	R_2 65% *ad libitum*, Ross females
7	405 (3×135	*ad libitum*, Cobb males
8	405 (3×135)	R_1 80% *ad libitum*, Cobb males
9	405 (3×135)	R_2 65% *ad libitum*, Cobb males
10	405 (3×135)	*ad libitum*, Cobb females
11	405 (3×135)	R_1 80% *ad libitum*, Cobb females
12	405 (3×135)	R_2 65% *ad libitum*, Cobb females

Table 2. Diet composition (%)

Ingredients	Starter (1-14 day)	Grower (15-28 day)	Finisher (29-35 day)
Wheat	38.27	46.75	52.90
Corn	20.00	15.00	12.00
Soybean meal (48%)	32.00	28.00	25.00
Fish meal	2.00	-	-
Rapeseed oil	4.00	6.00	6.50
L-Lysine Hcl (98%)	0.20	0.20	0.14
L-Threonin (98%)	0.06	0.06	0.05
DL-Methionine (99%)	0.10	0.1.0	0.05
Limestone	1.23	1.50	1.36
Salt	0.22	0.25	0.30
Monocalcium phosphate	1.32	1.35	1.10
Sodium bicarbonate	0.10	0.15	0.10
Vitamin-Mineral premix[1]	0.50	0.50	0.50
Nutrient composition			
Calculated			
CP (g/kg)	231.19	206.70	194.71
ME (MJ/kg)	12.57	13.06	13.27
Lysine (g/kg)	13.45	12.15	10.34
Methionine (g/kg)	6.62	5.80	4.78
Methionine+ Cystine (g/kg)	10.21	9.13	8.02
Ca (g/kg)	9.02	8.99	8.00
P (g/kg)	7.50	6.80	6.16
Na (g/kg)	1.50	1.55	1.61
Analysed			
Dry matter (g/kg)	906.4	899.4	907.2
Crude protein (g/kg)	233.9	208.9	199.8
Ether extract (g/kg)	63.5	80	82.9
Crude fibre (g/kg)	28.2	31.7	27.7
Ash (g/kg)	57.7	56.8	53.2
ME (MJ/kg)	12.64	12.58	13.17

[1]Composition of vitamin and mineral premix was according to commercial specifications.

Table 3. Environmental conditions

Age (day)	Temperature (° C)	Lighting program
1-7	33° C	23 hours light:1 hour dark
8-14	30 ° C	23 hours light:1 hour dark
15-21	27° C	19 hours light:5 hours dark
22-28	24° C	19 hours light:5 hours dark
29-35	21° C	23 hours light:1 hour dark

5. Results and Discussion

5.1. Daily weight gain

Results of growth of chickens expressed by daily weight gain are shown in Table 3 which describes main effects of this growth characteristic.

5.1.1. Effect of interaction between strain, sex and feeding group on daily weight gain

There was no 3-way meaningful interaction between strain, sex and feeding group in daily weight gain at all intervals of the growth. Generally, growth rate was satisfactory in Ross 308 and Cobb 500 chickens, with a daily weight gain reaching up to 57 and 58 grams, respectively, during the 35-day of the experiment.

Information is scarce regarding the effect of interaction between strain, sex and feeding group in daily weight gain. The lack of significant interaction among selected factors suggests that broiler chickens response to feeding regime in similar way. The lack of significant between strain or/and sex and feeding group in daily weight gain has been previously cited (Lippens *et al*., 2000; Dozier *et al*., 2003; Benyi *et al.*, 2009; Wijtten *et al*., 2010).

To sum up, our findings suggest that influences of strain, sex and feeding group on daily weight gain are independent of one another.

5.1.2. Effect of strain on daily weight gain

There were no significant differences in daily weight gain between Ross 308 and Cobb 500 chickens during the entire experiment.

The results are in agreement with the results of Haščík *et al*. (2010), Lewis *et al*. (2010) and Bjedov *et al.* (2011), who did not observe significant differences in daily weight gain between Ross 308 and Cobb 500 chickens. Haščík *et al*. (2010) showed that daily weight gain for Ross 308 chickens and Cobb 500 chickens over the 35-day of growth period were 45.11 and 45.64 grams, respectively. The findings are not surprising because these two fast-growing broiler chicken strains were selected in the same purposes (Sirri *et al*., 2010). As noted by Fernandes *et*

al. (2013), Cobb 500 (cockerels) showed the maximum weight gain at 47 days old, while Ross 308 had the inflection point of the growth curve at it was between 33 and 35 days old.

The temporary conclusion suggests that the daily weight gain was not significantly affected by strain. However, strain difference is one the most factors that is able to have significant influence on daily weight gain due to the effect of genetic differences in weight gain and in feed capacity and initial live weight.

5.1.3. Effect of sex on daily weight gain

Cockerels had significantly higher ($P \leq 0.001$) daily weight gain than pullets from day 1 to day 7. However, pullets showed meaningfully greater daily weight gain than cockerels during the period between 8 to day 14 days of age. Additionally, cockerels grew significantly higher ($P < 0.001$) than pullets from day 15 to day 28 days of age. Lastly, there were no meaningful differences between sexes in daily weight gain from day 29 to day 35.

The significant sex differences in daily weight gain (growth) are well documented and may result from the longer accelerating phase of growth in cockerels compared to pullets (Knížetová *et al*., 1985; Rehfeldt *et al*., 1997). It also has been established that cockerels normally show a significantly greater and more rapid gain in body weight than do the pullets and this is in agreement with these results of at the first week, third week and fourth week (Lippens *et al.,* 2000; Dozier *et al*., 2003; Novele *et al.,* 2009; Sam *et al*., 2010; Fernandes *et al.,* 2013). However, pullets had significantly higher daily weight gain compared to cockerels over the second week. The cause of significantly higher daily weight gain of pullets compared to cockerels over the second week is not clear. Nevertheless, this presumably due to the fact that cockerels may not consumed enough feed over the second week, so that they grew more slowly than pullets. According to Garcia (1992), cockerels have higher erythrocyte counts, hemoglobin and hematocrit, but lower leukocyte counts than pullets, suggesting that cockerels were more affected when submitted to duration of stress episode caused by feed restriction during the second week. Similarly, cockerels could have a more aggressive and alert behaviour than pullets, this higher sensibility to stressful conditions can lead to higher weight depression. In addition, Newcombe *et al*. (1992) reported that pullets displayed greater concentrations of IGF-1 than cockerels in plasma at 14 days of age. The higher IGF-1 titres in pullets at 14 days of age, may be

associated with a higher growth rate or growth spurt for pullets at this time. Also, increased pullets growth than cockerels during the second week could be attributed to higher physical activity cockerels compared to pullets. As suggested by Sam *et al*. (2010), the higher activity of the cockerels in comparison with the pullets need to higher feed intake of cockerels compared to pullets, but feed restriction during the second week may has been reduced the enough amount of feed for cockerels so that it caused higher growth depression in cockerels than in pullets. Obviously, the differences in cockerels and pullets growth might be exploited by raising pullets and cockerels separately.

Broiler chickens may be grown with cockerels and pullets fed separately or combined as straight-run flocks. Although pullets tend to have lower requirements for most nutrients than cockerels, the differences are minimal and typically not sufficient to warrant different formulations (Waldroup, 2011). Sam *et al*. (2010) also reported that there is no significant benefit in separating the broiler chickens into sexes during the eight weeks conventional period of raising. However, keep raising chicken beyond 8 weeks, sex separation become useful because it could be a better way to boost growth of separate chickens, especially the cockerels.

All in all, the immediate conclusion clearly demonstrates that the sex differences are significantly affect in daily weight gain because of higher growth rate, higher feed intake and higher nutrient utilization of cockerels compared to pullets (sexual dimorphism). So, results raises the possibility that sex separation may be suitable for obtaining maximum growth .

5.1.4. Effect of feeding group on daily weight gain

Prior to the initiation of feed restriction, daily weight gain did not differ ($P > 0.05$) among all groups (from day 1 to day 7). As anticipated, during 7-day feed restriction (from day 8 to day 14), the daily weight gain of R1 (80%-restricted chickens) and R2 (65%-restricted chickens) chickens was significantly lower ($P \leq 0.001$) compared to the ADL group. It is worthwhile to note that the retardation of growth was more pronounced for the group R2. No significant differences in daily weight gain between groups were seen at all intervals after feed restriction (realimentation period). However, in the last week of the experiment restricted chickens grew insignificantly faster than those of ADL. This faster growth might have been a sign of compensatory growth.

The growth and development of chicks depend on the uptake of nutrient. So, any limitation or reduction in receiving nutrients can lead to weight loss in chickens. During the feed restriction period (from day 8 to day 14 of age), ADL group grew faster than group R1 and R2. The degree of daily weight gain reduction was a direct effect of the intensity of the restriction. The significant decrease in growth as a consequence of feed restriction observed in this study is in agreement with those of Plavnik and Hurwitz (1985, 1990), Pinheiro *et al.* (2004), Khadem *et al*. (2006), Yang *et al*. (2009) and Butzen *et al*. (2013). In the present study, reduction of daily weight gain in R1 and R2 chickens could have been related to lower feed intake and hence, lower nutrient intake in these chickens. The more severe the feed restriction the lower was the daily weight gain attained over the period of feed restriction.

Whether significant compensatory growth can occur after a period of undernutrition has been the subject of controversy. In this experiment, feed restriction did not result in significant compensatory growth over the realimentation period**,** which is in agreement with the current results of Jang *et al.* (2009), Yang *et al*. (2009), Li *et al.* (2011), Jalal and Zakaria (2012) but in contrast to the previous results of Santoso *et al*. (2002) and Pinheiro *et al.* (2004), who found that feed restricted chickens during realimentation periods showed a statistically higher weight gain compared to *ad libitum* chickens. The main reason for the difference between this finding and those of Santoso *et al.* (2002) and Pinheiro *et al.* (2004) may be related to length of time allowed for refeeding. It has long been known that the length of time allowed for refeeding may influence compensatory growth (Plavnik and Hurwitz, 1985, 1991; Zubair and Leeson, 1996a). The experiment was ended at 35 days of age and this may not allow sufficient time to compensate fully in weight from a feed restriction. So, it is likely to infer that the feed-restricted chickens may have caught up if the growth period had extended beyond 35 days of age.

Particularly, no significant compensatory growth was observed during last week of experiment, which is consistent with the current results of Butzen *et al*. (2013), Mirshamsollahi (2013) and Nataraju (2013), who found an insignificant compensatory growth from day 29 to day 35 but different from those of Saleh *et al.* (1996), who indicated a compensatory growth between day 22 and day 35 after a discontinuous restriction of 20% or 40% of the *ad libitum* intake during the period of 8 to 14 days of age (day 10 and day 11 *ad libitum*) and Jalal and Zakaria (2012), who reported that weight gain were significantly greater for *ad libitum* group than restricted

groups. It is possible that differences between results may stem from different diets composition and different genetic makeup of chickens. The main logical difference between this finding and those of Saleh *et al.* (1996) may be attributed to broken or discontinuous restriction. This is likely the result of the dividing up of the period of restriction with short periods of *ad libitum*. In the discontinuous feed restriction, the broiler chickens eat to satiation in the *ad libitum* period and then do not loss much weight, causing greater weight gain. Also, complete compensatory growth may be more consistently realized if a number of short restriction periods are used rather than a long, continuous period, as the result of improvement in the efficiency of lean tissue deposition and energy retention (Farrell and Williams, 1989). In addition, the use of a discontinuous feed restriction, where periods of restriction are separated by periods of full feeding have been shown to produce similar results to a continuously applied restriction but with no evidence of chicken excitement. Excitement in commercial practice may result in deaths from crushing while feeding (Jones and Farrell, 1992). Therefore, it is plausible to assume that discontinuous restriction is more appropriate in order to induce compensatory growth. Conversely, Zubair and Leeson (1994b) indicated that varying the period of feed restriction may not offer more practical application than does continuous feed restriction.

Generally, it has been reported that early feed restriction for low intensity and/or shorter period induced a compensatory growth at later feeding period, but early feed restriction for high intensity and/or longer period stunted the growth and development of broiler chickens (Plavnik and Hurwitz, 1985, 1991; Mahmud *et al*., 2006; Lippens *et al*., 2000; Li *et al.*, 2011). Therefore, it is also reasonable to deduce that the feed-restricted chickens might have caught up if a milder intensity and/or a shorter period of feed restriction than that used here applied. The use of the milder restriction regimens could allow for a quicker compensatory growth at later feeding period, but would reduce the advantage of the effect on feed efficiency and probably also abdominal fat. These are drawbacks of the milder intensity of feed restriction.

An important factor influencing the contradictory observed in compensatory growth might be the nutrient composition of realimentation (Plavnik and Hurwitz, 1985, 1989; Jones and Farrell, 1992; Zubair and Leeson, 1996a; Lippens *et al*., 2000). The quality of the diet used during realimentation can greatly influence both magnitude and the efficiency of subsequent growth. For example, Gous (1977) found that the ability of the chicken to absorb some amino acids maybe increase as a result of prior feed restriction. Fjeld *et al*. (1989) and Fontana *et al*.

(1992) suggested that protein might be a limiting nutrient during the recovery after a period of restriction. The insignificant compensatory growth during the realimentaion period especially over the last week with both R1 and R2 group might be an indication of the need for higher levels of nutrients (e.g., energy and/or protein, amino acid) during the realimentaion period particularly over the last week. However, Leeson and Zubair (1997) and Acar *et al*. (2001) did not agree with manipulating diet formulation during realimentation of chickens previously nutrient-restricted.

Thus, the results indicated that quantitative feed restriction beginning from day 8 to day 14 reduced growth, however no significant compensatory growth was observed during the realimentaion period especially over the last week of experiment. On balance, whether market broiler chickens achieve total catch-up growth following an early feed restriction is open to question. Evidence provided by present research and by other researcher seems to answer "No"- but again factors such as nature, timing, duration and severity of feed restriction and length and nutrient composition of realimentation period as well as strain and sex may affect subsequent ability of broiler chickens to recover from a growth deficit.

5.1.5. Conclusion for individual effects affecting on daily weight gain

Taken together, these results indicate that influences of strain, sex and feeding group on daily weight gain are independent of one another. Also, these findings suggest the daily weight gain was not significantly affected by strain at any age. In general, significant differences were detected between cockerel and pullet in daily weight gain in all intervals except daily weight gain from day 29 to day 35. Clearly, the differences in cockerels and pullets growth might be exploited by raising pullets and cockerels separately. This research also showed that daily weight gain of feed-restricted broiler chickens during the period of restriction was reduced, however, feed restriction did not result in significant compensatory growth during refeeding period. It is likely to deduce that a shorter period of feed restriction or a more prolonged growth period may be beneficial in achieving any degree of success for compensatory growth.

Table 3. Daily weight gain (g)

	BW gain (1 to 7) (day)	BW gain (8 to14) (day)	BW gain (15 to 21) (day)	BW gain (22 to 28) (day)	BW gain (29 to 35) (day)
Strain					
Ross 308	18.40	43.00	63.41	76.64	82.77
Cobb 500	18.10	43.24	62.91	78.50	85.38
SEM	0.35	1.32	2.90	4.67	5.32
Sex					
Cockerels	18.93[a]	32.26[b]	65.15[a]	83.82[a]	85.36
Pullets	17.57[b]	53.97[a]	61.17[b]	71.32[b]	82.79
SEM	1.12	2.65	2.91	3.27	8.73
Feeding group[1]					
ADL	18.19	53.34[a]	62.82	78.36	81.57
R1	18.00	39.27[b]	63.94	77.46	85.94
R2	18.56	36.74[b]	62.72	76.89	84.71
SEM	1.21	2.66	3.23	4.35	10.71
Significance	P-value				
Strain	0.461	0.782	0.641	0.213	0.471
Sex	0.002	0.001	0.001	0.001	0.477
Group	0.527	0.001	0.596	0.709	0.593
Strain*Sex*Group	0.632	0.271	0.383	0.469	0.999

[a, b] Statistically significant differences ($P \leq 0.05$) on columns are indicated by different superscripts.

[1]ADL= Chickens received feed *ad libitum*. R1= Chickens received restricted feed to 80% *ad libitum* intake from day 8 to day 14; thereafter, they received feed for *ad libitum* consumption. R2= Chickens received restricted feed to 65% *ad libitum* intake from day 8 to day 14; thereafter, they received feed for *ad libitum* consumption. SEM= Standard error of the mean.

5.2. Carcass Composition

Findings of carcass composition of chickens expressed by carcass weight, breast muscles weight, thigh muscles weight and abdominal fat weight are presented in Table 4 which describes main effects of these carcass characteristics.

5.2.1. Effect of interaction between strain, sex, feeding group and age on carcass composition

There was no four-way significant interaction between strain, sex, feeding group and age in terms of carcass weight, breast muscles weight, thigh muscles weight and abdominal fat weight.

The absence of significant interaction on carcass composition indicated that the effect of feeding group (*ad libitum* or quantitative feed restriction) was essentially the same regardless of the strain and sex and differences between groups tended to be similar for different breed and sexes. Similarly, Attia *et al*. (1998) found no 3-way significant interaction between feeding group, strain and sex for carcass weight. Dozier *et al*. (2003) indicated that neither sex nor strain source interacted with feeding group to influence on carcass composition. Fernandes *et al*. (2013) reported that there were no significant interactions between strain, sex and age for carcass yield, breast muscle yield and thigh muscle yield.

Taken together, the findings suggest that the lack of 4-way interaction in carcass composition indicated that both strains and sexes responded similarly to intensity of feed restriction and advancing age.

5.2.2. Effect of strain on carcass composition

In spite of the fact that chickens for carcass analyses were selected on approximately similar live weight, significant differences were found between strains. These differences did not significantly affect carcass weight, abdominal fat weight, and breast and thigh muscles weight.

Carcass weight was not affected by strain. Kralik *et al*. (2007) made similar observations. They showed that carcass weight was not significantly different between Ross 308 and Cobb 500 strains. These findings are in agreement with the results of Kokoszyńs and Bernacki (2008),

Nikolova *et al*. (2008), Nikolova and Bogosavljevic (2011) and Indarsih and Tamsil (2012), who reported that carcass weight was not significantly affected by strain. However, other researchers suggested that carcass weight was significantly affected by strain (Rizzi *et al*., 2007, 2009; Fanatico *et al*., 2009; Lokman *et al*., 2011; López *et al*., 2011). These differences may come from differences in chicken strains which were used. Petričević *et al*. (2011) found that there was no significant difference between carcass yield in Ross 308 strain and Cobb 500 strain.

No differences were noted in breast muscles weight between strains. Similarly, Kralik *et al*. (2007) reported no significant differences in terms of breast weight between Ross 308 and Cobb 500 strains. The observation supports previous studies showing that breast weight in broiler chickens was not affected by strain (Kokoszyńs and Bernacki, 2008; Nikolova *et al*., 2008; Nikolova and Pavlovski, 2009; Indarsih and Tamsil, 2012). In addition, Olawumi and Fagbuaro (2011) indicated that there were no significant differences in breast muscle weight between Arbor acres strain and Hubbard strain. In contrast, Rizzi *et al*. (2007) and Rizzi *et al*. (2009) showed that there were significant differences in breast muscles weight between organic laying hens or between three Italian dual-purpose chicken breeds. The results were different from that reported by Fanatico *et al*. (2009), Indarsih (2009), Abdullah *et al*. (2010) and Lokman *et al*. (2011), who found that breast weight was affected by strain. In the experiment of Abdullah *et al*. (2010), Lohman strain and Hubbard strain were used. The differences between the results obtained in this experiment and those of Rizzi *et al*. (2007) and Fanatico *et al*. (2009), Indarsih (2009), Rizzi *et al*. (2009) and Abdullah *et al*. (2010) may stem from differences in genetic makeup of the chickens. Other researchers noted no strain impact on breast yield (Lippens *et al*., 2003; Wijtten *et al*., 2008, 2010).

There was no significant difference in thigh muscles weight in response to strain. The results are consistent with the results of other studies (Kokoszyńs and Bernacki, 2008; Nikolova *et al*., 2008; Nikolova and Pavlovski, 2009). Other studies (Rizzi *et al*., 2007; Rizzi *et al*., 2009), however, indicated a significant difference in leg (thigh and drumstick) muscles weight between chicken breeds. Differences in strain and type of chickens may explain the differences observed. Additionally, Lippens *et al*. (2000) and Fanatico *et al*. (2009) postulated that thigh and drumstick yield was significantly affected by strain.

Abdominal fat weight was unaffected by strain. These results match results found earlier by Kralik *et al*. (2007) and Petričević *et al*. (2011). Similarly, as noted by Lippens *et al*. (2003),

Olawumi and Fagbuaro (2011), Indarsih and Tamsil (2012), abdominal fat weight was not affected by strain. Also, Kokoszyńs and Bernacki (2008) indicated that there were no significant differences in abdominal fat weight between Ross 308 and JV broiler chickens. However, they showed that there were significant differences in abdominal fat weight between Ross 308 and Hubbard Evolution broiler chickens. Other studies showed significant differences between other commercial broiler chicken strains in abdominal fat weight (Smith and Pesti, 1998; Lippens *et al*., 2000; Kokoszyńs and Bernacki, 2008; Indarsih, 2009). Lokman *et al* (2011) showed that fat weight was significantly different between chicken strains. The contradictory between these results and the findings of this study could be related to the different strains which were used.

In conclusion, the results show that there is no significant difference between two strain Ross 308 and Cobb 500 in carcass weight, weight of breast and thigh muscles and abdominal fat weight.

5.2.3. Effect of sex on carcass composition

Although in order to carcass analysis, chickens with similar live weights were chosen, meaningful differences were found between genders. These differences greatly affect carcass weight, weight of thigh muscles but for weight of breast muscles and abdominal fat weight. Breast muscles weight of cockerels was slightly greater than those of pullets. Cockerels had higher carcass weight and thigh muscles weight when compared to pullets. In contrast, pullets had greater abdominal fat weight in comparison with cockerels.

Carcass weight was significantly affected by sex. Bogosavljevic *et al*. (2006) also found that carcass weight of cockerels was significantly greater than those of pullets, being the result of difference in live weight. The difference in carcass weight attributed to sex has been thoroughly documented (Bogosavljevic *et al*., 2006; Nikolova *et al*., 2008; Nikolova and Pavlovski, 2009; Saláková *et al*., 2009; Nikolova and Bogosavljevic, 2011). If the main criterion during raising chickens was the carcass weight indicator, it would be more useful to raised only cockerels. Many studies indicated that carcass yield was not significantly affected by sex (Lippens *et al*., 2000; Dozier *et al.*, 2003; Lippens *et al*., 2003; Olawumi and Fagbuaro, 2011; Brewer *et al*., 2012a). However, Ajang *et al*. (1993) indicated that carcass yield was significantly lower in pullets than in cockerels.

No differences were observed in breast muscles weight between sexes. These results are in line with the previous reports of Veerapen and Driver (1999), Teye *et al*. (2006), Olawumi and Fagbuaro (2011) and Butzen *et al*. (2013), who opined that there were no significant differences in breast muscles weight between sexes. In contrast to the results, other studies noted that breast muscles weight was significantly more in cockerels than in pullets (Grashorn and Clostermann, 2002; Elminowska-Wenda, 2007; Nikolova *et al*., 2008; Saláková *et al*., 2009; Indarsih and Tamsil, 2012). It seems that the difference between the results in this study and the previous ones might be due to genetic makeup of the chickens. Ricard (1988) and Lippens *et al*. (2000) showed that breast meat yield was unaffected by sex. However, Lippens *et al*. (2003) and Berri *et al*. (2007) reported that breast meat yield was significantly higher in pullets than in cockerels.

Thigh muscles weight was significantly higher in cockerels when compared to pullets. This agrees with the findings of Elminowska-Wenda (2007), Nikolova *et al*. (2008), Novele *et al.* (2008, 2009) and Nikolova and Pavlovski (2009), who demonstrated that weights of thigh and drumstick were significantly greater in cockerels in comparison with pullets. This difference in thigh muscles weight can be associated with the observed differences in carcass weights between sexes. Moreover, Lippens *et al*. (2000) and Dozier *et al.* (2003) reported that yields of thigh and drumstick were significantly greater in cockerel than in pullets.

As expected, abdominal fat weight was significantly higher for pullets compared to cockerels. Nikolova *et al*. (2007) also observed that weight of abdominal fat was significantly higher in pullets than in cockerels. The difference in abdominal fat weight related to sex has been completely cited (Veerapen and Driver, 1999; Albatshan *et al*., 2000; Lippens *et al*., 2000; Lippens *et al*., 2003; Indarsih and Tamsil, 2012). Generally, many researchers found that abdominal fat yield was significantly greater in pullets compared to cockerels (Attia *et al*., 1998; Dozier *et al.*, 2003; Rahimi *et al*., 2005; Berri *et al*., 2007). The results indicated a significant effect of sex on meat quality of broiler chickens (pullets are more fatty than the cockerels).

Pullets tend to deposit proportionally more fat in the carcass than cockerels, after about 30 days of age. Body fat takes nine times more feed energy to produce than does muscle (Leeson *et al*., 1988). For this reason it is usually uneconomical to grow pullets much beyond 45 days of age unless special emphasis is placed on reducing fat deposition (Leeson, 2001).

In summary, the data indicate that there are significant differences in carcass composition between sexes except breast muscles weight. The results also show a significant effect of sex on

meat quality of broiler chickens (cockerels has higher carcass weight and thigh muscles weight than pullets; pullets are more fatty than the cockerels). Presence of lower abdominal fat in cockerels makes it a product with leaner carcass. If the main criterion during raising chickens was the carcass weight indicator, it would be more useful to raised only cockerels. Clearly, slaughtering pullets at earlier ages may limit decreases in carcass value due to excess fat deposition.

5.2.4. Effect of feeding group on carcass composition

Despite the fact that selected chickens for carcass analysis had similar live weight, significant differences were observed between feeding groups. These differences significantly affected on carcass weight, weight of thigh muscles except for weight of breast muscles and abdominal fat weight. Group R1 chickens had the highest carcass weight among the feeding groups. Breast muscles weight was not significantly different between *ad libitum* chickens and feed restricted chickens. Group R2 had significantly lower thigh muscles weight than *ad libitum* group and group R1. In general, R1 and R2 chickens had significantly greater abdominal fat weight than *ad libitum* group. As matter of fact, broiler chickens on 65% of *ad libitum* feeding (R2 chickens) had significantly more abdominal fat weight in comparison with *ad libitum* group and group R1.

It has been shown that carcass weight was not affected by feed restriction (Camacho *et al.,* 2004; Baoming *et al*., 2006; Yagoub and Babiker, 2008; Li *et al.,* 2011; Sahraei and Mohammadi Hadloo, 2012). On the other hand, a significantly reduced carcass weight should be expected when restrictions are rather severe or longer (Acar *et al*., 2001; Urdaneta-Rincon and Leeson, 2002; Mohebodini *et al.,* 2009; Tesfaye *et al*., 2011; Sahraei and Mohammadi Hadloo, 2012). In the experiment a significant increase in carcass weight was found only with the less severe restriction (R1 chickens). However, no significant difference was detected between carcass weight of R2 chickens and those of *ad libitum* group who showed lower carcass weight in restricted chickens than *ad libitum* chickens.

In the study, breast muscles weight was not significantly affected by feed restriction which is consistent with the data reported by Baoming *et al*. (2006) but in contrast with the findings of Mcgovern *et al.* (1999) and Mohebodini *et al.* (2009) that found a lower breast muscles weight in restricted chickens. The contradictory between these findings and the findings

of this study could be related to severity and duration of undernutrition. For affecting of breast muscles weight by the feed restriction, it is difficult to see a clear picture. Most of the results in literature cannot demonstrate a significant affecting of breast muscles weight by feed restriction (Camacho *et al.,* 2004; Lien *et al*., 2008; Boostani *et al.,* 2010; Li *et al.,* 2011). Inducing a significant improvement of the breast muscles weight is rarely seen in literature (Li *et al.,* 2011). In contrast, lower breast muscles weight should be expected, when restrictions are rather severe or longer (Urdaneta-Rincon and Leeson, 2002; Velleman *et al*., 2010; Li *et al.,* 2011; Tesfaye *et al*., 2011; Butzen *et al*., 2013).

Thigh muscles weight was significantly lower in R2 group than ADL group which is consistent with the results of Mohebodini *et al.* (2009). Also, when restrictions are rather severe, lower thigh muscles weight should be happened (Saleh *et al.,* 2004, 2005; Tesfaye *et al*., 2011). Other researchers found that thigh muscles weight was not affected by feed restriction (Camacho *et al.,* 2004; Baoming *et al*., 2006; Lien *et al*., 2008; Li *et al.,* 2011).

Perhaps one of the most controversial aspects of early feed restriction programmes has been the lack of a consistent effect on abdominal fat. The findings emphasise that feed restricted chickens enhance fat deposits noticeably during the refeeding period. However, Jalal and Zakaria (2012) could not show a significant effect of feed restriction on abdominal fat weight of feed restricted chickens of 65% or 80% *ad libitum* feed intake from day 8 to 14 days of age. The differences between these results and results of this study could be related to slaughter age and genetic makeup of broiler chickens. The fact that there was a significant enlargement of abdominal fat deposition suggests that even feed restricted broiler chickens are still overeating and that the level of feed intake may control *de novo* lipogenesis (Rosebrough and McMurty, 1993). It also seems higher abdominal fat of feed restricted in the study probably due to super hypertrophy of the fat cells rather than hyperplasia over realimentation period. Cartwright *et al*. (1988) noted that the problem of fat deposition in broiler chicken was apparently related to factors which affected adipocyte hypertrophy or body composition and not adipocyte hyperplasia. It appears that a more severe and longer time of feed restriction is necessary to significantly reduce abdominal fat weight. A reduction in abdominal fat weight has been documented in previous and current studies (Mcgovern *et al.,* 1999; Mohebodini *et al.,* 2009). Jalal and Zakaria (2012) indicated that there was a significant reduction in abdominal fat weight of feed restricted chickens on 50% of *ad libitum* feed intake from day 8 to 14 days of age. The inconsistency

between the findings and these findings could be related to severity and duration of undernutrition. It is reasonable to assume that a more severe and slightly longer feed restriction is necessary to significantly reduce abdominal fat weight. However, others have failed to confirm this effect (Camacho *et al.,* 2004; Saleh *et al*., 2004, 2005; Lippens *et al.*, 2009; Li *et al.,* 2011). Thus, reports on the effect of feed restriction on abdominal fat are unequivocal. It is possible that these discrepancies may be due to nature, severity, timing, duration of under-nutrition of feed restriction and experimental conditions. Moreover, the contradictories in some cases may be ascribed to genetic makeup differences in strain and sex of broiler chicken which were used.

It is concluded that restricted chickens showed significantly higher abdominal fat weight than *ad libitum* chickens. Intensities of feed restriction had different effect on carcass and thigh muscles weight. Breast muscles weight was not affected by feed restriction. Feed restriction was so successful in maximizing carcass weight (only for R1 chickens) but no success in minimizing development of excess accumulation fat deposition. It is plausible that a more severe feed restriction is necessary to significantly reduce abdominal fat weight.

5.2.5. Effect of age on carcass composition

There were significant differences between ages for carcass weight. Carcass weight significantly increased with age, and at 35 days of age chickens had about 6.1-fold increase in carcass weight compared to carcass weight at 14 days. Furthermore, weight of breast and thigh muscles significantly increased. It is interesting to note that trends of an increase of breast and thigh muscles weight were about 2.4-fold at the 14, 22 and 28 days of age but about 1.5-fold at 35 days of age. In addition, there were significant differences for abdominal fat weight with advancing age. Abdominal fat weight greatly increased with age so that chickens at 35 days of age had about more than 7.4 times abdominal fat in comparison with abdominal fat of chickens at the age of 14 days. This indicates that the accumulation of fat in broiler chickens maybe begin at very early stage of growth period.

Carcass weight significantly increased with advancing of age. A significantly greater carcass weight was observed at the age of 35 days more than at the age of 28, 21 and 14 days old. Suryanto *et al.* (2009) also indicated that carcass weight significantly increased with advancing age. A significant increase in carcass weight with advancing age thoroughly cited (Perreault and

Leeson, 1992; Elminowska-Wenda, 2007; Lokman *et al*., 2011; Nikolova and Bogosavljevi-Bošković, 2011). Khantaprab *et al*. (1997) and Fernandes *et al*. (2013) showed that carcass yield was significantly different at different ages.

There was a significant enlargement in breast muscles weight with age. A significantly greater breast muscles weight was detected at the age of 35 days more than breast muscles weight at the age of 28, 21 and 14 days old. Suryanto *et al*. (2009) and Butzen *et al*. (2013) also reported that breast muscles weight significantly increased with advancing age. Increase in breast muscle weight was anticipated with the effect of advancing age were well documented (Perreault and Leeson, 1992; Santiago Anadón, 2002; Elminowska-Wenda, 2007; Nikolova and Pavlovski, 2009; Li *et al.*, 2011; Lokman *et al*., 2011). Khantaprab *et al*. (1997) and Fernandes *et al*. (2013) demonstrated that breast muscles yield was significantly affected by age.

A significant increase was observed in thigh muscles weight due to age. A significantly higher thigh muscles weight was observed at the age of 35 days more than at the age of 28, 21 and 14 days old. Suryanto *et al*. (2009) and Butzen *et al*. (2013) also opined that thigh muscles weight significantly increased with advancing age. A statistically increase in thigh muscles weight with advancing of age is seen in the previous studies (Perreault and Leeson, 1992; Santiago Anadón, 2002; Elminowska-Wenda, 2007; Nikolova and Pavlovski, 2009; Li *et al.* 2011). Furthermore, Khantaprab *et al*. (1997) and Fernandes *et al*. (2013) found that thigh muscles yield was significantly affected by age.

Abdominal fat weight significantly increased with advancing age. A statistically higher abdominal fat weight was detected at the age of 35 days more than at the age of 28, 21 and 14 days old chickens. Results are similar to data reported by Bartov and Plavnik (1998) that concluded older chickens prior to slaughtering had increased weight of abdominal fat. Likewise, Khantaprab *et al*. (1997), Giachetto *et al*. (2003) and Li *et al.* (2011) demonstrated that broiler chickens had significantly higher abdominal fat with advancing age. Also, Lokman *et al*. (2011) showed that fat weight in chicken increased with age. In principal, a higher abdominal fat weight is a disadvantage in extending growth period of broiler chicken (older chicken). Excess abdominal fat by the modern broiler chicken presents a two-fold problem.

Thus, the results show that advancing age in the chicken is associated with a noticeable increase in carcass composition.

5.2.6. Conclusion for individual effects affecting on carcass composition

These results emphasise that carcass composition was not affected by interaction between strain, sex, feeding group and age. Also, carcass weight, breast muscles weight, thigh muscles weight and abdominal fat weight were not significantly different between two strains. Likewise, no differences in breast muscles weight were noted between sexes. Overall, carcass weight and thigh muscles weight were significantly higher in cockerels compared to those of pullets. As expected, abdominal fat weight was significantly greater in pullets than in cockerels. Slaughtering pullets at earlier ages may limit decreases in carcass value due to excess fat deposition. The evidences from this study suggest that a significant improvement in carcass weight (only for R1 chickens) no negative effect on breast muscles weight when broiler chickens are given 80% or 65% *ad libitum* intake. Intensities of feed restriction had different effect on carcass and thigh muscles weight. In general, abdominal fat weight was significantly higher in restricted chickens (R1 and R2 chickens) compared to *ad libitum* chickens. It seems true that a more severe feed restriction is necessary to significantly reduce abdominal fat weight. Advancing age in the chicken is connected with a significant increase in carcass composition.

Table 4. Carcass composition (g)

	Live weight (g)	Carcass weight (g)	Abdominal fat weight (g)	Breast muscles weight (g)	Thigh (whole leg) muscles weight (g)
Strain					
Ross 308	1119[a]	745.94	14.19	184.22	148.43
Cobb 500	1099[b]	730.28	13.37	179.40	149.94
SEM	3.10	3.93	0.37	1.23	7.26
Sex					
Cockerels	1137[a]	747.47[a]	12.84[b]	182.11	154.21[a]
Pullets	1081[b]	728.75[b]	14.71[a]	181.51	144.16[b]
SEM	4.23	5.11	1.07	2.23	10.37
Feeding group[1]					
ADL	1118[a]	732.00[b]	12.62[c]	183.32	147.50[a]
R1	1113[a]	755.31[a]	13.84[b]	184.17	151.95[a]
R2	1096[b]	727.03[b]	14.87[a]	177.93	121.35[b]
SEM	3.43	4.12	0.31	1.37	9.11
Age (day)					
14	376[d]	222.29[d]	3.62[d]	43.97[d]	39.58[d]
21	762[c]	483.75[c]	8.47[c]	105.88[c]	94.58[c]
28	1351[b]	877.87[b]	16.05[b]	223.21[b]	180.10[b]
35	1947[a]	1368.54[a]	26.96[a]	354.16[a]	282.50[a]
Significance	P-value				
Strain	0.003	0.067	0.152	0.057	0.306
Sex	0.001	0.029	0.001	0.811	0.001
Group	0.020	0.016	0.006	0.093	0.030
Age	<0.001	<0.001	<0.001	<0.001	<0.001
Strain*Sex* Group*Age	0.131	0.816	0.987	0.468	0.596

[a, b, c, d] Statistically significant differences ($P \leq 0.05$) on columns are indicated by different superscripts.

[1]ADL= Chickens received feed *ad libitum*. R1= Chickens received restricted feed to 80% *ad libitum* intake from day 8 to day 14; thereafter, they received feed for *ad libitum* consumption. R2= Chickens received restricted feed to 65% *ad libitum* intake from day 8 to day 14; thereafter, they received feed for *ad libitum* consumption. SEM= Standard error of the mean.

5.3. Breast muscle fibre characteristics

Results of breast muscle fibre characteristics of chickens expressed by the cross-sectional areas, fibre densities, diameters, perimeters, lengths, widths and are shown in Table 5 which describes main effects of these carcass characteristics.

5.3.1. Effect of interaction between strain, sex, feeding group and age on breast muscle fibre characteristics

There was significant four-way interaction between strain, sex, feeding group and age in breast muscle fibre characteristics. The four-way significant interaction demonstrated that significant difference in breast muscle fibre characteristics exist between genotypes in response to feeding group, sex and age. Four-way meaningful interactions between strain, sex, feeding group and age for breast muscle fibre number density showed that limit Cobb 500 cockerels to 65% *ad libitum* regimen significantly increased breast muscle fibre density at any age but the opposite occurred for the Ross 308 pullets on 65% *ad libitum* regimen at any age. Significant interactions ($P \leq 0.001$) between strain, sex, feeding group and age for muscle fibre area and diameter suggested restricting Cobb 500 cockerels to 65% decreased breast muscle fibre area and diameter at any age whereas completely different occurred for the Ross 308 pullets at any age. The significant interactions ($P \leq 0.001$) between strain, sex, feeding group and age for breast muscle fibre perimeter, and length proposed that Cobb 500 cockerels on feed restriction or *ad libitum* regimens had meaningfully higher muscle fibre perimeter and length at any age while the opposite occurred for the Ross 308 pullets on feed restriction or *ad libitum* regimens at any age. The interaction ($P \leq 0.001$) between strain, sex, feeding group and age for muscle fibre circularity showed that Cobb 500 pullets and cockerels on 65% *ad libitum* regimen had decreased muscle fibre circularity at any age while Ross 308 pullets and cockerels on 80% *ad libitum* regimen had significantly increased muscle fibre circularity.

Thus, producing breast meat with good quality to fulfil consumer perceptions is dependent on adequately choosing a strain, sex, slaughter age and feeding group (*ad libitum* or quantitative feed restriction) in broiler chickens.

5.3.2. Effect of strain on breast muscle fibre characteristics

There were significant differences in muscle fibre density, area, diameter, perimeter, length, width and circularity between two strains. Breast muscle of Cobb 500 strain had significantly higher ($P \leq 0.001$) muscle fibre density than those of Ross 308 strain. On the other hand, breast muscle of Ross 308 strain had significantly greater ($P \leq 0.001$) muscle fibre area, diameter, perimeter, length, width and circularity than breast muscle of Cobb 500 strain.

Generally, some of the meat quality and quantity traits are affected by muscle fibre characteristics strongly determined by strain. However, there is scare information related to effect of strain or genotype on breast muscle fibre characteristics of broiler chickens. Significant difference between Cobb 500 and Ross 308 strains in terms of breast muscle fibre characteristics could be related to their physical activity and/or to other factors remains unclear. It has been shown that Cobb 500 and Ross 308 strains have significant differences in physical activity (Sosnówka-Czajka *et al*., 2006). Activity-induced muscle growth has been reported to be accompanied by changes in muscle fibre size and number in quails (Rehfeldt *et al.*, 2004) and in chickens (Alves *et al*., 2012). Chen *et al*. (2013), however, have reported that the breast muscle fiber characteristics of slow-growing broiler chickens were unaffected by outdoor access (higher physical activity). Differences in strain of chickens may explain the differences observed. Modern broiler chicken strains seem also to have enhanced muscle fibre cross-sectional area (Iwamoto *et al.*, 1993; Remignon *et al.*, 1994, 1995; Burke and Henry, 1997), thus different muscle fibre density.

Meaningful effect of strain was detected on muscle fibre density. There is lack of information related to effect of strain on breast muscle fibre density of broiler chickens. A significant effect of strain on muscle fibre density was also reported by Chen *et al*. (2007) and Zhao *et al*. (2012). Conversely, Sarsenbek *et al*. (2013) indicated that breast muscle fiber density of Baicheng-You chickens and Arbor Acres broiler chickens were not significantly different. Differences in histological analysis, slaghuter age and genotype of chickens probably explain the differences showed. A common belief that muscles with larger fibre area have low muscle fibre density was clearly supported by data in the present study. Because larger fibres need more space, their densities are less than smaller fibres.

Significant effect of strain was observed on muscle fibre area and diameter. This significantly larger fibre area and diameter suggest that the increase of breast muscle weight was due to muscle hypertrophy. Sogunle *et al.* (2010) and Guan *et al*. (2013) also reported that breast muscle fibre diameter was significantly different due to genotype. Similarly, MacRae *et al*. (2006), Chuaynukool *et al*. (2007), Jaturasitha *et al*. (2008b), Branciari *et al*. (2009b) and Petracci *et al*. (2013) indicated that chicken genotype had a significant effect on musculus *pectoral major* muscle fibre area or diameter. In addition, the differences in musculus *pectoral major* and musculus *pectoralis superficialis* muscle fibre density and muscle fibre size (e.g., area or diameter) related to chicken breed have been documented (Chen *et al*., 2007; An *et al.*, 2010; Zhao *et al*., 2011; Zhao *et al*., 2012; Asadi Khoshoii, 2013; Verdiglione and Cassandro, 2013). Furthermore, An *et al*. (2013a) found that commercial crossbred chicken breed had significantly higher *pectoralis* muscle fibre diameter and lower muscle fibre density compared to Chinese native chicken breed. An *et al*. (2013b) indicated that musculus *pectoralis major* muscle fibre diameter of Beijing-You chicken was 31.42 μm and it had no significant difference with Leghorn chicken, but it had significant difference with Arbor Acres broiler chickens whose muscle fibre diameter was 45.03 μm. It is of interest to note that Choi *et al*. (2013) observed significant differences in breast muscle fibre area of different Japanese quail lines. However, Radu-Rusu *et al*. (2009) pointed out that musculus *biceps brachialis* (wing muscle) of Ross 308 strain had significantly higher muscle fibre area and diameter compared to those of Cobb 500. Werner *et al*. (2008) reported that British United Turkeys Big 6 and Kelly Broad-Breasted Bronze turkey had no significant differences in musculus *pectoralis superficialis* muscle fibre diameter. Dračková *et al*. (2010) indicated that musculus *biceps femoris* and musculus *pectoralis major* muscle fibre diameters were not significantly different in Moravia BSL in comparison with Moravia Barred. Sarsenbek *et al*. (2013) found that breast muscle fibre diameter of Baicheng-You chicken and Arbor Acres broiler chickens were not significantly different. The contradictory between theses finding and the findings of this study might be interpreted to considering different muscle type, different fibre type composition of muscle, different histological analysis, different age at slaughter, different genotype and different species.

Circularity indicates the shape of the cell by comparing the perimeter of fibre with perimeter of a circle of the same area, so that: circularity = 4π area/ (perimeter) ^{2}. According to Round *et al*. (1982), most normal muscle cells cut in true cross section give a circularity of 0.8

with a range of 0.85 to 0.75. This observation was in satisfactory because it showed that both mean circularity muscle fibres of Cobb 500 and Ross 308 were 0.79.

One of the most common issues facing the meat and poultry industries today is the incidence of tough meat. For example, in broiler chicken muscles with a larger fibre size exhibit tougher meat than muscles with smaller fibre size (Chen *et al*., 2007). Previous researches suggested that muscles with higher numbers of medium size (e.g., area or diameter) fibres tend to exhibit good meat quality and quantity (Witkiewicz, 1999; Rehfeldt *et al*., 2000; Rehfeldt *et al.*, 2004; Bünger *et al*., 2009; Choi and Kim, 2009). The most important reasons for these advantages are high tenderness and low giant fibre in these muscles. In general, the smaller the diameter of muscle fibres, the more tender the meat (Lepetit *et al*., 2008; Choi and Kim, 2009; An *et al*., 2010; Bízková and Tůmová, 2010). Also, occurrence of giant fibre will be decreased. Similarly, as noted by Saxena *et al.* (2009), selection for higher muscle fibre numbers of moderate size is more advantageous in rendering both high meat content and good quality. They also

Therefore, it seems reasonable to deduce that tenderness of breast muscles of Cobb 500 chickens might be better than that of Ross 308 chickens because of significantly higher breast muscle fibre density and smaller muscle fibre size (e.g., area or diameter) than Ross 308 chickens. This possibility will be explored at the future. So, the challenge from the genetics point of view will be to find the right balance between fibre size and fibre number for the optimal meat characteristics. In addition to optimizing muscle fibre development, there is an going demand to maximizing growth of lean tissue and to minimizing development of excess accumulation of body and carcass fat.

In summary, these results indicate that strain has a significant effect on breast muscle fibre characteristics due to genetic variation, different physical activity between strains and/or to other factors remains unclear. It seems plausible to assume that breast muscles of Cobb 500 chickens are more tender than that of Ross 308 chickens because of significantly higher breast muscle fibre density and smaller muscle fibre size (area and diameter) than Ross 308 chickens. This possibility will be discovered at the future.

5.3.3. Effect of sex on breast muscle fibre characteristics

There were significant differences ($P < 0.001$) in muscle fibre density, area, diameter, perimeter, length and width between sexes. Breast muscle of cockerels had significantly higher

muscle fibre density than pullets. On the other hand, pullets had significantly greater ($P < 0.001$) muscle fibre area, diameter, perimeter, length and width in breast muscle than cockerels. There were no significant differences between cockerels and pullets in circularity of breast muscle fibre.

Chicken meat quality and quantity are affected by many factors and muscle fibre characteristics are one of the factors. The results confirmed the effect of sex (sex dimorphism) of the broiler chickens and its marked effects on muscle fibre characteristics. There are few results on the effect of sex on breast muscle characteristics. The response difference in breast muscle fibre characteristics between cockerels and pullets might be due to difference in physical activity, to difference in plasma androgen hormones especially testosterone concentration and/or to other factors remains unclear. It has shown that there is difference between cockerels and pullets in physical activity, in plasma androgen hormones and/or to other factors remains unclear (Lin and Hsu, 2002; Rehfeldt *et al.*, 2004).

A significant effect of sex on muscle fibre density was reported by An *et al*. (2010) and An *et al*. (2013a). A greater muscle fibre density in cockerels is consistent with previous observation in broiler chicken (Scheuermann *et al*., 2003). On the other hand, Asadi Khoshoii *et al*. (2013) and Verdiglione and Cassandro (2013) indicated that sex did not affect *pectoralis* muscle fibre density of chickens. These differences may come from different histological analysis, different method raising and feeding, different age at slaughter and different genetic makeup of chickens.

A significant impact of sex was found on muscle fibre area and diameter in broiler chickens (Scheuermann *et al.*, 2003; Berri *et al.*, 2007; An *et al*., 2010; Verdiglione and Cassandro, 2013) and the results were confirmed by these results. This suggests a greater total muscle number in cockerels as already reported by (Rehfeldt *et al*., 1997; Henry and Burke, 1998; Scheuermann *et al.*, 2003; Berri *et al.*, 2007) and ducks (Baéza *et al*., 1999) and pigs (Petersen *et al*., 1998). However, Radu-Rusu *et al*. (2008) indicated that there were no significant differences in musculus *pectoralis superficialis* muscle fibre area and diameter between cockerels and pullets. They also reported that there were profound differences in musculus *pectoralis profundis* muscle fibre area and diameter between cockerels and pullets. Teuşan *et al*. (2009), Dračková *et al*. (2010) and Teuşan *et al*. (2012) showed that muscle fibre diameters were significantly higher in male than in female. Others found that there were no significant sex

related differences in *pectoralis* muscle fibre size (Iwamoto *et al*., 1998; Radu-Rusu *et al*., 2008; An *et al*., 2013a; Asadi Khoshoii *et al*., 2013). These differences may stem from different histological analysis, different muscle type and different fibre type composition of muscle, different method raising and feeding, different age at slaughter and different genetic makeup of chickens.

In conclusion, the data suggest that sex has a profound effect on breast muscle fibre characteristics due to difference in physical activity, to difference in plasma androgen hormones especially testosterone concentration and/or to other factors remains unclear. Larger breast muscle fibre area and diameter pullets than cockerels may induce meat toughening. Thus, it seems reasonable to deduce that cockerel meat is more tender than pullet meat because of significantly higher breast muscle fibre density and smaller muscle fibre size (area and diameter) than pullet. This fact will be discovered at further studies.

5.3.4. Effect of feeding group on breast muscle fibre characteristics

Group R2 had greater ($P \leq 0.001$) breast muscle fibre number density than ADL (*ad libitum*) group and group R1. On the other hand, R2 chickens had smaller ($P \leq 0.001$) breast muscle fibre area and diameter than ADL group and group R1. No meaningful differences were observed between ADL group and restricted groups in muscle fibre perimeter and length. Group R1 had significantly greater muscle fibre width than ADL group and group R2. Breast muscle of both groups R1 and R2 had smaller ($P \leq 0.001$) muscle fibre circularity than ADL group.

High genetics selection and improved nutrition has increased muscle mass, especially breast muscle mass via enhance hypertrophy of muscle fibres. Nonetheless, this increase of muscle development is associated with low meat quality. As we stated above muscles with higher numbers of low or medium size (area or diameter) fibres tend to exhibit good meat quality and quantity. As nutrition is one of the key factors of muscle fibre development, muscle fibre size can reduced by feed restriction. Currently, there is limited information regarding the effect of feed restriction on breast muscle fibre characteristics. The majority of studies in chicken describing the effect of feed restriction of different timing, period, or severity have been focused on growth performance and carcass composition. After hatch the increase in muscle fibre size is thought to be determined by the rates of protein synthesis and protein degradations - or protein turnover. Satellite cell proliferation supports protein turnover. It seems that feed restriction exclusively

affect fibre size by means of reduced nuclear and protein accumulation. As mentioned earlier it is type of muscle and type of feed restriction (i.e, nature, timing, duration and severity of the feed restriction) that will determine whether or not a muscle will respond with a reduction in fibre size under feed restriction.

The difference in breast muscle fibre size (e.g., area or diameter) attributed to feed restriction has been documented in broiler chickens (Mozdziak *et al*., 2002a; Roy *et al*., 2006; Velleman *et al.,* 2010) and turkey pullet (Mozdziak *et al*., 2002b). Similarly, Marcu *et al*. (2013) showed that qualitative feed restriction significantly reduced breast muscle fibre area or diameter in broiler chickens. Present findings pointed out that growth stunting of breast muscle fibre R2 chickens is due to restricting growth of both fibre width (R2 group) and circularity with the majority effect associated with reductions in muscle fibre area and diameter. These evidence shows that only the 65%-*ad libitum* intake (R2 group) can reduce the muscle fibre area or diameter of *pectoralis major* muscle. Therefore, it confirms that it is severity of the feed restriction that will determine whether or not a muscle will respond with a reduction in fibre size under feed restriction.

R2 chickens had higher muscle fibre density than *ad libitum* group. A reduction in muscle fibre size reflects more muscle fibre density, this may suggest improved balance between muscle fibre number and muscle fibre size and in turn subsequently affect meat quality (e.g., more tender meat). Therefore, it is quite reasonable to regard that the tenderness of R2 chickens breast might be better than R1 and ADL chickens' counterparts. This possibility will be explored in further studies. The challenge from the nutrition point of view will be to find the right balance between fibre size and fibre number (optimizing muscle fibre development) accompany with maximizing growth of lean tissue and to minimizing development of excess accumulation of body and carcass fat for the optimal meat characteristics.

The results confirm that only the 65%-*ad libitum* intake (R2 group) can significantly reduce the muscle fibre area, diameter, width and circularity of *pectoralis major* muscle. Therefore, it is likely to assume that the tenderness of R2 chickens breast might be better than R1 and ADL chickens.

5.3.5. Effect of age on breast muscle fibre characteristics

Breast muscle fibre number density significantly decreased with age so that the largest breast fibre density and the smallest breast fibre density were observed at the age of 14 and 35

days, respectively. Area and diameter of breast muscle fibres were increased ($P \leq 0.001$) with advancing age so that at the age of 35 days, breast muscle had about 3.72-fold and 1.92-fold, increase in fibre area and diameter compared to 14 days of age, respectively. Perimeter, length and width of breast muscle fibres were increased ($P \leq 0.001$) with advancing age so that the biggest perimeter, length and width of breast fibres and the smallest perimeter, length and width of breast fibres were observed at the age of 35 and 14 days, respectively. The largest fibre circularity and the smallest breast fibre circularity were observed at the age of 21 and 35 days, respectively.

Muscle fibres formed during embryonic development exhibit hypertrophy, or increase in size, in posthatch growth. This hypertrophy is due to incorporation of nuclei from satellite cells resulting in increased DNA content and concomitant increased protein synthesis. There are scarcely any data concerning the changes of muscle fibre characteristics induced by increasing age. Significant age effect illustrated larger muscle fibre area, diameter, perimeter, length and width, and lower muscle fibre density for the older chickens than the younger chickens. This significantly larger fibre area, diameter, perimeter, length and width with advancing age suggest that the development of breast muscle fibres was due to muscle hypertrophy. In fact, the difference in posthatch muscle growth of young and old chickens includes differences in hypertrophy. Differences in amount and time course of hypertrophy posthatch indicate either a difference in satellite cell proliferation and incorporation, resulting in increased nuclei per muscle fibre and correlated increased protein synthesis, or if satellite cell proliferation and incorporation is the same, a difference in protein expression or degradation irrespective of the number of nuclei. The difference in chicken muscle fibre area attributed to age has been thoroughly documented (Moss *et al*., 1968; Remignon *et al*., 1995; Burke and Henry, 1997; Baéza *et al*., 2012; Bowker *et al*., 2012; Teuşan *et al*., 2012). As reported by MacRae *et al*. (2006), mean muscle fibre diameter of the *Pectoralis major* muscle significantly increased with age in Cobb 500 chickens line. In addition, older broiler chickens have been shown to have significantly larger breast muscle fibre area and diameter, and lower muscle fibre density compared to younger broiler chickens (Chen *et al*., 2007). Also, An *et al*. (2010) observed similar results in white Leghorn chickens. Muscle fibre density were 1213 and 455 at the age of 14 and 28 days, respectively. These results compared to 4496 and 3043 at the age of 14 and 28 days, respectively, reported by Chen *et al*. (2007), for Arbor Acres chickens. These differences may result from

different histological analysis and different chicken strains. The breast muscle fibre area was 2625 μm^2 at 35 days of age compared to 2170 μm^2 at 37 days of age reported by Papinaho *et al*. (1996) and 2430 μm^2 at 35 days of age found by Baéza *et al*. (2012). This difference may stem from the genetic makeup of chickens. Fibre diameters in breast muscle were 28.86, 48.82 and 55.62 μm at the age of 14, 28 and 35 days, respectively. These finding compared to 24.19 and 32.01 μm at the age of 14 and 28 days, respectively, reported by Chen *et al*. (2007), for Arbor Acres chickens. Previous fibre diameter measurements for breast muscle were 59.7 μm at 37 days for mixed-sex Ross 208 chickens (Papinaho *et al*., 1996). These differences may come from differences in uncontrollable factors such as chicken strains. It is worthwhile to note that Miraglia *et al*. (2006) reported that fibre diameter in breast muscle was 69.4 μm at the age of 57 days for Ross chickens. Smith *et al*. (1993) observed that fibre area in breast muscle of broiler chickens was 3346 μm^2 at the age of 49 days. Branciari *et al*. (2009ab) showed that breast muscle fibre area were 4876 μm^2 for Ross chickens at the age of 85 days and 5713 μm^2 for Ross 208 chickens at the age of 81 days. Asadi Khoshoii *et al*. (2013) pointed out that the mean musculus *pectoralis superficialis* muscle fibre diameters in native chickens ranged from 29-52.5 μm, whereas in Ross commercial broiler chickens ranged from 31-39 μm at the age of 8-10 weeks.

In summary, the data indicate increasing breast muscle fibre characteristics with advancing age except breast fibre density and circularity. So, tenderness seems better in young chickens breast in comparison with old chickens ones. Thus, it is quite reasonable to assume that the tenderness of chicken's breast might reduce with advancing age.

5.3.6 Conclusion for individual effects affecting on breast muscle fibre characteristics

It should be noted that there were significant interactions between strain, sex, feeding group and age for breast muscle fibre characteristics. For example, four-way interactions ($P \leq 0.001$) between strain, sex, feeding group and age for muscle fibre area suggested restricting Cobb 500 cockerels to 65% decreased breast muscle fibre area at any age whereas the opposite occurred for the Ross 308 pullets at any age. As a result, producing breast meat with good quality to fulfil consumer perceptions is dependent on adequately choosing a strain, sex, slaughter age and feeding group (*ad libitum* or quantitative feed restriction) in broiler chickens. These finding indicated that not only there were significant differences between Cobb 500 chickens and Ross 308 chickens in breast muscle fibre characteristics but also between *ad libitum* and restricted chickens in breast muscle fibre characteristics especially muscle fibre area, diameter, width, fibre

density and circularity. Sex had a significant effect on breast muscle fibre characteristics except circularity. In addition, significant differences were found in breast muscle fibre characteristics with advancing age.

Table 5. Breast muscle fibre characteristics

	Muscle fibre density[1]	Area (μm^2)	Diameter (μm)	Perimeter (μm)	Length (μm)	Width (μm)	Circularity
Strain							
Ross 308	657[b]	1735[a]	44.29[a]	158.54[a]	55.45[a]	28.85[a]	0.787[a]
Cobb 500	701[a]	1573[b]	42.08[b]	151.69[b]	53.08[b]	26.72[b]	0.779[b]
SEM	2.274	7.0730	0.1011	0.4040	0.1651	0.0717	0.0010
Sex							
Cockerels	734[a]	1579[b]	42.07[b]	150.94[b]	52.77[b]	26.82[b]	0.782
Pullets	623[b]	1728[a]	44.31[a]	159.30[a]	55.77[a]	28.25[a]	0.783
SEM	2.274	7.0730	0.1011	0.4040	0.1651	0.0717	0.0010
Feeding group[2]							
ADL	671[b]	1655[a]	43.18[a]	154.37	54.07	27.65[b]	0.790[a]
R1	671[b]	1674[a]	43.55[a]	155.66	54.45	27.81[a]	0.786[b]
R2	694[a]	1632[b]	42.82[b]	155.32	54.28	27.14[c]	0.771[c]
SEM	1.8606	5.7869	0.0827	0.3308	0.1352	0.0588	0.0009
Age (day)							
14	1213[a]	705[d]	28.86[d]	103.02[d]	36.33[d]	18.44[d]	0.796[b]
21	697[b]	1299[c]	39.44[c]	139.71[c]	48.95[c]	25.41[c]	0.800[a]
28	455[c]	1980[b]	48.82[b]	173.61[b]	60.32[b]	31.58[b]	0.791[c]
35	350[d]	2625[a]	55.62[a]	204.13[a]	71.47[a]	34.72[a]	0.742[d]
Significance	P-value						
Group	<.001	<.001	<.001	0.081	0.074	<.001	<.001
Strain	<.001	<.001	<.001	<.001	<.001	<.001	0.013
Sex	<.001	<.001	<.001	<.001	<.001	<.001	0.756
Age	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Strain*Sex *Group*Age	<.001	<.001	<.001	<.001	<.001	<.001	<.001

[a, b, c, d] Statistically significant differences ($P \leq 0.05$) on columns are indicated by different superscripts.[1]Muscle fibre density = number of muscle fibres per mm^2 area of breast muscle). [2]ADL=.Chickens received feed *ad libitum.* R1= 80% *ad libitum* intake from day 8 to day 14. R2= 65% *ad libitum* intake from day 8 to day 14. SEM= Standard error of the mean.

5.4. Thigh muscle fibre characteristics

Results of thigh muscle fibre characteristics of chickens expressed by the cross-sectional areas, fibre densities, diameters, perimeters, lengths, widths and circularities are summarised in Table 6 which describes main effects of these muscle fibre characteristics.

5.4.1. Effect of interaction between strain, sex, feeding group and age on thigh muscle fibre characteristics

There were significant four-way interactions between strain, sex, feeding group and age in thigh muscle fibre characteristics. The four-way significant interaction demonstrated that significant difference in thigh muscle fibre characteristics exist between genotypes in response to intensity of feed restriction, sex and age. Four-way meaningful interactions between strain, sex, feeding group and age for thigh muscle fibre number density showed that limit Ross 308 cockerels to 65% *ad libitum* regimen increased significantly higher thigh muscle fibre density at any age but the opposite occurred for the Cobb 500 pullets on 65% *ad libitum* regimen at any age. The significant interactions ($P \leq 0.001$) between strain, sex, group and age for muscle fibre area, diameter, perimeter, length and suggested restricting Cobb 500 pullets to 80% increased thigh muscle fibre area, diameter, perimeter length and width at any age whereas completely different occurred for the Ross 308 cockerels at any age. The interactions ($P \leq 0.001$) between strain, sex, group and age for muscle fibre circularity indicated that Ross 308 cockerels on feed restriction regimen had significantly increased muscle fibre circularity at any age while Cobb 500 pullets on feed restriction regimen had significantly decreased muscle fibre circularity. So, it is essential to choose a suitable strain, sex, slaughter age and group (*ad libitum* or feed restriction) in order to produce thigh meat with good quality because these probably affect consumer perceptions.

5.4.2. Effect of strain on thigh muscle fibre characteristics

There were significant differences in muscle fibre density, area, diameter, perimeter, length, width and circularity between two strains. Thigh muscle of Cobb 500 strain had significantly higher ($P \leq 0.001$) muscle fibre area, diameter, perimeter, length and width than those of Ross 308 strain. However, thigh muscle fibre density and circularity were smaller ($P \leq 0.001$) in Cobb 500 chickens than in Ross 308 chickens.

Little information is available about the effect of strain on thigh muscle fibre characteristics of the broiler chickens. Significant differences between Cobb 500 chickens and Ross 308 chickens in term of thigh muscle fibre characteristics could be related to their physical activity and/or to other factors remains unclear. Sosnówka-Czajka *et al*. (2006) demonstrated that Ross 308 chickens had significantly greater physical activity than Cobb 500 chickens. Physical activity has been shown to increase muscle fibre density of musculus *plantaris* muscle (leg muscle) and reduce muscle fibre size in guinea pig (Rehfeldt *et al.*, 2004). As mentioned earlier, breast muscle fibre characteristics (e.g., area or diameter) were significantly higher in Ross 308 chickens compared to Cobb 500 chickens but not muscle fibre density. One could extrapolate from these results that such responses are dependent on muscle type. Chen *et al*. (2013), however, have reported that the thigh muscle fiber characteristics of slow-growing broiler chickens were unaffected by outdoor access (higher physical activity). Differences in strain of chickens and type of muscle may explain the differences observed.

Previous studies demonstrated the close relationship between the muscle fibre characteristics (including muscle fibre density and diameter) and tenderness of meat: thinner muscle fibre, more density, better tenderness (Sifre *et al*., 2005; Chen *et al*., 2007; Sogunle *et al.*, 2010). Tenderness has been noted as the most important quality attribute in determining consumers' ultimate satisfaction with a whole chicken muscle cut. The results from this study showed that Ross 308 chickens had significantly higher thigh muscle fibre density and smaller muscle fibre size (area and diameter) than Cobb 500 chickens. Therefore, it would be reasonable to deduce that the tenderness of Ross 308 chicken thigh muscle might be better than that of Cobb 500 chicken thigh muscle.

As mentioned earlier, most normal muscle cells cut in true cross section give a circularity of 0.8 with a range of 0.85 to 0.75 (Round *et al*., 1982). These observations were in satisfactory because it showed that both mean circularity muscle fibres of Ross 308 and Cobb 500 were 0.802 and 0.789, respectively.

Significant effect of strain was detected on muscle fibre density. Other studies also reported that chicken genotype had a significant effect on musculus *bicep femoris* muscle fibre density (Zhao *et al*., 2012; An *et al*., 2013a). On the other hand, Sarsenbek *et al*. (2013) found that thigh muscle fibre density of Baicheng-You chickens and Arbor Acres broiler chickens were

not significantly different. Differences in histological analysis, slaghuter age and genotype of chickens probably explain the differences showed.

Meaningful effect of strain was observed on muscle fibre size. The difference in muscle fibre diameter of the musculus *biceps femoris* muscle related to genotype has been cited (MacRae *et al*., 2006; Chuaynukool *et al*., 2007). Sogunle *et al.* (2010) also reported that thigh muscle fibre diameter was significantly different due to genotype. Similarly, other studies indicated that chicken genotype had a significant effect on musculus *bicep femoris* muscle fibre diameter and area (Jaturasitha *et al*., 2008b; Zhao *et al*., 2012; An *et al*., 2013a; Guan *et al*., 2013). Also, Mobini and Asadi Khoshoii (2013) showed that the domestic fowls had more musculus *Quadiceps femoris* muscle fibre percentage than the Ross broiler chickens. It is of interest to note that Kulíšek *et al*. (2009) and Bízková (2011) observed significant differences in thigh muscle fibre characteristics of different rabbit genotypes. However, Radu-Rusu *et al*. (2009) opined that musculus *biceps brachialis* (wing muscle) of Ross 308 strain had higher muscle fibre area and diameter compared to those of Cobb 500 strain. As suggested by Sandercock *et al*. (2009), there is high genetic variation between chicken lines in wing muscle and drumstick muscles. Dračková *et al*. (2010) demonstrated that musculus *biceps femoris* and musculus *pectoralis major* muscle fibre diameters were not significantly different in Moravia BSL compared to Moravia Barred. Sarsenbek *et al*. (2013) indicated that thigh muscle fibre diameter of Baicheng-You chickens and Arbor Acres broiler chickens were not significantly different. It seems that the difference between these results and those of Radu-Rusu *et al*. (2009) and Dračková *et al*. (2010) are related to genetic variation in muscles and muscles type as well as different histological analysis and different age at slaughter.

In conclusion, these results demonstrate that strain has a significant influence on muscle fibre characteristics due to genetic variation, different physical activity between strains and/or to other factors remains unclear. It is plausible to assume that thigh muscles of Ross 308 are more tender than Cobb 500 because of significantly higher thigh muscle fibre density and smaller muscle fibre size (area and diameter) than Cobb 500. This possibility will be examined in further studies.

5.4.3. Effect of sex on thigh muscle fibre characteristics

There were significant differences in thigh muscle fibre characteristics between sexes. Cockerels had significantly higher thigh muscle fibre density and circularity than pullets thigh muscle fibre density and circularity. However, pullets showed significantly greater thigh muscle fibre area, diameter, perimeter, length and width than cockerels thigh muscle fibre area, diameter, perimeter, length and width.

There is very little information in literature about the effect of sex on musculus *biceps femoris* muscle fibre characteristics in broiler chickens. Significant differences in thigh muscle fibre characteristics were observed between cockerels and pullets. The response difference in thigh muscle fibre characteristics between cockerels and pullets might be due to difference in physical activity, to difference in plasma androgen hormones especially testosterone concentration and/or to other factors remains unclear. It has shown that there is difference between cockerels and pullets in physical activity, in plasma androgen hormones and/or to other factors remains unclear (Lin and Hsu, 2002; Rehfeldt *et al.*, 2004).

The density of muscle fibres was significantly higher in cockerels than in pullets. On the other hand, Teuşan *et al*. (2011) opined that pullets had significantly higher thigh muscle fibre density than cockerels. Chiang *et al*. (1995), An *et al*. (2013a) and Mobini and Asadi Khoshoii (2013) suggested that the sex of the chickens virtually had no influence on density of muscle fibres. These differences can be attributed to factors that include genetic makeup, muscle type and so on.

The relationship between gender and muscle fibre size is debatable. According to Lawrence and Fowler (2002), male chickens usually have larger musculus *extensor hallucis longus* muscle fibres than females and castrated males. Also, Dračková *et al*. (2010) indicated that musculus *biceps femoris* and musculus *pectoralis major* muscle fibre diameters were significantly higher in cocks than in hens. However, Biesiada-Drzazga *et al.* (2006) found that male geese compared to female geese showed significantly smaller diameters of musculus *biceps femoris* type I and type II muscle fibres. Radu-Rusu *et al.* (2008) reported that musculus *gastrocnemius* and musculus *semimembranosus* muscle fibre area and diameter were significantly higher in the pullets than in the cockerels. On the other hand, Chiang *et al*. (1995), Rehfeldt *et al.* (1997), An *et al*. (2013a) and Mobini and Asadi Khoshoii (2013) suggested that

the sex of the chickens virtually had no influence on density, area or diameter of thigh muscle fibres. These differences may stem from different histological analysis, different muscle type, different fibre type composition of muscle, different age at slaughter, different method raising and plane of nutrition and different species and strain.

Overall, the results indicate the presence of significant sex effects on thigh muscle fibre characteristics due to difference in physical activity, to difference in plasma androgen hormones especially testosterone concentration and/or to other factors remains unclear. It would be reasonable to suppose that thigh muscles of cockerels are more tender than those of pullets because of significantly higher thigh muscle fibre density and smaller muscle fibre size (area and diameter). This fact will be determined in further studies.

5.4.4. Effect of feeding group on thigh muscle fibre characteristics

Groups R2 had significantly more muscle fibre density than ADL group and group R1. Group R1 had significantly greater muscle fibre area than groups ADL and R2. No meaningful difference was observed between groups ADL and R2 in muscle fibre area. Groups R1 and R2 had greater ($P \leq 0.001$) muscle fibre diameter, perimeter, length, width and circularity than *ad libitum* group.

As mentioned earlier, muscles with higher numbers of medium size (area or diameter) fibres tend to exhibit good meat quality and quantity (Rehfeldt *et al*., 2000; Rehfeldt *et al.*, 2004). Because feed restriction could be used as a management technique in poultry production, by far the most interest has stemmed from the endeavour to reduce muscle fibre size (area or diameter) by feed restriction. There are few results on the effects of feed restriction on thigh muscle fibre area or diameter as well as the other fibre characteristics. Most studies of early feed restriction in broiler chickens have been focused on growth and carcass composition. These results suggest that chickens in group R1 have shown significantly increased muscle fibre area, diameter, perimeter, length, width and circularity than ADL group. In addition R2 chickens showed a meaningful increase in muscle fibre density, perimeter, length, width and circularity than ADL group. Based on these results, one could hypothesise that feed restriction had no effect on the reduction of muscle fibre size in the *biceps femoris* muscle. White muscle fibres of *pectoralis* muscle respond

markedly to nutritional level of the feed (Henkel, 1991; Tesseraud *et al*., 1996; Velotte and Crasto, 2004; Roy *et al*., 2006). Also, as mention earlier, the 65%-*ad libitum* intake (R2 group) can reduce the muscle fibre area or diameter of *pectoralis major* muscle, as previously mentioned. In broiler chicken, the musculus *iliotibialis lateralis* muscle in the thigh also contains a high proportion of white fibres ensuring its rapid growth in chickens on a high nutritional plane (Iwamoto *et al*., 1997, 1998; Roy *et al*., 2007). Therefore, it plausible to deduce that muscle fibre respond to feed restriction is dependent on muscle type. Furthermore, it confirms that it is muscle type that will determine whether or not a muscle will respond with a reduction in fibre size under feed restriction.

Whether feed restriction can reduce muscle fibre size of broiler chickens is open to question. No effect of feed restriction was observed on musculus *flexor hallucis longus* muscle fibre area of broiler chickens by Sartori *et al.* (2001). However, Sartori *et al.* (2003) reported that feed restriction reduced the size of IIA fibres in this muscle. Other researchers found that area of musculus *gastrocnemius* type IIB muscle fibre was significantly reduced by feed restriction in broiler chickens (Li *et al*., 2006; Li *et al*., 2007). Thus, it is quite reasonable to assume that not only are such responses dependent on muscle type but they are also dependent on typology of fibres in muscle. Gondret *et al.* (2000) showed that there were no significant differences between areas of musculus *biceps femoris* fibres of rabbit. On the other hand, previous research proposes that feed restriction significantly elevated muscle fibre area of *longissimus* and *tibialis cranialis* muscles of pig and musculus *biceps femoris* of rabbit (Lefaucheur and Gerrard, 2000; Dalle Zotte *et al*., 2004, 2005). It seems that these discrepancy may reflect differences in species, genetic makeup, muscle type, typology of fibres in muscle, nature, timing, severity and duration of feed restriction which may affect the response to feed restriction.

A growing interest in obtaining meat products with specific quality traits has been observed lately among poultry producers. It was shown that chicken with larger muscle fibre area or diameter often associated with meat toughening (higher shear force; Tang *et al*., 2009). The problem toughening of meat seems to stem from higher consumer demand for increasingly processed products, which in turn forces producers to increase production. Larger fibre have a higher water content and may be more likely to lose this water during processing and cooking (high drip losses), whereas muscles containing smaller fibres will have more connective tissue which could lead to tougher meat. This suggests that muscle fibres may become more densely

packed due to muscle fibre hypertrophy (e.g., larger muscle fibre area), which may bring about a toughening effect (Gwartney *et al*., 1992). Smaller fibre diameters may make a higher packing density possible and increase toughness of the meat (Dransfield and Sosnicki, 1999). Therefore, tenderness differences of meat are not only the manifestation of larger fibres but also the existence of smaller fibres. For these reasons, muscles with higher numbers of medium size (area or diameter) fibres tend to exhibit good meat quality and quantity. Again, a reduction in muscle fibre size reflects more muscle fibre density, this may suggest improved balance between muscle fibre number and muscle fibre size and in turn subsequently affect meat quality (e.g., more tender meat). Research is needed to evaluate the specific role of fibre size as well as other fibre characteristics in determining the textural properties of chicken meat. This possibility will be explored in further studies.

On balance, the data demonstrates that feeding group (*ad libitum* or quantitative feed restriction) has a profound influence on thigh muscle fibre characteristics. This evidence suggests that feed restriction resulted in increasing the thigh muscle fibre characteristics. Therefore, it is quite reasonable to deduce that thigh muscles of *ad libitum* and R2 chickens are more tender than R1 chickens because of significantly higher thigh muscle fibre density and smaller muscle fibre size (area and diameter) than Cobb 500. In addition, it seems true to assume that chicken muscle response to plan of nutrition (e.g., *ad libitum* or feed restriction) depends on the type of muscle. These possibilities will be examined in further studies.

5.4.5. Effect of age on thigh muscle fibre characteristics

Number of thigh muscle fibre density decreased significantly with age so that the largest fibre number density and the smallest fibre number density were observed at the age of 14 and 35 days, respectively (thigh muscle fibre density. Area and diameter of thigh muscle fibres was increased ($P \leq 0.001$) with advancing age, and at 35 days thigh muscle had approximately 3.45-fold and 1.83-fold increase in cross-sectional area and diameter of muscle fibres compared to 14 days of age. Perimeter, length and width of thigh muscle fibres was increased ($P \leq 0.001$) with advancing age so that the biggest fibre perimeter, length and width and the smallest fibre perimeter, length and width were observed at the ages of 35 and 14 days, respectively. The biggest fibre circularity and the smallest fibre circularity were observed at the age of 21 and 35 days, respectively.

Postnatal growth of skeletal muscle is driven by hypertrophy of the existing fibre. This requires both of an increase in myonuclear content, and the acceration of muscle proteins (Burrin and Mersmann, 2005). Thus, fibre diameter and area of thigh muscles will increase considerably as the chicken become older agree with the results reported by Remignon *et al*. (1994), Chiang *et al*. (1995), Dransfield and Sosnicki (1999), Dahmane Gošnak *et al*. (2010) and Alves *et al*. (2012) and the present study. Also, MacRae *et al*. (2006) found that mean muscle fibre diameter of the musculus *biceps femoris* muscle significantly increased with age in Cobb 500 chickens line. This significantly larger fibre area, diameter, perimeter, length and width with advancing age suggest that the development of breast muscle fibres was due to muscle hypertrophy. In fact, the difference in posthatch muscle growth of young and old chickens includes differences in hypertrophy. Differences in amount and time course of hypertrophy posthatch indicate either a difference in satellite cell proliferation and incorporation, resulting in increased nuclei per muscle fibre and correlated increased protein synthesis, or if satellite cell proliferation and incorporation is the same, a difference in protein expression or degradation irrespective of the number of nuclei. Fibre densities in thigh muscle were 1439, 849, 570 and 425 at the age of 14, 21, 28 and 35 days, respectively. These finding compared to the breast muscle fibre densities, 1213, 697, 455 and 350, respectively. Thigh muscle fibre areas were 584, 1003, 1492 and 2016 μm^2 at the age of 14, 21, 28 and 35 days and 705, 1299, 1980 and 2625 μm^2 for breast muscle fibre areas at the same ages, respectively. These differences may stem from the differences in type of muscle. It is reasonable to assume that thigh muscle fibre areas are always less than breast muscle fibre areas with advancing age.

The average diameter of chicken musculus *biceps femoris* muscle fibres has been variously reported as 34.1 μm (MacRae *et al*., 2006), 18 to 22 μm (Wattanachant *et al*., 2005). Mobini and Asadi Khoshoii (2013) pointed out that the mean musculus *quadiceps femoris* muscle fibre diameters in domestic fowls ranged from 34.5-51.5 μm, whereas in Ross broiler chickens ranged from 37-45 μm at the age of 8 weeks. These differences may come from the differences in genetic makeup. Furthermore, these differences in muscle fibre diameter may have been due to the differences in age, rate of rigor onset, and degree of sarcomere shortening (Smith and Fletcher, 1988; Wattanachant, 2008). It is worthwhile to note that Stojanović *et al*. (2009) showed that muscle fibre diameter of the musculus *biceps femoris* muscle Ross 308 increased

with advancing age in prenatal development of muscle. In general, the significantly larger fibre area, diameter, perimeter, length and width with age suggest that the development of thigh muscle fibres was due to muscle hypertrophy.

Taken together, there were significant differences in different ages for thigh muscle fibre characteristics of broiler chickens. Since broiler chickens had significantly larger fibre area, diameter, perimeter, length and width with age, it is quite plausible to deduce that the tenderness of chickens' thigh might be reduce with advancing age. It is also reasonable to assume that the tenderness of chickens' thigh might better than chickens' breast with with advancing age.

5.4.6. Conclusion for individual effects affecting on thigh muscle fibre characteristics

These results demonstrated that there were highly significant interactions between strain, sex, feeding group and age for thigh muscle fibre characteristics. For example, four-way significant interactions ($P < 0.001$) between strain, sex, feeding group and age for thigh muscle fibre area and diameter suggested restricting Cobb 500 pullets to 80% increased thigh muscle fibre area, diameter, perimeter, length and width at any age whereas completely different occurred for the Ross 308 cockerels at any age. It should be noted that not only there were significant differences between Cobb 500 and Ross 308 in thigh muscle fibre characteristics but also between *ad libitum* and restricted chickens in thigh muscle fibre characteristics especially (area, fibre density and circularity). In addition, sex has a significant effect on thigh muscle fibre characteristics. Also, significant differences are between different ages in thigh muscle fibre characteristics. Thus, it is necessary to select a proper strain, sex, slaughter age and feeding group (*ad libitum* or feed restriction) in order to produce thigh meat with good quality because these probably affect consumer perceptions.

Table 6. Thigh muscle fibre characteristics

	Muscle fibre density[1]	Area (μm^2)	Diameter (μm)	Perimeter (μm)	Length (μm)	Width (μm)	Circularity
Strain							
Ross 308	825[a]	1227[b]	34.60[b]	123.21[b]	43.41[b]	22.11[b]	0.802[a]
Cobb 500	816[b]	1320[a]	37.85[a]	135.72[a]	47.64[a]	24.16[a]	0.789[b]
SEM	1.9545	5.3636	0.1018	0.3847	0.1510	0.0680	0.0009
Sex							
Cockerels	897[a]	1159[b]	35.16[b]	125.08[b]	44.08[b]	22.47[b]	0.799[a]
Pullets	743[b]	1388[a]	37.29[a]	133.85[a]	46.97[a]	23.80[a]	0.792[b]
SEM	1.9545	5.3636	0.1018	0.3847	0.1510	0.0608	0.0009
Feeding group[2]							
ADL	816[b]	1246[b]	35.29[b]	126.73[b]	44.61[b]	22.47[b]	0.794[c]
R1	794[c]	1317[a]	36.81[a]	131.24[a]	46.10[a]	23.56[a]	0.798[a]
R2	852[a]	1258[b]	36.56[ab]	130.42[a]	45.87[a]	23.38[a]	0.796[b]
SEM	1.5925	4.3703	0.0814	0.3111	0.1230	0.0554	0.0008
Age (day)							
14	1439[a]	584[d]	26.18[d]	93.05[d]	32.72[d]	16.82[d]	0.801[b]
21	849[b]	1003[c]	33.33[c]	117.85[c]	41.37[c]	21.48[c]	0.808[a]
28	570[c]	1492[b]	37.22[b]	132.57[b]	46.57[b]	23.84[b]	0.802[b]
35	425[d]	2016[a]	48.15[a]	174.38[a]	61.45[a]	30.41[a]	0.771[c]
Significance	P-value						
Group	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Strain	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Sex	<.001	<.001	<.001	<.001	<.001	<.001	0.011
Age	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Strain*Sex *Group*Age	<.001	<.001	<.001	<.001	<.001	<.001	<.001

[a, b, c, d] Statistically significant differences ($P \leq 0.05$) on columns are indicated by different superscripts. [1](Muscle fibre density= number of muscle fibres per mm^2 area of thigh muscle). [2]ADL= Chickens received feed *ad libitum*. R1= 80% *ad libitum* intake from day 8 to day 14. R2= 65% *ad libitum* intake from day 8 to day 14. SEM= Standard error of the mean.

6. Conclusion

The objectives of the research reported herein were to examine the effects of strain, sex, feeding group, age and their possible interactions as a means of improving growth and carcass composition as well as mainly modification of subsequent muscle fibre development in broiler chickens during growth period.

The results show that daily weight gain was significantly affected only by sex not by strain. In general, cockerels exhibited significantly more daily weight gain compared to pullets for the majority of the growth periods in the experiment. Obviously, the differences in cockerels and pullets growth might be exploited by raising pullets and cockerels separately. Feed restriction significantly reduced daily weight gain over feed restriction period, however, no significant compensatory growth was observed during refeeding period. Based on results, it is possible to deduce that attainment of growth compensation by feed-restricted broiler chickens requires a shorter period of feed restriction or a more prolonged growth period than that used here. Daily weight gain was unaffected by interaction between strain, sex and feeding group.

Carcass weight, abdominal fat weight, breast muscles weight and thigh muscles weight were not significantly different between two strains. Likewise, no differences in breast muscles weight were noted between sexes. On the other hand, carcass weight and thigh muscles weight were higher ($P < 0.001$) in cockerels in comparison with pullets. As expected, abdominal fat weight was significantly greater in pullets than in cockerels. Slaughtering pullets at earlier ages may limit decreases in carcass value due to excess fat deposition. Intensities of feed restriction had different effect on carcass and thigh muscles weight. The evidences from this study suggest that a significant improvement in carcass weight (only for R1 chickens) has no negative effect on breast muscles weight when broiler chickens are given 80% or 65% *ad libitum* intake. Abdominal fat weight was significantly higher in restricted chickens (R1 and R2 chickens) compared to *ad libitum* chickens. Advancing age in the chicken is connected with a significant increase in observed traits of carcass composition. Carcass composition was not affected by interaction between strain, sex, feeding group and age.

Breast muscle fibre characteristics were significantly affected by strain. Significant sex effect was observed for breast muscle fibre characteristics apart from circularity. There was significant difference in breast muscles fibre characteristics with advancing age. Significant differences were observed between feeding groups except for perimeter and length of breast muscle fibres. For instance, R2 chickens showed significantly higher breast muscle fibre number density and smaller muscle fibre area and diameter than do *ad libitum* chickens and R1 chickens. It is intersting to note that restricting chickens to 65% *ad libitum* intake had a positive impact on breast muscle fibre characteristics (e.g., area, diameter and number density), suggesting this severisty of feed restriction might have been sufficient to modify muscle fibre development. There were the significant 4-way interactions between strain, sex, feeding group and age for breast muscle fibre characteristics. Indeed, restricting Cobb 500 cockerels to 65% decreased breast muscle fibre area and diameter at any age whereas the opposite results occurred for the Ross 308 pullets at any age. Overall, producing breast meat with good quality to fulfil consumer perceptions is dependent on choosing a suitable strain, sex, slaughter age and feeding group (*ad libitum* or quantitative feed restriction) of broiler chickens.

Significant strain effect was observed for thigh muscle fibre characteristics. There was significant difference in thigh muscles fibre characteristics in response to sex. Thigh muscle fibre characteristics were significantly affected with advancing age. Also, significant differences between thigh muscle fibre characteristics were noted between feeding groups. For example, R1 chickens had significantly higher muscle fibre area and diameter than do *ad libitum* chickens. It is worthwhile to note that restricting chickens to 80% *ad libitum* intake had not a positive impact on thigh muscle fibre characteristics (e.g., area, diameter and number density), indicating this intensity of feed restriction might have not been sufficient to modify muscle fibre development. In addition, restricting chickens to 65% *ad libitum* intake had not a positive impact on thigh muscle fibre characteristics (e.g., area and diameter). Therefore, on the basis of the data presented the conclusion is made that it may reasonable to assume that thigh muscle fibre might not be sensetive to quantitative feed restriction. The significant strain by sex by feeding group by age interaction was detected for thigh muscle fibre characteristics. Certainly, restricting the Cobb 500 pullets to 80% significantly increased thigh muscle fibre area, diameter, perimeter, length and width at any age whereas completely different findings occurred for Ross 308 cockerels at any age. Thus, it is essential to choose a proper strain, sex, slaughter age and feeding group (*ad*

libitum or quantitative feed restriction) in order to produce thigh meat with good quality because these probably affect consumer perceptions.

The hypotheses of the study were whether feed restriction would affect selected characteristics of muscle fibres development and there might be interaction of strain, sex and feeding group (*ad libitum* or quantitative feed restriction) affecting selected traits of muscle fibre development. Based on the results, these hypotheses were confirmed. It is important to note that breast muscle fibres had significantly better response to feed restriction compared to thigh muscle fibres in terms of reducing muscle fibre size.

While at first it might appear that feed restriction improve muscle fibre development, a closer look reveals that relationship between feed restriction and muscle fibre is open to question, especially due to difference between strain, sex, implementing methods of feed restriction, muscle type and slaughter age, as previously mentioned. Thus, results from the study suggest that segregation of chickens by strain, sex, plane of nutrition (e.g., *ad libitum* or feed restriction) and slaughter age could be used to optimize muscle fibre development and this may suggest improved balance between muscle fibre number and muscle fibre size and in turn subsequently affect meat quality and quantity.

In summary, this research emphasises the impact of strain, sex and feeding group (*ad libitum* or quantitative feed restriction) on chicken growth, carcass composition and muscle fibre characteristics. However, further research comparing broiler chickens with different strain, sex and age at slaughter should be conducted to examine differences in growth, carcass composition, muscle fibre characteristics and subsequent meat quality that may exist in different plan of nutrition (e.g., *ad libitum* or feed restriction).

7. References

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