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Full Length Article

Electron Microscopic Changes in Muscle and Liver of Feed Restricted Growing Lambs

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Abstract

Fifteen Najdi male lambs, 30.1±0.3 kg live weight and 3.5 months of age, were used to evaluate the effect of feed restriction on the muscle fiber diameter, sarcomere length, tissues architecture, glycogen content and ultra-structure changes in the muscle of *Longissimus dorsi* and liver. Lambs were randomly allocated to one of three groups (5 lambs each) throughout 35 days experimental trial. Feeding treatments were *ad libitum* (Control, C), 75% (25% feed restriction, 25R) and 60% (40% feed restriction, 40R) of the *ad libitum* C intake, respectively. Significant smaller fiber diameter was associated with restricted feeding groups. Control group liver samples contained more glycogen than either 25R or 40R groups. The main ultra-structural changes were associated with swollen mitochondria; reduce glycogen granules and lipid concentration in the liver samples especially in 40R group. Myofibril diameter of the muscle *L. dorsi* of C group was significantly larger (13.5 µm) than 25R (12.5 µm) and 40R (11.5 µm) groups. Also, C group had significant longer sarcomere (2.03 µm) compared to the 25R (1.87 µm) and 40R (1.93 µm) groups.

Keywords: Electron microscope; Restriction; Fiber; Lamb

Introduction

It is widely accepted that muscle fiber composition is an important source of variation in meat quality (Wojtysiak *et al*., 2010; Javaid *et al*., 2012). Different production factors such as breed, age, diet, growth rate, or slaughter weight (Hocquette *et al.*, 2007) affect sensory, biological and histological characteristics of muscles. Postnatal muscle growth and properties can be altered by nutrition as reported by Greenwood *et al.* (2000). Adequate nutrition is essential for normal skeletal muscle growth. Malnutrition during the postnatal growth reduces body weight and skeletal muscle weight (Rehfeldt *et al.*, 1999). Feed restriction, both in quantity and quality leads to decrease in muscle fiber diameter in sheep (Joubert, 1956). The latter also stated that the super-maintenance treatment resulted in minor changes in diameter of muscle fibers, while prolongation of the sub-maintenance treatment caused considerable shrinkage of individual muscle fibers.

It has been reported that liver and internal organs are the first to respond to feed restriction, while skeletal muscles are less affected. Ultra-structural changes in muscle cannot be observed in ruminant until higher levels of tissue nutrients are imposed to mobilization due to feed deprivation (Brandstetter *et al.*, 1998). Changes in skeletal muscle may be due to depletion of muscle mass and proteins.

Several researchers tested the effect of feed restriction on growth performance and carcass characteristics (Hicks *et al.*, 1990; Murphy and Loerch, 1994; Choat *et al.*, 2002; Eila *et al.*, 2012; Anjum *et al.*, 2012). However, its effect on muscle fiber and liver ultra-structural changes of growing lambs in arid and semi-arid regions is not clear. Therefore, this study was designed to investigate the effects of feed restriction regimes on the ultra-structural and histological characteristics of *Longissimus dorsi* muscle and liver of growing Najdi lambs.

Materials and Methods

Animals and Feeding System

Fifteen Najdi male lambs of about 3.5 months of age (30.1±0.3 kg live weight) were used to evaluate the effects of feed restriction on muscle fiber diameter, sarcomere length, tissues architecture, glycogen content and ultra-structure changes in the muscle of *L. dorsi* and liver. Lambs were randomly allocated to one of the three groups (5 lambs each) throughout the 35 days experimental trial. Feeding treatments were *ad libitum* fed (Control) on a commercial diet (14.53% CP, 1.16% EE, 24.91% NDF, 14.22% ADF,

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7.46% ash and 2.78 Mcal ME kg^{-1} DM), 75% (25% feed restriction, 25R) and 60% (40% feed restriction, 40R) of the *ad libitum* control intake, respectively.

Sampling and Transmission Electron Microscopy

At the end of the feeding trial (Approximately 4.5 months of age), lambs were slaughtered after 18 hrs fasting period. The mean weights of the cold carcasses were 17.36, 16.15 and 14.84 kg, for Control, 25R, and 40R groups, respectively. Samples of the muscle *L. dorsi* (12-13 ribs) and liver were immediately sliced into pieces of 1 mm in diameter and fixed in 3% buffered glutaraldehyde (sodium cacodylate buffer, pH 7.2) for 4 h at 4ºC. Tissues specimens were then post-fixed in 1% osmium tetaroxide, in cacodylate buffer pH 7.2 for 2 h at 4ºC. The fixed tissues were dehydrated using ascending grades of ethanol and transferred to epoxy resin via propylene oxide; thereafter, specimens were embedded in the epoxy resin pending sectioning (Reynolds, 1963). Semi thin sections (1 µm thickness) were prepared and stained with toluidine blue. Accordingly, ultra-thin sections of silver shades (70-80 nm) were cut on an ultra-microtome (Leica, UCT) and stained with uranyl acetate and lead citrate. The stained sections were viewed by using a JEOL JEM 1011 CX transmission electronic microscope at 100 kv.

Sarcomere length and myofibril diameter were measured using electron micrograph images of longitudinal sections of *L. dorsi* muscle from three treatments. Sarcomere length and myofibril diameters were calculated using a micron ruler and the values were converted using the calculated magnification factor. The mean of 100 measurements per sample was calculated.

Histological Examination

Core samples obtained from the liver and muscle of *L. dorsi* between the 12^{th} and 13^{th} ribs were removed and cut into 3×1 cm pieces and immediately immersed in liquid nitrogen, then vacuum packaged and stored at -20ºC. The muscle samples were then embedded in a tissue-freezing medium (Jung), and a cryostat from Leica (CM 1850, Leica) was used to cut the samples into 7-µm-thick sections. The procedure of Bancroft and Cook (1984) (using a Leica Auto Stainer; ST 5020, Leica) was used for staining the sections by H and E (Hematoxylin and Eosin) and PAS (Periodic Acid Schiff) reaction. The stained sections were viewed under a Dm-3000 light microscope (Leica Microsystems AG, Heerbrugg, Switzerland) at a magnification of 20X. Images were taken using a DFC 420 camera (Leica).

Statistical Analysis

The collected data of muscle fiber diameter and sarcomere length were statistically analyzed by GLM procedures of SAS (2002). Duncan's multiple range test was used to separate the means when significant ($p<0.05$) main effects were found for fiber diameter and sarcomere length.

Results and Discussion

Ultra-structural Changes in the Muscle of *L. dorsi*

The ultra-structural analysis of fixed and sectioned tissues may identify markedly different behavior patterns of muscle fiber (Bellairs *et al.*, 1980). The feeding regimens had an impact on the ultra-structure of *L. dorsi* muscle (Fig. 1). Among the restricted regimens, there is a trend of decrease in muscle fiber size. The small, but significant, differences between the 25R and C groups might be related to a short of the experimental period and slow response of the muscle development to the 25% restricted diet, which may require several days before an effect could be expected on myofibrillar fragilization (Therkildsen *et al.*, 2002).

Hammond (1932) stated that the muscles that showed the greatest increase in weight also developed the largest mean fiber size. Animal's plane of nutrition affects the redial growth of its muscle fibers. In sheep on a high plane of nutrition, Joubert (1956) found that muscle fiber diameters increased in proportion to increases in the total muscle mass of the carcass. The increase in radial growth due to super maintenance feeding was limited if the treatment was extended for a long time. Muscle fiber diameters decreased when the sheep were placed on a sub-maintenance plane of nutrition. Asghar and Yeates (1979) showed that sub-maintenance feeding results in degeneration of actin and myosin filaments. In normal muscle fibers, the sarcoplasm between myofibrils makes an important contribution to the radial dimensions of muscle fibers.

The transformation of muscle fibers from one physiological type to another complicates studies on the redial growth of fibers. For example, fiber transformation from a type with a large diameter to a type with a small muscle fiber diameter might increase the mean diameter of the small-diameter type. This would be difficult to distinguish from redial growth. In a number of ovine muscles, White *et al.* (1978) and Moody *et al.* (1980) found an increase in percentage of fibers with strong ATPase and weak aerobic enzyme activity.

Ultra-structural Changes in Liver Tissue

Most of the liver mitochondria of the three groups were spherical; few were rod-shaped. Glycogen was quantitatively more in the control group, less in the 25R group and much less in the 40R group. In 25R group, some of the mitochondria were swollen and became granular with no distinct inner membrane and cristae (Fig. 2).

In 40R group, some of the mitochondria were highly swollen with the disappearance of some granules and no

Table 1: Effect of feed restriction on myofibril diameter and sarcomere length of Najdi lambs

Item	C*	25R	40R	SEM
n	5	5	5	
Myofibril diameter, um	13.5[a]	12.5[b]	11.5[c]	0.32
Sarcomere length, um	2.03[a]	1.87[b]	1.93[c]	0.007

*C: control 0%, 25R: 25% and 40R: 40% feed restriction groups.
[a,b,c] Means at the same raw with different superscript significantly differ (P<0.05)

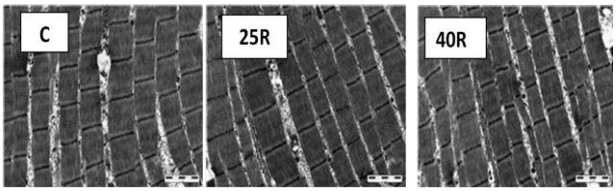

Fig. 1: Electron micrograph of a longitudinal section of *L. dorsi* muscles of Najdi lambs. C: Control, 25R: 25% and 40R: 40% feed restriction groups (magnification 315000X)

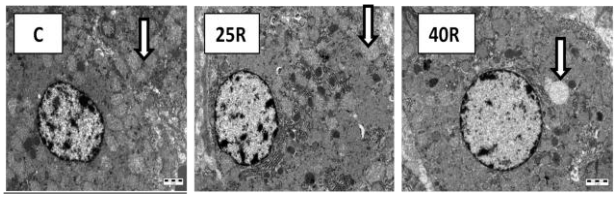

Fig. 2: Transmission electron microscopy of Najdi lambs liver (white arrow: mitochondria).C: Control 0%, 25R: 25% and 40R: 40% feed restriction groups (magnification 315000X)

distinct inner membrane and cristae. The main ultra-structural changes were associated with swollen mitochondria; reduce glycogen granules and lipid concentration in the liver of 40% feed restriction group. In agreement with the current study, Asghar and Yeates (1979) found that sub-maintenance feeding decreased the number of mitochondria and glycogen granules, whereas the transverse tubular system and sarcoplasmic reticulum were unaffected.

Myofibril Diameter and Sarcomere Length

Means of myofibril diameter and sarcomere length of the C, 25R and 40R groups are presented in Table 1. Myofibril diameter of the muscle *L. dorsi* of C group was significantly larger (13.5 µm) than 25R (12.5 µm) and 40R (11.5 µm) groups. Significant difference between the myofibril diameter of 25R and 40R groups was also found. Regarding the sarcomere length of the three groups, C group had significant longer sarcomere (2.03 µm) compared to the 25R (1.87 µm) and 40R (1.93 µm) groups. Also, the difference between the 25R and 40R groups was statistically significant. The present results are in the line of Yambayamba and Price (1991) findings, who found that fiber diameter of Herford crossbred heifers after two months

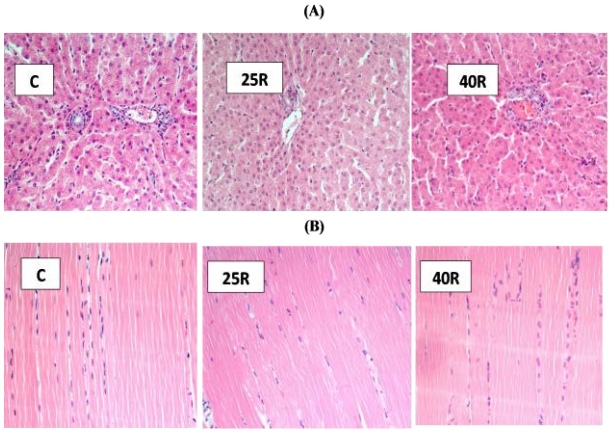

Fig. 3: Light microscope liver sections (C, 25R, and 40R) depicted no histopathologic differences (A), and longitudinal sections of *L. dorsi* muscle (B) of Najdi lambs stained with HandE, C: Control, 25R: 25% and 40R: 40% feed restriction groups (magnification 20X)

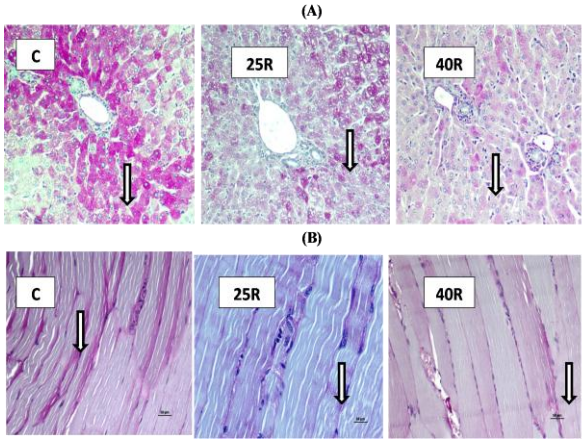

Fig. 4: Light microscope liver sections (C) depicted high glycogen content (arrows) compared to the other livers (25R and 40R); (A), and longitudinal sections of *L. dorsi* muscle (B) of Najdi lambs depicted high glycogen content (arrows) compared to25R: 25%, and 40R: 40% feed restriction groups.(stained with PAS, magnification 20X)

of feed restriction was smaller (P<0.05) than in the *ad libitum* fed animals. Joubert (1956), Yambayamba and Price (1991), and Wojtysiak *et al.* (2010) concluded that there is a direct and positive relationship between fiber size and diameter, and live and carcass weight. The previous conclusion may explain the results of the present study, as the carcasses of C group were heavier than 25R and 40R groups. On the contrary of the present results, Solomon *et al.* (1981) found that sarcomere lengths were not affected by breed, slaughter weight or sex of lambs.

Histological Changes in Liver and Muscle Tissues

Liver sections showed normal lobular architecture and

normal portal areas. No degenerative of fibrotic changes are seen in hepatocytes, no cellular infiltration, sinusoidal distension or increased Kuppfer cell reactivity. *L. dorsi* muscles were also showed normally striated muscle fibers with no evidence of pathological abnormality was observed.

Glycogen deposition in liver samples apparently varied according to the treatments, judging by PAS color intensity, the highest intensity is seen in C group followed by 25R group. Lowest intensity is recorded in 40R group. This suggests less glycogen deposition in the liver in 40R group compared to C and 25R groups. In *L. dorsi* muscle, same as above the most dense glycogen deposition, judging by color intensity, was in C group compared to 25R and 40R groups. Similar results were reported by Holness *et al.* (1988), Nur *et al.* (1995) and Kokubun *et al*. (2009) who found that limiting the energy intake significantly reduced hepatic glycogen stores and tended to decrease skeletal muscle glycogen.

Conclusion

Limitation in the rate and development of sheep muscle fiber would be a result of feed restriction. Electron microscopic changes in muscle and liver of restricted feeding lambs involved progressive cell degeneration demonstrated by reducing muscle fiber size, swollen mitochondria, and reduce glycogen granules in the liver and muscles.

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References

Anjum, M.I., A. Azim, M.A. Jabbar, M. Anwar and I.H. Mirza, 2012. Age and weight at puberty in Nili-Ravi buffalo heifers reared on three dietary energy restriction periods followed by compensatory growth. *Pak. Vet. J*., 32: 367–371

Asghar, A. and N.T.M. Yeates, 1979. Muscle characteristics and meat quality of lambs grown on different nutritional planes—III. Effect on muscle ultrastructure. *Agric. Biol. Chem.*, 43: 445–453

Bancroft, J.D. and H.C. Cook, 1984. *Manual of Histological Techniques*. Churchill Livingstone, Longman Group Limited, London, UK

Bellairs, R., E.J. Sanders and P.A. Portch, 1980. Behavioral properties of chick somatic mesoderm and lateral plate when explanted *in vitro*. *J. Embryol. Exp. Morph.*, 56: 41–58

Brandstetter, A.M., B. Picard and Y. Geay, 1998. Muscle fiber characteristics in four muscles of growing male cattle. II. Effect of castration and feeding level. *Livest. Prod. Sci.*, 53: 25–36

Choat, W.G., C.R. Krehbiel, M.S. Brown, G.C. Duff, D.A. Walker and D.R. Gill, 2002. Effects of restricted versus conventional dietary adaptation on feedlot performance, carcass characteristics, site and extent of digestion, digestion kinetics, and ruminal metabolism. *J. Anim. Sci.*, 80: 2726–2739

Eila, N., A.G. Lavvaf, T. Farahvash and A. Zarei, 2012. Influence of various growth patterns on performance of Ross 308 broilers. *Pak. Vet. J.*, 32: 372–374

Greenwood, P.L., A.S. Hunt, J.W. Hermanson and A.W. Bell, 2000. Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. *J. Anim. Sci.*, 78: 50–61

Hammond, J., 1932. *Growth and Development of Mutton Qualities in the Sheep*. A survey of the problems involved in meat production. Oliver and Boyd, London

Hicks, R.B., F.N. Owens, D.R. Gill, J.J. Martin and C.A. Strasia, 1990. Effects of controlled feed intake on performance and carcass characteristics of feedlot steers and heifers. *J. Anim. Sci.*, 68: 233–244

Hocquette, J.F., S. Tesseraud, I. Cassar-Malek, Y. Chilliar and I. Ortigues-Marty, 2007. Responses to nutrients in farm animals: implications for production and quality. *Animal*, 1: 1297–1313

Holness, M.J., M.J. L. Schuster-Bruce and M.C. Sugden, 1988. Skeletal-muscle glycogen synthesis during the starved to fed transition in the rat. *Biochem. J.*, 154: 855–859

Javaid, S., M.I. Anjum and M. Akram, 2012. Effect of dietary protein and energy level on proximate composition of breast and thigh meat in white leghorn layers at molt and post molt production stages. *Pak. Vet. J*., 32: 483–488

Joubert, D.M., 1956. A study of pre-natal growth and development in the sheep. *J. Agric. Sci. (Cambridge)*, 47: 382–428

Kokubun, E., S.M. Hirabara, J. Fiamoncini, R. Curi and H. Haebisch, 2009. Changes of glycogen content in liver, skeletal muscle, and heart from fasted rats. *Cell Biochem. Funct.*, 27: 488–495

Moody, W.G., J.D. Kemp, M. Mahyuddin, D.M. Johnston and D.G. Ely, 1980. Effect of Feeding Systems, Slaughter Weight and Sex on Histological Properties of Lamb Carcasses. *J. Anim. Sci.*, 50: 249–256

Murphy, T.A. and S.C. Loerch, 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics and cmposition. *J. Anim. Sci.*, 72: 2497–2507

Nur, T., I. Sela, N.J.G. Webster and Z. Madar, 1995. Starvation and refeeding regulate glycogen synthase gene expression in rat liver at the posttranscriptional level. *J. Nutr.*, 125: 2457–2462

Rehfeldt, C., N. Stickland, I. Fiedler and J. Wegner, 1999. Environmental and genetic factors as source of variation in skeletal muscle fiber number. *Basic Appl. Myol.*, 9: 235–253

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.*, 17: 208–212

SAS, 2002. *SAS User's Guide: Statistics*. SAS Institute Inc., Cary, North Carolina, USA

Solomon, M.B., W.G. Moody, J.D. Kemp and D.G. Ely, 1981. Effect of breed, slaughter weight and sex on histological properties of ovine muscle. *J. Anim. Sci.*, 52: 1019–1025

Therkildsen, M., L.M. Larsen and M. Vestergaard, 2002. Influence of growth rate and muscle types on muscle fiber type characteristics, protein synthesis capacity and activity of the calpain system in Friesian calves. *Anim. Sci.*, 74: 243–251

White, N.A., M.D. McGavin and J.E. Smith, 1978. Age-related changes in percentage of fiber types and mean fiber diameters of the ovine quadriceps muscles. *Amer. J. Vet. Res*., 39: 1297–1302

Wojtysiak, D., U. Kaczor, K. Połtowicz and K. Krzysztoforski, 2010. The effects of sex and slaughter weight on muscle fibre characteristics and physico-chemical properties of lamb *longissimus thoracis* muscle. *Anim. Sci. Pap. Rep*., 28: 61–69

Yambayamba, E. and M.A. Price, 1991. Fiber-type proportions and diameters in the longissimus muscle of beef heifers undergoing catch-up (compensatory) growth. *Can. J. Anim. Sci.*, 71: 1031–1035

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