EFFECTS OF CIMATEROL ON CARCASS AND SKELETAL MUSCLE CHARACTERISTICS UNDER AD LIBITUM AND RESTRICTED FEEDING CONDITIONS IN LAMBS

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Summary

Fifty-two wether lambs weighing 30 kg were randomly assigned to 5 treatment groups; 1) initial slaughter, 2) control-maintenance (CON-MT), 3) control-ad libitum (CON-AL), 4) cimaterol-maintenance (CIM-MT) and 5) cimaterol-ad libitum (CIM-AL). Ad libitum-fed animals had free access of a high-concentrate diet, whereas maintenance animals were restricted in feed intake to maintain the initial weight of 30 kg for 90 days. Cimaterol was administered in the feed at 10 mg/kg. Regardless of feeding level, the administration of CIM improved carcass weight ($p < .05$), dressing % ($p < .01$), longissimus muscle area ($p < .01$), leg conformation and muscling ($p < .01$), USDA yield and quality grades ($p < .01$) and protein concentration ($p < .01$) in carcass as well as in muscle. Cimaterol feeding decreased organ wt ($p < .01$), backfat depth ($p < .01$), intramuscular fat and overall fatness. Cimaterol was effective for muscle accretion even under restricted feeding condition. The greater accretion of muscle was the result of the hypertrophy of both type I and type II muscle fibers but the hypertrophy of type II fiber (110%) was much greater than that of type I fiber (37%). Cimaterol feeding decreased muscle DNA concentrations but the number of nuclei per muscle fiber was not changed, indicating that the lower DNA concentration was due to the dilution effect caused by the hypertrophy of muscle fiber. As evidenced by lower flank streaking, lower marbling and darker muscle, CIM feeding adversely affected meat quality. Meat tenderness was also adversely affected, resulting in significantly (p H .01) tougher meat in CIM-fed animals.

(Key Words: Cimaterol, Carcass Characteristics, Meat Quality, Fiber Type, DNA, Restricted Feeding)

Introduction

It has been demonstrated that the beta-adrenergic agonists improve animal growth performance and carcass composition in finishing lambs (Baker et al., 1984; Beermann et al., 1986; Kim et al., 1987), broilers (Dalrymple et al., 1984), swine (Jones et al., 1985) and cattle (Ricks et al., 1984). Cimaterol, a β-agoinst, also improved dressing percent and increased longissimus muscle area, overall muscling and the yield of four lean cuts in lambs (Kim et al., 1987). The increase of muscle mass in cimaterol-fed lambs was primarily attributed to the hypertrophy of type II fibers.

All these data were obtained with meat animals fed β-agonists under ad libitum feeding conditions and no information is available on the response of animals to β-agonists under restricted feeding conditions. Since the muscle accretion and protein synthesis is a very active and energy demanding process (Reeds et al., 1982), normal response of animals to restricted energy intake would be a delay of muscle tissue growth and the conservation of energy for essential physiological functions.

Therefore, it would be interesting to test if cimaterol can function as a repartitioning agent even under restricted energy intake. As part of a feeding trial (Kim et al., 1989), the effects of cimaterol on carcass characteristics, skeletal muscle fiber types and meat quality in lambs subjected to two feeding conditions, restricted feeding to main-

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tain constant body weight and ad libitum feeding for maximal weight gain, were investigated.

Materials and Methods

Fifty-two predominantly Targhee wether lambs, weighing approximately 30 kg, were used for comparative slaughter feeding trial. A completely mixed high concentrate diet (table 1) was used with or without 10 mg/kg cimaterol during the experiment and the animals were either ad libitum fed or limited fed twice daily.

TABLE 1. COMPOSITION OF DIET[1]

Ingredient	%
Corn	31.25
Barley	20.00
Alfalfa hay	15.00
Oat hay	15.00
Soybean meal	8.00
Molasses	10.00
Trace mineral salt	.5
Limestone	.25

[1]The diet contained 13.5% crude protein and 2.70 Mcal metabolizable energy/kg feed. Moisture content was 11%.

At the beginning of the experiment, eight lambs were killed to establish initial carcass and muscle composition and muscle fiber characteristics. The remaining animals were randomly divided into 4 treatment groups (table 2), then housed in individual pens and fed for 90 days. Since the original experiment was to study the effects of cimaterol on nitrogen retention and energy utilization as reported elsewhere (Kim et al., 1989), the number of animals was not completely balanced in the experimental design. Animals were weighed every 3 weeks after an overnight withdrawal of feed and water (shrunk weights). Feed allowances for maintenance groups were initially calculated for individual animal according to the formula of 115 kcal ME/kg $EB^{0.75}$/d (EB = empty body weight), and then they were adjusted at the time of weighting (every 3 wk) to maintain the initial body weights.

TABLE 2. EXPERIMENTAL DESIGN

Group	Level of feeding	No. of animals
Initial Slaughter		8
Control	Maintenance (CON-MT)	8
Control	Ad libitum (CON-AL)	14
Cimaterol	Maintenance (CIM-MT)	8
Cimaterol	Ad libitum (CIM-AL)	14

After 90 days in the feedlot, all animals were shipped to the university abattoir, held overnight with water only and slaughtered according to the standard commercial procedure. Animals were handled carefully to minimize undue stress prior to slaughter. After 24 h chilling of carcasses in a 1°C cooler, the following carcass measurements were made: chilled carcass weight, liver and heart weight, dressing percent, maturity, depth of fat and longissimus muscle area at the 12th rib, kidney and pelvic fat, leg conformation, flank streaking, and color and firmness of longissimus muscle at the 12th rib. Firmness was subjectively measured on an 8-point numerical value system described by Boggs and Merkel (1979). Color was also subjectively determined on a 5-point scale; 1=pale, 2=slightly pale, 3=bright red, 4=slightly dark, and 5=dark. USDA quality and yield grades were determined as described by Boggs and Merkel (1979).

For the measurement of tenderness, two and a half cm thick loin chops (5 chops per animal) were broiled in an oven until the internal temperature reached 70°C. After cooling the cooked chops to 25°C, a core sample of 2 cm diameter was drilled parallel to the muscle fiber from each chop and Warner-Bratzler (WB) shear force was measured on an Instron at a crosshead speed of 20 cm/min.

Carcass composition was chemically determined by grinding the right side of each carcass and analyzing fat, moisture, protein and ash by AOAC method (1984). Muscle composition was also chemically determined by grinding the center portion of longissimus muscle (15-20g) at the 12th rib and analyzing fat, moisture and protein.

For the cytological analysis, the longissimus dorsi (LD) muscle sample taken from the geometrical center at the 12th rib was frozen in dry ice-acetone. Sections were cut in a cryostat and then stained for myofibrillar adenosine triphosphatase (ATPase) after acid incubation at pH 4.2, as des-

cribed by Guth et al.(1970). The stained sections were projected on a projection microscope[2] and all the fibers in several microscopic fields were traced. Approximately 500 fibers were traced for a given muscle sample. During the tracing, type I (slow-contracting, oxidative) and type II (fast-contracting, mixed glycolytic/oxidative) fibers were identified. The proportions of fiber types were calculated by counting the number of each fiber type from the tracings. Individual fiber cross-sectional area was also determined from the tracings using a Zeiss MOP Digital Image Analyzer[3] and average cross-sectional area was calculated. Another thin sections were stained with hematoxylin and eosin, and the number of nuclei inside the basement membrane was counted.

For the analysis of DNA and RNA concentrations, about 5 grams of LD muscle samples were frozen liquid nitrogen and pulverized according to the method of Bochert and Briskey (1965). A .2- to .25-g sample of pulverized muscle was homogenized in 10 ml of .9% NaCl solution for 1 min at high speed using a polytron homogenizer. Duplicate 1 ml homogenate samples were drawn and added to .5 ml of ice-cold .6 N perchloric acid. DNA and RNA were separated by the modified Schmidt – Thannhauser method (Munro and Fleck, 1966). DNA concentration was determined using the Ceriotti procedure (Ceriotti, 1952), while RNA concentration was spectrophotometrically determined at 260 nm. Statistical analysis of all data was conducted using two-tailed Student's t-test of Statistical Analysis System (SAS, 1982).

Results and Discussion

The effects of cimaterol on carcass and organ weights are summarized in table 3. Carcass weight and dressing percentage were higher ($p < .01$) in the CIM group for both maintenance and ad libitum fed animals. CIM-maintenance (CIM-MT) animals had a 5.6 percentage point higher dressing percent than control-maintenance (CON-MT) animals at an identical live weight. This higher dressing percentage was attributed to lower ($p < .01$) organ weight and heavier muscling. The results are consistent with those of a previous study with lambs (Kim et al., 1987) which showed lower liver and heart weights and higher dressing percent in CIM-fed animals. Duquette et al. (1987) also reported that the weights of hide, liver and heart were significantly lower in steers fed β-agonist. In rats, however, the administration of β-agonists increased heart weight and did not affect the weights of other internal organs (Deshaies et al., 1981; Reeds et al., 1986; Kim, 1988). At present, we have no explanation for the apparent species differences in the growth response of internal organs to β-agonists.

TABLE 3. EFFECTS OF CIMATEROL ON CARCASS AND ORGAN WEIGHTS

Item	Initial slaughter		Maintenance			Ad libitum		
	Mean	SE	Control (CON-MT)	CIM (CIM-MT)	SE	Control (CON-AL)	CIM (CIM-AL)	SE
No. of animals	8		8	8		14	14	
Live wt(kg)	30.1	.63	29.7	29.6	1.06	49.9	52.3	1.29
Total wt gain(kg)			0	0	0	20.3	22.5	1.4
Total feed intake(kg)			50.4	47.7	1.8	146.7	148.5	4.5
Carcass wt(kg)	13.5	.38	13.7	15.3*	.81	26.7	29.3**	.96
Organ wt(g)								
Liver			428	316**	10.2	871	806*	26.7
Heart			148	126**	4.6	221	187**	10.5
Dressing %	44.9	.70	46.1	51.7**	.74	53.5	56.0**	.70

*$p < .05$, differs from control.
**$p < .01$, differs from control.

[2]Reichert Visopan, C. Reichert Optical Co., Wein, Austria.
[3]Mop Digital Image Amalyzer, Carl Zeiss Inc., New York, NY.

Table 4 and figures 1-3 summarize the effect of cimaterol on carcass characteristics and meat quality. Variables indicating the fatness of the carcass, flank streaking, backfat thickness and kidney and pelvic fat showed lower values in the CIM groups regardless of feeding level. The backfat depth of CIM-ad libitum (CIM-AL) group was 63% ($p < .01$) that of control-ad libitum (CON-AL) group, whereas the backfat depth of CIM-MT animals was unmeasurably thin. The LD muscle areas of CIM-MT and CIM-AL groups increased by 48 and 30% over the respective control animals, and the average LD area of CIM MT was as large as that of CON-AL group (table 4 and figure 3). CIM-fed carcasses were shorter and broader (figures 1 and 2) and had one grade higher leg conformation scores than the respective controls. Because of the greater muscling and higher conformation scores, the USDA yield grade and final quality grades were also significantly ($p < .01$) improved in the CIM-fed animals. These results clearly indicated that cimaterol was remarkably effective for muscle accretion even under severely restricted feeding conditions.

The data further demonstrated that cimaterol enhanced the efficiency of muscle accretion per kg feed to a greater extent at lower feeding level. Further study is warranted to determine the optimal energy and protein intake for maximal efficiency of muscle accretion in the presence of cimaterol.

In contrast to increased yield of lean meat,

TABLE 4. EFFECTS OF CIMATEROL ON CARCASS CHARACTERISTICS AND MEAT QUALITY

Item	Initial slaughter		Maintenance			Ad libitum		
	Mean	SE	Control	CIM	SE	Control	CIM	SE
Maturity score	A		A	A		A	A	
Flank streaking[1]	–		2.1	2.0	.13	4.6	3.8**	.23
Firmness[2]	–		2.3	3.4**	.24	3.6	4.6**	.17
LD muscle color[3]	–		3.5	3.1	.24	3.1	4.4**	.13
Backfat, mm	1.2	.08	1.4	NM[6]	.34	8.2	5.2**	.78
KP fat (%)	–		1.0	0.6**	.11	1.1	0.8*	.35
LD area (cm^2)	8.7	.43	8.3	12.3**	.75	12.6	16.4**	.34
Leg conformation[4]	–		6.3	9.6**	.65	10.9	13.6**	.25
USDA yield grade	–		2.0	1.2**	.11	4.2	3.2**	.27
Quality grade[5]	–		4.8	6.8**	.31	11.0	12.8**	.30
WB(shear kg/core)	–		5.2	10.2**	1.0	5.2	8.4**	.71

[1] Small = 5, slight = 4, trace = 3, practically devoid = 2.
[2] Tend to be moderately firm =3, moderately firm = 4, tend to be firm = 5.
[3] Dark = 5, medium = 3, pale = 1.
[4] $Prime^{+}$ =15, $Prime^{0}$ = 14, $Prime^{-}$ = 13, $Choice^{+}$ = 12, $Choice^{0}$ = 11, $Choice^{-}$ = 10, $Good^{+}$ = 9, $Good^{0}$ = 8, $Good^{-}$ = 7.
[5] Prime = 13, $Choice^{+}$ = 12, $Choice^{0}$ = 11, $Choice^{-}$ = 10, $Good^{+}$ = 9, $Good^{0}$ + 8, $Good^{-}$ = 7, $Standard^{+}$ = 6, $Standard^{0}$ = 5, $Standard^{-}$ = 4.
[6] Not measurable.
*$p < .05$, differs from control. ** $p < .01$, differs from control.

meat quality was adversely affected by cimaterol. CIM-AL animals showed lower flank streaking ($p < .01$), darker muscle color ($p < .01$) and firmer muscle texture ($p < .01$) than CON-AL lambs. Warner Bratzler shear values of LD muscles from CIM-MT and CIM-AL animals were higher ($p < .01$) than the respective control animals.

Other studies also showed a decrease in flank streaking and marbling in clenbuterol or cimaterol-fed lambs and cattle (Baker et al., 1984; Hamby et al., 1986; Kim et al., 1987; Miller et al., 1988). Since β-agonists are lipolytic, a decrease in marbling would be expected. Though the results are not always consistent depending on the animal species and ante mortem stress, some reports also indicated a trend of darker muscle in cimaterol-fed animals (Allen et al., 1985; Lee et al., 1988). Because cimaterol is a substituted catecholamine, it would stimulate glycogenolysis and cause darker color in stressed animals as reported by Lee et al. (1988). The meat toughening effect of β-agonists

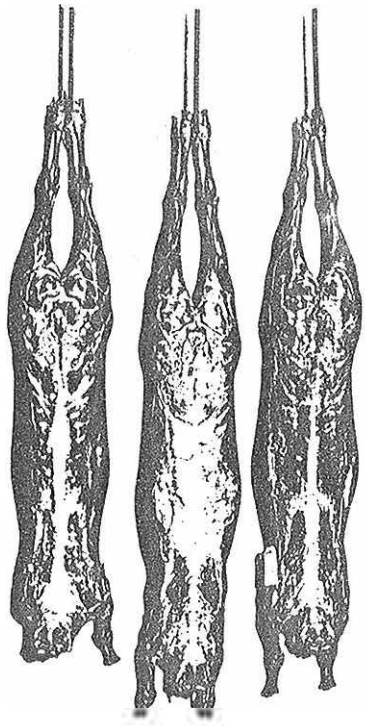

Figure 1. Carcass conformation of maintenance-fed lambs. Middle carcass is control and left and right carcasses are CIM-fed.

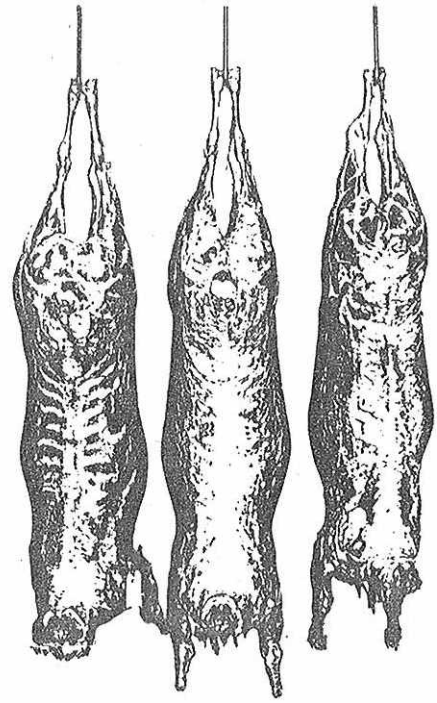

Figure 2. Carcass conformation of ad libitum fed lambs. Middle carcass is control and left and right carcasses are CIM-fed.

has been recently reported by several investigators in lambs (Hamby et al., 1986; Lee et al., 1988; Wang et al., 1988), in cattle (Schiavetta et al., 1988), in poultry (Morgan et al., 1988) and in swine (Jones et al., 1985). No differences in tenderness were also observed in some studies with swine (van Weerden, 1987; Merkel, 1988). One week withdrawal of cimaterol prior to slaughter greatly improved the tenderness of treated lambs (Lee et al., 1988).

The mechanisms of meat toughening by cimaterol are not known, but early onset of rigor and heat shortening during slaughter, intermediate ultimate pH, greater compactness of muscle (greater protein content and less water), lower proteolytic capacity and higher calpastatin activity have been suggested as possible causes (Kretchmar et al., 1988; Lee et al., 1988; Morgan et al., 1988).

In carcass composition (table 5), CIM-fed animals showed higher water and protein content ($p < .01$) and lower fat ($p < .01$) than the control animals. Of particular interest is the change of carcass composition in the maintenance-fed animals compared to the composition of initial slaughter animals. CON-MT lambs showed a 48% increase in fat and 9 and 12% decrease in water and protein content, respectively. It suggested that control animals maintained at constant weight tended to deposit more fat and less water and protein. Contrary to the control animals, CIM-MT lambs maintained a carcass composition that was almost identical to those in the initial slaughter group. In longissimus muscle composition, CIM-MT group showed lower water ($p < .01$) and higher protein content ($p < .01$) than CON-MT animals, whereas CIM-AL group showed higher water and protein ($p < .01$) and lower fat content ($p < .01$) compared to CON-AL group. The data indicated that CIM feeding increased muscle protein concentration and decreased intramuscular fat content, which agreed well with the increased compactness (firmness) of muscle texture and decreased marbling as discussed previously.

It has been well documented that under the normal feeding conditions, β-agonists increase lean muscle mass and decrease fat deposition in rats (Berne et al., 1985; Rothwell and Stock, 1985) and in domestic animals (Dalrymple et al., 1985;

TABLE 5. EFFECTS OF CIMATEROL ON CARCASS AND MUSCLE COMPOSITION (%)

Item	Initial slaughter		Maintenance			Ad libitum		
	Mean	SE	Control (CON-MT)	CIM (CIM-MT)	SE	Control (CON-AL)	CIM (CIM-AL)	SE
Carcass composition[1]								
Water	62.6	.6	57.2	61.7**	1.1	48.3	52.7**	1.0
Fat	15.8	.9	23.4	16.7**	1.3	34.5	28.3**	1.4
Protein	16.4	.3	14.5	16.8**	.3	13.0	15.2**	.3
Ash	4.2	.1	4.3	4.0	.2	3.6	3.3	.1
Muscle composition[2]								
Water	76.7	.2	78.3	75.7**	.4	73.7	74.2	.3
Fat	2.0	.2	2.2	1.7	.3	5.0	2.6**	.3
Protein	20.2	.2	18.5	21.4**	.3	20.2	22.2**	.2

[1]Measured by chemical analysis of ground half carcass.
[2]Longissimus dorsi was analyzed.
*$p < .05$, differs from control.
**$p < .01$, differs from control.

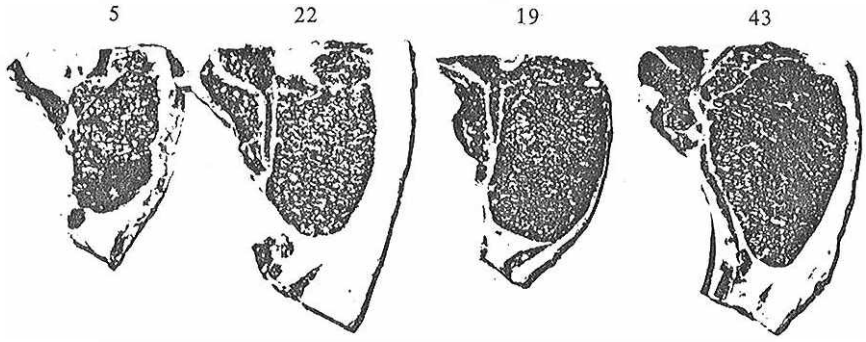

Figure 3. Comparison of longissimus muscle area and backfat depth at the 12th rib. 5=CON-MT, 22=CON-AL; 19=CIM-MT; 43=CIM-AL

Jones et al., 1985; Beermann et al., 1986; Kim et al., 1987). No information has been available on the response of animals to β-agonists under restricted feeding conditions and the present study demonstrates that the accretion of lean tissue and muscle protein is greatly enhanced by cimaterol even under severely restricted feeding situations.

The greater muscle and protein accretion in CIM-fed animals was found to be the result of hypertrophy of both type I and type II fibers (table 6 and figure 4). However, the hypertrophy of type II fibers was much greater than that of type I fibers. The cross-sectional area of type I fibers was only 38 and 36% greater in CIM-MT and CIM-AL lambs compared to the respective controls. In contrast, the cross-sectional area of type II fibers was 115 and 110% greater in CIM-MT and CIM-AL lambs compared to the respective controls (table 6). Furthermore, mean type II fiber area of CIM-MT group (3014 μm^2) was greater than that of CON-AL group (2210 μm^2). The data clearly demonstrate that type II fibers have hypertrophied to a greater extent than type I fiber regardless of feeding levels. CON-MT group showed smaller fiber size than initial slaughter group, indicating that muscle fibers atrophied under the maintenance feeding regimen. As discussed previously, CON-MT had decreased carcass and muscle protein content (table 5), which agreed well with the atrophied muscle fibers. In contrast, CIM-MT group showed 65% increase in type II fiber size with smaller (5%) increase in type I fiber size

TABLE 6. EFFECTS OF CIMATEROL ON FIBER TYPE PERCENTAGE AND FIBER SIZE[1]

Item	Initial slaughter		Maintenance			Ad libitum		
	Mean	SE	Control (CON MT)	CIM (CIM-MT)	SE	Control (CON-AL)	CIM (CIM-AL)	SE
No. of animals	5		4	4		4	4	
Type I fiber (%)	11.5	1.6	19.0	15.2	3.3	11.4	11.5	2.6
Type I fiber, area (μm^2)	1834	122	1388	1922*	236	2842	3858*	476
Type II fiber area (μm^2)	1831	153	1403	3014**	363	2210	4633**	403

[1]Longissimus dorsi was analyzed. Fiber type classification was based on acid-stable ATPase reaction.
*$p < .05$, differs from control.
**$p < .01$, differs from control.

compared to the initial slaughter group.

Another significant difference among treatments was the proportion of type I fibers. As evidenced in figure 4, CON-MT group showed a greater incidence (19%) of type I fibers, whereas no differences were observed among the other three treatments. The proportion of type I fiber in CON-MT was higher than that of initial slaughter group, suggesting that restricted feeding might have reverted type II fibers to type I fibers.

The present study confirmed the findings of Kim et al. (1987) and the conclusions of Beermann et al. (1987) that cimaterol-induced muscle hypertrophy was principally the result of increased radial growth of type II fibers. Furthermore, cimaterol greatly enhanced the growth of type II fibers even under severely restricted feeding conditions.

Beermann et al. (1985) also reported that cimaterol reduced the incidence of type I fibers in the semitendinosus muscle but not in the longissimus muscle. In this study cimaterol reduced the incidence of type I fiber in LD muscle of the maintenance-fed animals but not in ad libitum-fed animals. We have no explanation for the apparent differences in the response between the two different feeding levels.

DNA and RNA concentrations and the number of nuclei per muscle fiber are summarized in table 7. A marked difference ($p<.01$) was found in DNA concentration between control and CIM groups for both maintenance and ad libitum-fed animals. Regardless whether it is expressed per gram of wet muscle or per gram of muscle protein, DNA concentrations of CIM-MT and CIM-AL muscles were approximately 50% and 30% less than those of the respective control muscles. However, the number of nuclei per muscle fiber was almost identical except in the CON-MT group. Thus, the DNA data suggest that the lower concentration of DNA in

TABLE 7. EFFECTS OF CIMATEROL ON DNA AND RNA CONCENTRATION IN LONGISSIMUS MUSCLE

Item	Initial slaughter		Maintenance			Ad libitum		
	Mean	SE	Control (CON-MT)	CIM (CIM-MT)	SE	Control (CON-AL)	CIM (CIM-AL)	SE
No. of animals	8		8	8		8	8	
DNA(μg/g wet muscle)	445	24.0	590	322**	56.2	355	251**	16.1
DNA(mg/g protein)	2.21	.13	3.20	1.50**	.32	1.75	1.13**	.10
RNA(μg/g wet muscle)	420	14.0	342	284	45.1	318	351	13.3
RNA (mg/g protein)	2.08	.07	1.86	1.32	.26	1.57	1.58	.06
RNA/DNA ratio	.95	.04	.58	.91**	.09	.90	1.44**	.11
No. of nuclei/fiber[1]			.9	1.4*	.16	1.3	1.4	.16

[1]The number of nuclei/fiber was obtained by counting the number of nuclei in cross-sections of muscle fibers.
*$p < .05$, differs from control.
**$p < .01$, differs from control.

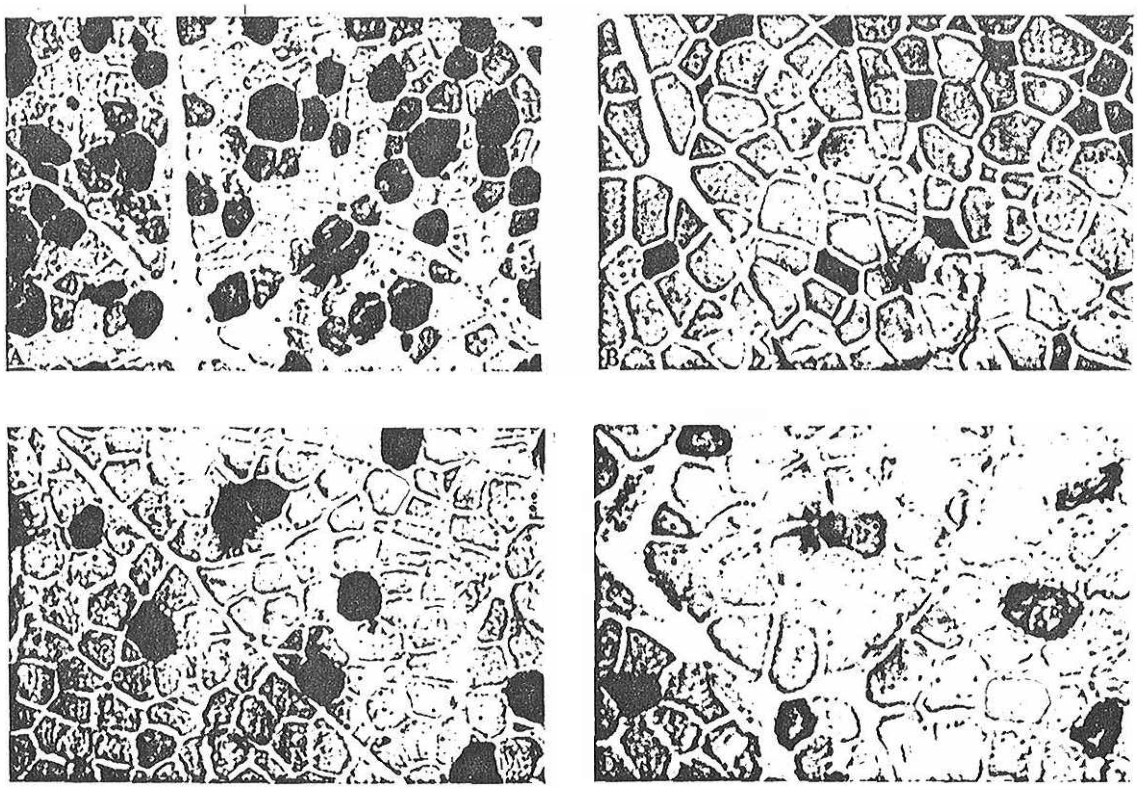

Figure 4. ATPase staining of longissimus muscle fibers (magnifications X 160): A=CON-MT; B=CIM MT; C=CON AL; D=CIM-AL.

CIM-fed animals is the result of the dilution effect caused by the hypertrophy of muscle fiber rather than the actual change of DNA content per muscle fiber. It also indicates that the hypertrophy of skeletal muscle induced by CIM does not require the proliferation and incorporation of satellite cells into the muscle fiber as suggested previously (Beermann et al., 1987; Kim et al., 1987). The higher concentration of DNA and yet lower number of nuclei within muscle fibers in CON-MT group could be the result of muscle fiber atrophy and a greater contribution of extracellular connective tissue nuclei to the total DNA concentration of muscle tissue.

No differences were found in muscle RNA concentrations between control and CIM-fed lambs. However, total RNA content in the muscle would be greater in CIM group because the muscle weight was greatly increased. Beermann et al. (1987) also reported a greater RNA content in the semitendinosus muscle of CIM-fed lambs. The increased RNA/DNA ratio observed by us and by Reeds et al. (1986) and Beermann et al. (1987) suggest that capacity for protein synthesis is increased. It was interesting to note that CIM-MT group having the same LD cross-sectional area as CON-AL group (table 4) had a similar RNA/DNA ratio. Whether all the increased RNA is mRNA specific for muscle protein synthesis is unknown but recent studies with ractopamine (Garcia et al., 1988) and clenbuterol (Smith et al., 1987) have shown that these β-agonists significantly increase the relative concentrations of myosin light chain – 1/3 mRNA and actin mRNA. Such results suggest that β-agonists may cause the muscle hypertrophy by increasing the expression of genes encoding myofibrillar proteins.

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