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THE SHORT TERM EFFECTS OF RACTOPAMINE SUPPLEMENTATION ON PROTEIN TURNOVER IN GROWING SWINE

By

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ABSTRACT

THE SHORT TERM EFFECTS OF RACTOPAMINE SUPPLEMENTATION ON PROTEIN TURNOVER IN GROWING SWINE

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To determine if Ractopamine (RAC) affects skeletal muscle protein synthesis and degradation rates within one week, a study was performed using 22 crossbred barrows (68 ± 4 kg). Six barrows were slaughtered on d Ø to determine composition of the longissimus (LD) and semitendinosus (ST) muscles. Four barrows received a control (C) diet and 4 barrows received the control diet supplemented with 2Ø ppm RAC for 14 d. To determine whole body protein synthesis (WBPS) and degradation (WBPD), 8 barrows were fed RAC or C (n=4/trt) and continuously infused for 6 h with N-15 lysine on d 7. To determine fractional synthesis rates (FSR) of the LD and ST, the same barrows were infused on d 8 with C-14 tyrosine for 6 h. On d 14, remaining barrows were slaughtered and the left LD and ST were removed. Differences in protein content in LD and ST between d Ø and d 14 were used to calculate fractional accretion rate (FAR). No differences between C and RAC in LD and ST FAR, FSR, WBPS and WBPD were observed. DNA concentrations (ug/g tissue) were lower in RAC than C on d 7 and d 14. The data show that one week of RAC has no effect on protein metabolism.

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INTRODUCTION

Increased health risks associated with excess dietary fat and cholesterol have led to an increased consumer demand for lean meat products. One of various approaches which may satisfy this demand is the feeding of a class of compounds referred to as beta adrenergic agonists (B-agonists) to our meat producing livestock. Beta agonists improve carcass traits by promoting muscle accretion and depressing fat deposition. The increase in muscle protein accretion may be the consequence of inhibition of protein degradation, as in the case of Clenbuterol, or by stimulation of protein synthesis as in the case of Ractopamine. Further research needs to be conducted, however, to determine the optimal doses of the compounds as well as to determine the stage of maturity at which the B-agonists are most effective.

The purpose of this study was to determine if the beta agonist, Ractopamine, can stimulate protein synthesis after one week and protein accretion after two weeks of administration when fed at the level of 20 ppm to growing pigs. To demonstrate the effects of Ractopamine on protein metabolism, skeletal muscle fractional protein synthesis and accretion and whole body lysine flux were determined.

LITERATURE REVIEW

Protein turnover has been defined by Waterlow et al. (1978) as the renewal or replacement of a protein molecule. Consistent with this definition, Reeds and Palmer (1985) termed protein turnover as the continual degradation and resynthesis of cellular proteins. This process is generally discussed in terms of protein synthesis and protein degradation. In the steady state, protein synthesis is equal to protein degradation; however, in the non-steady state, protein synthesis may exceed degradation and vice versa. In the case of net protein accretion, synthesis exceeds degradation. Prolonged starvation is representative of a non-steady state when protein degradation exceeds protein synthesis (Millward and Waterlow, 1978).

DEVELOPMENTAL CHANGES

The chief developmental change in muscle protein is a decline in overall fractional turnover rate with age (Millward and Waterlow, 1978; Millward et al., 1978; Mulvaney et al ., 1985; Skjaerlund et al 1987). Early in life, fractional synthesis and degradation rates are higher than the fractional rates found in adult subjects (Millward et al., 1975; Munro, 1976). As the subject matures, fractional synthesis rates begin to regress (Waterlow et al., 1978; Kelly et al., 1984). Millward and Waterlow (1978) credit the fall in synthesis rate to an increase in DNA unit size or protein:DNA ratio. This phenomenon, refered

to as hypertrophy, is characterized by a relative increase in muscle mass compared to the DNA content. The increase in total DNA per muscle, which occurs postnatally, is a result of satellite cell proliferation and fusion to existing myotubes (Allen et al., 1979).

While DNA dictates the amount of protein synthesized at the transcriptional level, RNA concentration determines the amount of protein synthesis at the translational level. Young (1974) reported few developmental changes in the ratio of RNA:DNA. A constant RNA:DNA ratio during development indicates that as the protein:DNA ratio increases (i.e., hypertrophy) there must be a decline in muscle ribosome concentration (Millward and Waterlow 1978). Further support of the decline in ribosomal RNA, is evident by the decrease in synthesis rates per unit of RNA with age (Garlick et al., 1973; Millward et al., 1975).

Similar to fractional synthesis rates, fractional degradation rates exhibit depressed rates with increasing stage of development (Waterlow et al., 1978; Kelly et al., 1984; Lewis, 1984; Goldspink and Lewis, 1985). Fractional degradation rates are calculated as the difference between fractional synthesis and accretion rates, therefore, the parallelism is not surprising. Direct measurement of protein degradation using tracer methodology is difficult due to the high degree of recycling of the tracer amino acid.

Swick (1982) stated that muscle growth is limited by the fractional degradation rate (FBR). It was postulated that the FBR regulates the ultimate DNA unit size (Millward and Waterlow,

1978). Young animals exhibit a smaller DNA unit size, or protein:DNA ratio, than older animals. Lower protein:DNA ratios are found during rapid growth when both FSR and FBR are elevated (Millward and Waterlow, 1978). FBR may also play a key role in the remodeling of the muscle fiber during growth (Millward et al., 1975). Both of these theoretical regulatory mechanisms of FBR may have their effects mediated in part by catheptic enzymes. Goldspink et al. (1982) reported a decline in the activity of cathepsins B, D and H/mg protein with age.

Van Es (1980) and Reeds et al. (1980) emphasized that protein turnover is a wasteful process. Reeds et al. (1980) concluded that a large proportion of whole body protein synthesis in growing animals is associated with the maintenance of existing proteins. Further increased protein deposition in growing animals is associated with an increase in protein synthesis above maintenance levels. The efficiency of protein deposition decreases with age as a greater amount of protein synthesis is required per unit of protein deposition in comparison to young animals. To demonstrate the wasteful nature of protein turnover, attempts have been made to estimate the contribution of energy expenditure in protein synthesis. The results from a number of studies compiled by Reeds et al. (1985) suggest that protein synthesis accounts for 11 to 15% of the total energy expenditure when the subject is in energy equilibrium. Compilation of various studies involving rat skeletal muscle show that skeletal muscle protein synthesis is a major component of total ATP utilization. The enhanced heat production associated with an

increase in protein deposition cannot be attributed solely to an increase in protein synthesis (Reeds et al., 1985).

FACTORS AFFECTING PROTEIN TURNOVER

Protein accummulation in skeletal muscle during growth is due to the dynamic nature of protein turnover. Skeletal muscle protein turnover is the continual degradation and resynthesis of muscle proteins (Reeds and Palmer, 1985). Many factors regulate the balance between protein synthesis and breakdown by affecting either synthesis, degradation or both. The primary factors affecting these processes are sex and genotype, degree of work imposed on the muscle, nutrient availability and hormones (Waterlow et al., 1978; Reeds and Palmer, 1985)

1. Hormonal Effects

Steroids, thyroid hormone, insulin and growth hormone are the primary hormones affecting protein turnover. Though they all play key regulatory roles, insulin is probably the single most important hormone in the maintenance of positive nitrogen balance (Fulks et al., 1975; Rannels et al., 1975). Fulks et al. (1975) incubated rat diaphragm muscles and noted that in the presense of insulin, amino acid release was inhibited, thereby, indicating a depression in protein degradation. Jefferson et al. (1974) demonstrated that not only was protein degradation inhibited by insulin in skeletal muscle perfusions, but protein synthesis was also stimulated. Recent work by Goldberg et al., (1985) and Skjaerlund et al., (1987) provided further evidence for insulin's ability to stimulate protein synthesis and inhibit protein degradation. Both groups reported an increase in amino acid

uptake as well as a decrease in amino acid release from muscle strips incubated in an insulin supplemented media.

Although both facets of protein turnover, i.e., synthesis and degradation, are influenced by the presence of insulin, protein synthesis is affected to a greater degree than degradation (Li, 1980). Myofibrillar protein synthesis in white muscle fibers is particularly affected by insulin (Tomas et al., 1984). In the absence of insulin, white fibers exhibit a lower capacity and efficiency for protein synthesis (Jefferson, 1980; Pain et al., 1983). Both groups reported an increase in peptide chain initiation which results in an increase in protein synthesis. In order to increase the number of peptide chains, an increase in the amount of RNA must first occur. Li (1980) was able to demonstrate an increase in RNA concentration by insulin. Elevated RNA and DNA levels have been attributed as the primary factors associated with an 170% increase in fractional synthesis rate in dystrophic chicken breast muscle (Kang et al., 1984).

As stated earlier, protein synthesis is affected by insulin to a larger extent than protein degradation but protein degradation rates are lowered by insulin. Li (1980) reported an elevation in protein degradation rates in the absenc e of insulin associated with an increase in lysosomal cathepsins B and D. When insulin was added back into the system, protein degradation rates were lowered. Insulin also reverses the catabolic effects of glucocorticoids on muscle protein (Tomas et al., 1984). This indicates the importance of the corticosterone:insulin ratio as a regulator of protein accretion.

Unlike insulin, glucocorticoids produce an overall effect of lower protein synthesis rates with no effect on protein degradation (Millward, 1976; Kelly and Goldspink, 1982; Odedra and Millward, 1982). Although no effect on protein degradation by glucocorticoids was found by these groups, Tomas et al. (1979) and Santidrian et al. (1981) reported higher 3-methylhistidine excretion in rats following pharmacological doses of corticosterone. The increase in 3-methylhistidine, a modified amino acid which is not reutilized following its release upon the degradation of actin and myosin, suggests an enhanced rate of protein degradation. Odedra et al. (1983) also reported elevated 3-methylhistidine excretion during the first four days of glucocorticoid treatment after which concentrations returned to normal. This indicates that the initial catabolic effect may be transient with skeletal muscle adapting to the high glucocorticoid concentrations.

The effects of glucocorticoids on muscle protein synthesis are regarded as inhibitory with respect to total and contractile protein (Millward et al., 1976; Rannels and Jefferson, 1980; Odedra and Millward, 1982; Odedra et al., 1983). Elevated concentrations of glucocorticoids found during starvation decrease contractile protein synthesis (Bates, 1983; Bates and Millward, 1983). With regard to the contractile proteins, actin synthesis rates are particularly depressed following glucocorticoid treatment (Odedra et al., 1983). The primary cause for the lower synthetic rate in skeletal muscle is a loss of RNA content (Odedra et al., 1983).

Subjects suffering from trauma are in a state of negative nitrogen balance (Clowes et al., 1980; Long et al., 1981). This condition, characterized by a loss of body protein (Clowes et al., 1980; Long et al., 1981; Clowes et al., 1983), results from an increase in skeletal muscle proteolysis as evidenced by an increase in 3-methylhistidine excretion (Long et al., 1981). Because prostaglandins are involved in the inflammatory response associated with trauma, it was postulated that they may also be responsible for promoting protein degradation (Rodemann and Goldberg, 1981). By incubating rat diaphragm muscles in media containing arachidonate, the precursor for prostaglandins, a 20 to 40% increase in net protein degradation resulted (Rodemann and Goldberg, 1981). Furthermore, rat diaphragm muscles incubated with cyclooygenase inhibitors, which prevent the conversion of arachidonate to prostaglandin PGH2, prevented the net increase in arachidonate stimulated protein degradation. Because PGH2 is a short lived intermediate which gives rise to PGE2, Goldberg et al. (1980) concluded that PGE2 causes an enhanced rate of protein degradation. The proposed mechanism by which PGE2 affects protein degradation is via stimulation of the lysosomal pathway (Goldberg et al., 1980). Lysosomal inhibitors are able to block PGE2 stimulation of protein degradation (Goldberg et al., 1980). In addition, Mortimore (1982) reported an increase in autophagic vesicle formation by PGE2. These findings combined with skeletal muscles ability to synthesize PGE2 (Rodeman and Goldberg, 1982),suggest that PGE2 may play a role in regulating protein catabolism during growth and development.

Palmer and Wahle (1987), Reeds and Palmer (1985) and Rodemann and Goldberg (1982) have shown that PGF2a can stimulate protein synthesis. Palmer et al. (1983) demonstrated an increasee in protein synthesis of 70% by intermittant stretching with a concommitant 105% increase in PGF2a release. By addition of indomethacin, which inhibits cyclooxygenase conversion of arachidonate to prostaglandin, stretch-induced protein synthesis was inhibited (Smith, 1983; Palmer et al., 1983).

The actual mechanism by which PGF2a acts to cause an increase in protein synthesis is unknown. Reeds (1984) and Reeds et al. (1983) suggest that insulin activates phospholipase A2 causing release of arachidonic acid, the precursor of PGF2a. Increased formation of PGF2a in turn leads to an increase in protein synthesis. When isolated muscles are incubated with insulin, protein synthesis and PGF2a release are stimulated (Smith et al., 1983; Palmer, 1987). In further support of this proposed mechanism of action, insulin -stimulated protein synthesis can be blocked by inhibitors of prostaglandin biosynthesis (Reeds et al., 1983).

The administration of exogenous growth hormone as a growth promoting agent has received much attention. Growth hormone promotes an increase in muscle mass and feed efficiency concurrent with a decrease in adipose tissue mass (Machlin, 1972; Chung et al., 1985; Etherton et al., 1986). The significance of these improvements indicates that the biological potential for maximal growth performance under contemporary conditions has not been reached. A commercial line of growth hormone based products

will likely improve the economic efficiency of livestock production (Etherton, 1984).

A regulatory hormone is a hormone in which an increase in its plasma concentration is associated with an increase in growth rate, carcass composition or efficiency (Etherton, 1984). Growth hormone is a regulatory hormone as evidenced by the fact that daily intramuscular injections of growth hormone in swine resulted in improved growth rates and feed efficiency (Machlin, 1972; Chung et al., 1985; Etherton et al., 1986). Carcasses of GH treated pigs possess a lower lipid content and a higher protein content (Etherton, 1987). The increase in lean tissue mass may be attributed to the ability of growth hormone to stimulate muscle protein synthesis (Etherton, 1982; Etherton and Kensinger, 1984). However, growth hormone does not stimulate protein synthesis in muscle cell cultures (Harper et al., 1986). The lower adipose tissue mass can be attributed to the lipolytic action of growth hormone. In cattle (Friesen, 1980; Bauman et al., 1982) and swine (Etherton et al., 1984), exogenous growth hormone stimulates lipolysis In vitro, GH has not been shown to be lipolytic, thereby, indicating that the in vivo stimulation of lipolysis is occurring indirectly. It has been suggested that the administration of growth hormone to improve carcass composition may prove to be the most beneficial when given to animals at the stage of development when adipose tissue mass is accummulating rapidly (Etherton, 1984). Etherton et al., (1987) reported a decrease in lipid content by 20% in 60 kg pigs receiving growth hormone, lending further support to his earlier

speculation.

Growth hormone releasing factor (GRF), a hypothalmic peptide known to stimulate the release of growth hormone, has been studied as a possible growth promotant (Etherton et al., 1986). When human pancreatic GRF [hpGRF-(1-44)-NH2]is administered to growing barrows, an increase in the plasma concentration of endogenous growth hormone is reported. The elevated concentration of growth hormone is associated with improved growth performance comparable to barrows receiving exogenous GH (Etherton et al., 1986). GRF administration produces similar results in cattle (Moseley et al., 1984), sheep (Hart et al., 1984) and rats (Spiess et al., 1983).

2. Exercise Effects

The effect of exercise on skeletal muscle protein turnover is inconclusive at best. Results vary from an increase in protein degradation, as determined by a higher 3-methylhistidine excretion (Rennie et al., 1981; Dohm et al., 1985) to no change in protein degradation (Wolfe et al., 1984; Davis and Karl, 1986). Using isotopically labeled amino acids, Rennie et al. (1981) and Wolfe et al. (1982) reported an apparent increase in whole body protein degradation following exercise. However, Rennie and Millward (1985) questioned the interpretation of the above data as whole body protein degradation may not accurately represent the effect of exercise on skeletal muscle protein degradation. Assuming the apparent increase in protein degradation following exercise is a real event, Kasparek (1985) proposed that the response is a result of increased proteolysis

of damaged tissue. Using a hindquarter perfusion system, Bylund-Fellenius (1984) reported a decrease in 3-methylhistidine release into the media following a 10 minute bout of maximal contraction suggesting an inhibition of protein degradation.

Exercise training leads to a larger muscle mass and is also termed work induced hypertrophy. Neither protein synthesis nor degradation appear to be affected by exercise training (Tapscott et al., 1982; Davis et al., 1985). However, rats subjected to acute bouts of exercise following prior exercise training, demonstrate a decreasee in protein synthesis (Davis and Karl, 1986).

3. Nutritional Effects

The effects of nutrition on protein turnover are well documented. Accurate interpretation of the data require the values to be expressed in operational terms of protein:DNA and RNA:protein ratios. This helps in the elimination of bias due to sex and strain differences and stage of development. Protein:DNA ratio, also termed DNA-unit, has been defined by Cheek et al. (1971) as an imaginary volume of cytoplasm managed by a single nucleus. By expressing muscle RNA concentrations as the ratio of protein:RNA, the value can be multiplied by the fractional synthesis rate to obtain synthesis per unit of RNA, i.e., the efficiency of protein synthesis (Millward and Waterlow, 1978).

Diet affects two parameters of protein synthesis; capacity and activity. Capacity for protein synthesis is reflected by the concentration of ribosomes, whereas, the activity of these ribosomes indicates the extent to which capacity is realized

(Millward and Waterlow, 1978). A loss of protein synthesis capacity is the immediate response to fasting (Millward et al., 1974). Loss of RNA is rapid and continues throughout the duration of the fast. Millward et al (1975) reported that the losses are extensive. Following consumption of a protein deficient diet or protein free diet, rats exhibited a 60% reduction in RNA:protein. While loss of capacity is an on going process throughout the restriction period, Millward and Waterlow (1978) found a decrease in RNA activity in isolated rat muscles after one day of starvation but the activity level remained fairly constant thereafter. A similar maintenance of reduced activity levels in skeletal muscle occurs in rats consuming a protein-free diet. Prolonged starvation or protein deficiency results in a loss of ribosome number and activity leading to a depression in fractional synthesis rate (Bergen, 1974; Millward and Waterlow, 1978; Li, 1979).

Nutritional deprivation results in an immediate depression in synthesis rate with a slight reduction in protein degradation (Emery et al., 1986). These changes result in an initial conservation of protein reserves as little body protein is lost. Further demonstration of a protein sparing effect is an increase in blood lipid levels indicating depletion of body fat stores (Goodman, 1980). Later after continued starvation or protein deficiency, blood lipid levels decrease and fractional breakdown rates increase. A 30 to 50% increase in degradation rates during starvation is associated with an increase in cathepsin D activity (Li, 1979). The combination of elevated fractional

degradation rates and depressed fractional synthesis rates promote a rapid loss of protein (Li and Goldberg, 1976; Millward and Waterlow, 1978).

Branched chain amino acids (BCAA) have been implicated as key regulators of protein turnover. Fulks et al. (1975) and Buse and Weigand (1977) documented the ability of leucine to inhibit protein degradation in vitro. Chua (1979) and Tischler and Goldberg (1980) reported that leucine must be transaminated to its keto acid to produce the inhibitory effect. The keto acid of leucine when administered to fasting man results in a nitrogen sparing effect (Mitch, 1981). The same effect was not observed when leucine was administered even though the body can metabolize leucine to its keto acid. Wolfe (1981) showed no improvement in nitrogen balance in starved dogs following infusion of BCAA or their keto acids. McNurlan et al. (1982) reported that leucine had no direct stimulatory effect on muscle protein synthesis in starved or protein deprived animals. However, the length of the leucine administration period may have been too short. It appears that the nutritional effects on the regulatory processes of protein turnover occur primarily through alterations in protein synthesis by mechanisms which are unlikely to involve BCAA.

PROTEIN TURNOVER METHODS

The methods to measure protein turnover fall into two general categories: 1. whole body protein turnover methods and 2. fractional protein turnover rate methods (Waterlow et al., 1978). The measurement of whole body protein turnover is based upon the apparent irreversible loss of labeled amino acids from the

metabolic (free) amino acid pool, i.e., flux. On the other hand, fractional rates involve the measurement of the incorporation of a labeled amino acid isotope into a particular tissue protein or the loss of labeled amino acid from the protein (Waterlow et al., 1978).

Both areas of protein turnover measurement involve the administration of an isotopically labeled amino acid as a metabolic tracer of protein metabolism. The common modes of isotope administration are single injection, continuous infusion, priming dose-continuous infusion or flooding dose (Bergen et al., 1987). The single injection dose method is by far the easiest, however, a large number of animals is required for the estimation of precursor pool specific activity (Zak, 1979).

The method generally employed is the continuous infusion method developed by Waterlow and Stephan (1968) as modified by Garlick et al. (1973). The method involves the determination of tissue protein synthesis rates at the completion of the infusion. To accomplish this task in skeletal muscle, a muscle tissue sample is obtained and tissue free pool (Si) and protein bound pool (Sb) specific activities of the tracer amino acid are measured.

Precise measurement of the precursor pool is a necessity for an accurate estimation of synthesis rates. By continuous administration of a labeled amino acid, the specific activity of the precursor pool reaches a plateau and remains constant. After attaining the plateau, only one sample needs to be obtained for protein synthesis measurement (Waterlow et al., 1978; Bergen et

al., 1987).

Both plasma and tissue free pool plateau specific activity can be used to calculate fractional synthesis rates. Plasma specific activity at plateau can be used to calculate fractional synthesis rate when tissue biopsies are unattainable as well as for the calculation of amino acid flux. Flux values are used in the estimation of whole body protein synthesis rate. Fractional synthesis rates tend to be lower when plasma plateau specific activity is used as the precursor pool. This stems from the higher specific activity of plasma than that of the tissue free pool specific activity. Tissue free pool specific activity is lower due to dilution of labeled amino acid with nonlabeled amino acid entering the pool from protein degradation (Waterlow et al., 1978). Gan and Jeffay (1967) estimated that 30% of the free amino acid pool arises from tissue catabolism. Because skeletal muscle is the largest free amino acid reservoir, 50 to 80% of the total free amino acids in the body arise from this tissue in comparison to .2 to 6% arising from plasma (Reeds, 1985). An approximation of plasma contribution to intracellular amino acids derived from protein degradation can be calculated by the formula: contribution = 1-(Simax/Spmax) where Simax is the tissue free pool specfific activity and Spmax is the plasma specific activity at plateau (Waterlow et al., 1978).

Waterlow and Stephen (1968) and Garlick et al. (1973) determined that the time course to plateau specific activity in the tissue free pool parallels the specific activity time course in blood. Therefore, an equation expressing this course of

events is Si=Simax[1-e-li(t)] where li(t) is the rate constant to reach plateau in the precursor pool. A drawback to this formula is the large number of animals required to attain an adequate measure of the rate constant, li. The obvious solution to this problem is to estimate the value. Waterlow et al. (1978) discribes two possible estimations of li for use in muscle tissue. The first of these assumes that the rate constant is equal to the ratio of the protein bound amino acid to the free amino acid multiplied by the fractional synthesis rate. This correction is used in cases where tyrosine, lysine or leucine are infused. In the second case, in which glycine is infused, li=PRks, where P=Spmax/Spmax-Simax, R=bound amino acid/free amino acid and ks=fractional synthesis rate. The additional correction factor, P, allows for the plasma contribution to the rapidly turning over glycine pool.

The calculation of fractional protein synthesis rate also involves measurement of specific activity in protein at the end of the infusion. Substituting the equation for the rate of increase in intracellular specific activity into the differential equation for the rate of increase in protein specific activity, one obtains the formula Sb/Si=[R/R-1][1-e-ks(t)/1-e-(R)(ks)(t)]-[1/R-1] for the protein synthesis rate of a particular muscle tissue (Waterlow et al., 1978). No amount of rearrangement will allow the formula to be solved. The formula may be solved graphically, however, computer iteration commonly provides a solution to the equation (Mulvaney, 1981). The formula (Sb/Si)t=ks, where Sb/Si is the specific activity of the bound

pool divided by the precursor pool specific activity, can also provide an estimate of fractional synthesis rate.

The priming dose-continuous infusion method is essentially the same as the continuous infusion method with the exception of administration of a priming dose of label in order to ensure that the specific activity in the precursor pool reaches a plateau sooner. The flooding dose method of Garlick et al. (1980) involves giving a large dose of unlabeled amino acid with the labeled tracer in a manner such that the specific activity in the blood and muscle is overwhelmed and reflects the injected material.

When applying whole body protein turnover methods, the following assumptions are made: 1) the free pool or precursor pool is homogenous, 2) the loss of amino acids is partitioned between protein synthesis and catabolism and 3) the isotope can enter the precursor pool only by administration during the measurement period (Reeds et al., 1980). Similar to the measurement of fractional synthesis rates, the method of determining whole body protein turnover generally involves the continuous infusion of a labeled amino acid over a period of 6 to 8 hours during which time blood samples are collected as well as urine or expired CO2 samples (Waterlow et al., 1978; Bergen et al., 1987). Using the model of Picou and Taylor-Roberts (1969), flux equals protein catabolism plus dietary N intake which equals protein synthesis plus irreversible catabolism of the tracee. Measurement of flux, intake and label excretion allows for the calculation of whole body protein synthesis and degradation

(Picou and Taylor-Roberts, 1969; Waterlow et al., 1978; Reeds et al., 1980).

Whole body protein turnover measurements involving infusion of a stable amino acid have become increasingly popular. The use of noninvasive methods of stable amino acid infusion eliminates the problems associated with radioactive sample handling and disposal. Measurement of stable isotope enrichment involves gas chromatography-mass spectrometry. With the development of selected ion monitoring gas chromatography-mass spectrometry (SIM-GCMS), infusion of stable isotopes has become even more practical than radioisotope infusion. SIM-GCMS offers the advantages of 1) gas chromatographic resolution of individual components in a sample 2) small amounts of sample material are required and 3) the mass spectrometer can generate ions which are nearly unique for the compound of interest (Matthews and Bier, 1983; Rennie and Halliday, 1984; Bier et al., 1985; Watson, 1985). However, a detection limit of .1 atom percent excess may be deemed a disadvantage (Bier et al., 1981).

The major advantage of N-15 labelled amino acids over C-13 labelled amino acids for use with the above techniques is the ease of urine sampling relative to expired breath sampling (Matthews and Bier, 1983). However, infusion of 13C leucine with expired breath collection has been performed in humans (Conway et al., 1980; Matthews et al., 1980; Motil et al., 1981; Rennie et al., 1982) and sheep (Flakoll et al., 1987) On the other hand, Krishnamurti and Schaefer (1987) infused N-15 leucine and reported whole body protein synthesis and whole body protein

degradation rates with no direct measurement of endproduct formation. They measured flux and N retention and used estimates for amino acid oxidation to calculate whole body protein turnover rates.

Direct measurement of protein synthesis rates using stable isotopes has been reviewed by Matthews and Bier (1983). Halliday and McKeran (1975) measured fractional synthesis rates in man by removing muscle biopsies following a continuous infusion of N-15 lysine. Halliday and Read (1981), LeMaster and Richards (1982) and Rennie et al (1982) have infused C-13 leucine and measured its subsequent incorporation into protein.

Stable isotopes have not been as widely employed in large animal whole body protein turnover studies as they have been in humans. However, whole body protein turnover studies using radioactively labeled amino acids have been conducted in growing lambs (Davis et al., 1981), cattle (Lobley et al., 1981) and swine (Garlick et al., 1976; Simon et al., 1978; Reeds et al., 1981). Reeds et al. (1981) found the whole body protein synthesis to be 5.4 g N/kg of metabolic body weight per day in 30 kg growing pigs, while whole body protein degradation was calculated as 4.4 g N/kg of metabolic body weight per day. In larger pigs averaging 75 kg, Garlick et al. (1976) reported whole body protein synthesis rates of 9 g/kg/d. The percent contribution of skeletal muscle protein synthesis to whole body protein synthesis was found to be 33% in 30 kg pigs and 26% in 75 kg pigs (Lobley et al., 1980). Further analysis of the data indicates that the efficiency of muscle protein deposition,

calculated as g deposited/ g synthesized, is higher in skeletal muscle than in other major tissues such as the liver and gastrointestinal tract. This is due primarily to the rapid fractional turnover rates found in liver and gastrointestinal tract tissues (Lobley et al., 1980).

BETA ADRENERGIC AGONISTS

In the past, efficient red meat production has involved selective mating of animals exhibiting rapid growth rates. This process has shown an increase in protein deposition but a concommitant increase in fat deposition is also observed. Recent interest has focused upon a group of compounds which promote protein deposition without causing an increase in fat deposition apparently by directing substrates away from adipose tissue to muscle accretion. These compounds, classified as B-adrenergic agonists, are also referred to as repartitioning agents due to their substrate shunting properties (Ricks et al., 1985). By increasing the rate of skeletal muscle accretion concurrent with a decrease in the rate of adipose accretion, the overall efficiency of growth should be improved assuming the maintenance requirement remains unchanged.

A variety of B-agonists have been administered to food producing animals with similar results across species. Dietary supplementation of the beta agonist Cimaterol (American Cyanamide Co.) results in improved carcass traits and composition in finishing swine (Dalrymple et al., 1984; Moser et al., 1986; Jones et al., 1983) and wether lambs (Beerman et al., 1986). When growing wether lambs received 10 ppm Cimaterol per day for

11 weeks, no improvement in average daily gain or feed to gain ratio was observed. However, when the same level of Cimaterol was supplemented into the diets of growing ram lambs for 5 weeks, an improved average daily gain and feed to gain ratio was recorded (Beerman et al., 1986). This implies that sex plays a role in determining the effectiveness of the B-agonist. Moser et al. (1986) and Dalrymple et al. (1984) reported no improvement in feed conversion or daily gains in finishing pigs fed Cimaterol at levels ranging from 25 to 100 ppm. The lack of improved feed efficiency may be a consequence of increased basal metabolic rate. Support of this theory is found in the observed increase in heart rate and blood flow in lambs fed Cimaterol (Beerman, D. unpublished results reported to Ricks, C. A.). Also contributing to the lack of improved performance may be a higher maintenance cost associated with 1 kg of muscle as opposed to 1 kg of adipose on a dry matter basis.

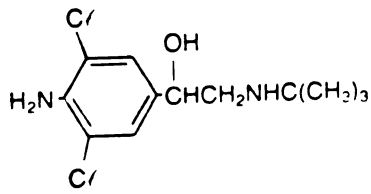
The most dramatic effect of Cimaterol is on carcass characteristics. Longissimus area was increased by 30% with a decrease in 12th rib fat thickness by 50 to 65% in lambs (Beerman et al., 1986). Moser et al. (1986) was only able to demonstrate a 4% increase in longissimus muscle area and a 16.5% decrease in 10th rib fat thickness in finishing pigs fed Cimaterol at 50 ppm. Dalrymple et al. (1984) found results consistent with the above in chickens.

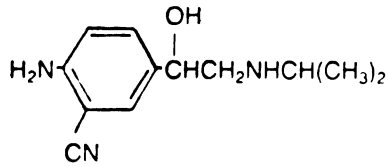
Clenbuterol, a structural analog of epinephrine, produces results similar to Cimaterol. Clenbuterol supplemented diets improves carcass composition and feed conversion in lambs (Baker

et al., 1984; Bohorov et al., 1987), chickens (Dalrymple et al., 1983; Muir et al., 1985) and pigs (Ricks et al., 1984). Ricks et al. (1984) was unable to detect any changes in performance in steers.

Ractopamine (Eli Lilly Co.), a phenethanolamine with B-agonist activity, produces a repartitioning effect similar to other beta agonists. While its structure is unlike the above B-agonists (Figure 3), it does mediate its affects through the B-adrenergic receptor (Hausman et al., 1987). In finishing swine fed 2Ø ppm Ractopamine in 16% crude protein diets, an increase in muscle accretion with a decrease in fat deposition was noted (Anderson et al., 1987). These workers attributed the increased muscle accretion to an apparent increase in fractional synthesis rate coupled with a possible decrease in fractional breakdown rate. Carcass characteristics were improved as evidenced by a 16% increase in 1Øth rib loin eye area and a 13% decrease in 1Øth rib fat depth (Anderson et al., 1987; Veenhuizen et al., 1987). A stimulation of lipolysis and inhibition of lipogensis have been reported (Hauseman et al 1987; Merkel et al., 1987). Ractopamine also reduces preadipocyte proliferation in rat primary cultures (Jones et al., 1987).

The obvious increase in muscle mass by the B-agonists suggests alterations in protein synthesis and/or degradation rates. Reeds et al. (1986) demonstrated an increase in protein and RNA accretion in the gastrocnemius and soleus muscles of male rats receiving Clenbuterol with no change in protein synthesis rate. Bohorov et al. (1987) also failed to find an increase in protein





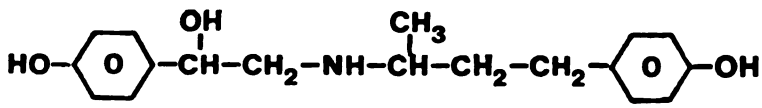


FIGURE 1. CHEMICAL STRUCTURES FOR THE B-AGONISTS, CLENBUTEROL, CLIMATEROL AND RACTOPAMINE

synthesis in wethers receiving Clenbuterol. Maltin et al. (1987) and Zeman et al. (1987) both reported a reversal of denervation induced muscle atrophy via Clenbuterol. This demonstrates the inhibitory effect of Clenbuterol on protein degradation. Bohorov et al. (1987) reported an increase in protein accretion in Clenbuterol treated wethers with no change in protein synthesis, thereby, indicating a depressed rate of protein degradation. Summary of the above results indicates the increase in muscle mass can be attributed to inhibition of protein degradation with no effect on protein synthesis. It should be noted, however, that in all of the above studies, protein degradation was never measured directly.

The increase in muscle mass by Clenbuterol and Cimaterol is associated with an increase in muscle fiber area not by an increase in muscle fiber number. Hypertrophy of type II muscle fibers is responsible for this increase in fiber area (Coleman et al., 1986; Hamby et al., 1986; Kim et al., 1986; Wu et al., 1986). In contrast, Beerman et al. (1986) reported hypertrophy of both type I and II fibers in Cimaterol treated wethers. Maltin et al. (1986) demonstrated similar findings in rats fed Clenbuterol. In a personal communication to Ricks (1987), Romsos rationalized the type I hypertrophy by stating that an increase in free fatty acid uptake could account for the additional ATP required for type I fiber hypertrophy. Similarly, Ricks (1987) suggested that an increase in glycogenolysis in white muscle following beta receptor stimulation would provide more ATP which would support an increase contraction rate. The increased

contractile activity would promote hypertrophy of type II fibers.

At the cellular level, following activation of the B-agonist-receptor complex, adenylate cyclase, protein kinase and triacylglycerol lipase are stimulated. With the activation of adenylate cyclase, there is a concommitant increase in cAMP levels. High levels of cAMP are positively correlated with increased lipolysis (Dalton et al., 1974). The increase in lipolysis is probably due to the cAMP cascade effect leading to the induction of triacylglycerol lipase (Mersmann, 1987). Several studies suggest that the increased cAMP stimulates cAMP-dependent protein kinases which in turn phosphorylate specific enzymes. Acetyl CoA carboxylase is a key enzyme involved in fatty acid synthesis. Increase phosphorylation of this enzyme by a cAMP-dependent protein kinase leads to its inhibition (Grifforn and Katz, 1984). Fatty acid synthetase can also be inhibited by high levels of cAMP (Lakshmanan et al., 1972). These findings suggest that B-agonists exert their effects on both lipolysis and lipogenesis. In fact, Clenbuterol appears to be a 5 to 10 times more potent antilipogenic agent than lipolytic agent in vitro (Ricks et al., 1987). The mechanism of enzyme phosphorylation is the same for both pathways but phosphorylation of lipogenic enzymes leads to their inhibition, whereas, phosphorylation of lipolytic enzymes leads to their activation.

It is possible that the stimulation of intracellular release of cAMP by the B-agonists may also affect energy metabolism. The major organ of concern is the liver. Liver contains beta-2 receptors and is directly involved in glucose metabolism.

Stimulation of adenylate cyclase by the receptor complex leads to increased cAMP. Through the cascade of events described earlier, glycogen phosphorolysis is initiated resulting in elevated blood glucose levels. If B-agonists exert an effect on energy metabolism, increased blood glucose and blood lactate levels should be found. Swine infused with Clenbuterol exhibit elevated blood glucose and lactate levels. However, these levels are not of the same magnitude as the levels found after administration of epinephrine (Mersmann, 1987). The high glucose levels may arise from liver glycogenolysis alone. Stimulation of muscle glycogen reserves may provide a direct energy source for this tissue, therby, sparing the amino acids for utilization in protein synthesis. Using a perfused rat hemicorpus model, increased lactate production and muscle glycogen breakdown have been demonstrated, consistent with the proposed mode of action. In the same system, however, an increase in protein synthesis was not evident but a decrease in protein degradation was observed (Li and Jefferson, 1977).

B-agonists may also affect the growth processes by manipulating hormone concentrations. The two hormones of significant importance to growth are insulin and growth hormone. Insulin is believed to be the single most influential factor involved in the regulation of skeletal muscle protein balance (Young, 1970). Insulin induced inhibition of protein degradation has been documented in various species. B-agonists stimulate the release of insulin after binding to the B-receptors on the pancreas. The increased insulin is not the mediator of the

increased muscle hypertrophy as Deschaies et al. (1981) infused insulin deficient rats with a B-agonist and observed the increased muscle mass (Deschaies et al., 1981).

Growth hormone has received much attention due to its ability to promote muscle accretion. It is possible that B-agonists may induce growth hormone release which in turn stimulates protein accretion. Perkins et al. (1983) established that B-agonists cause the release of growth hormone from rat pituitary cells following stimulation of the beta receptor. However, Ricks et al. (1984) state that the increased growth hormone release should be regarded only as an acute effect. In summary, the major anabolic hormones, insulin and growth hormone, show an increase in release but are apparently not the primary regulators of the B-agonist affect.

Beta agonists may exert their effect at the genomic level via their stimulation of cAMP. cAMP has been shown to inhibit glucokinase mRNA transcription (Sibrowski and Seitz, 1984). Beale et al. (1984) has shown that cAMP induces transcription of phosphoenol pyruvate carboxylase in rat liver. These findings suggest that cAMP does affect transcription and the affect may be inhibitory or stimulatory. Translation rates can also be influenced by cAMP. Roper and Wicks (1978) discovered that cAMP can accelerate the elongation rate of the enzyme, tyrosine aminotransferase. Snoek et al. (1981) found that cAMP also increased the number of nascent chains formed of this enzyme. It is possible that B-agonists act at the level of translation in a positive manner indirectly via an increase in cAMP

concentrations.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROTOCOL

Twenty-two crossbred barrows (68 ± kg) were randomly assigned to either an initial slaughter group, an experimental group for isotope infusion or a feeding trial group. Six barrows assigned to the initial slaughter group were killed on day Ø and the left side longissimus and semitendinosus muscles were removed, weighed and subsampled. The infusion barrows were fed a control corn-soybean meal diet (16% crude protein) (n=4) or the control diet supplemented with 2Ø ppm Ractopamine (n=4) for one week. N-15 lysine was continuously infused into the eight infusion barrows on day 7 for whole body protein turnover measurement. On day 8, the eight barrows were continuously infused with C-14 tyrosine. After completion of the infusion, the barrows were slaughtered and longissimus and semitendinosus samples removed for determination of fractional protein turnover rates. The remaining feeding trial barrows were fed either a control diet (n=4) or the control diet supplemented with 2Ø ppmm Ractopamine (n=4). On day 14, the barrows were slaughtered and the left side longissimus and semitendinosus muscles removed, weighed and subsampled.

INITIAL SLAUGHTER GROUP

Six barrows (68 ± kg) were slaughtered and longissimus and semitendinosus muscles were removed from the left side. Fat was trimmed from each muscle and lean tissue weights were recorded.

A subsample from the center portion of each muscle was obtained and frozen in a dry ice-isopentane solution. The subsamples were powdered with dry ice by the procedure of Mulvaney et al. (1985) and stored frozen at -7Ø C.

Moisture and ether extractable fat was determined on each powdered muscle subsample according to AOAC (1975) methods. Nitrogen determination was performed on each sample by the micro-Kjeldahl method described by American Instrument Company (1961). RNA and DNA content was determined according to Munro and Fleck (1969) as modified by Mostafavi (1978).

EXPERIMENTAL GROUP

Ten days prior to isotope infusion, each infusion barrow was anesthetized with phenobarbitol and placed on his back for the insertion of venous catheters. Fifteen centimeters of a Tygon tubing catheter with a silastic tip was inserted into the right jugular vein. The remaining 5Ø cm portion of the catheter was threaded under the skin and exteriorized at the top of the neck. The left jugular vein was catheterized in a similar fashion. Both catheters were flushed daily with heparinized saline (2Ø units/ml) and packed with a heparinized saline solution (2ØØ units/ml). Mushroom head bladder catheters were inserted into the bladder, exteriorized through the belly wall and allowed to drain freely.

All barrows were individually housed in farrowing crates. Three days post catheterization, the eight barrows were placed on either the control diet (n=4) or the control diet supplemented with 2Ø ppm Ractopamine (n=4). On day 7 of the trial, each

barrow was used in a priming dose-continuous infusion of the stable amino acid (N-15 lysine) to measure whole body protein synthesis and degradation. Proper isotope infusion levels were calculated according to the method of Dietz et al., (1982) (Appendix A). The priming dose consisted of 101 mg of alpha N-15 lysine and 41 mg (N-15)2 urea in 5 ml of sterile saline. The continuous infusion solution was composed of 347 mg of alpha N-15 lysine in the appropriate volume of saline such that each barrow was infused at the rate of 2.20 ml/h via a Harvard infusion pump. The priming dose was administered through the left jugular catheter and flushed with 2 ml of the infusion solution to ensure entry into the blood system. The infusion lines were then connected to the Harvard pump and the alpha N-15 lysine solution was continuously infused for six hours. Ten milliliter blood samples were collected through the right jugular catheter into heparinized tubes at 15 minute intervals for 2 hours and 30 minute intervals for the remaining 4 hours. The blood was centrifuged at 1,000 x g to obtain plasma which was subsequently stored at 0 C until further analysis. Urine samples were collected at 15 minute intervals for 1 hour, 30 minute intervals for the next hour and then every hour for the remainder of the infusion. Due to infection problems, urine samples were often hard to obtain. Particular emphasis was placed on obtaining urine samples at hourly intervals during the last 4 hours of the infusion. Urine samples were stored at 0 C until analysis.

On day 8, the barrows were continuously infused with C-14

tyrosine. C-14 tyrosine was diluted with sterile saline such that each pig received 4 uCi/kg of body weight at a rate of 2.2Ø ml/h. Ten milliliter blood samples were collected at 15 minute intervals for the first 2 hours and at 3Ø minute intervals for the remaining 4 hours. Blood samples were centrifuged at 1,ØØØ x g to obtain plasma which was subsequently stored at Ø C. Following completion of the infusion, the barrows were euthanized and longissimus and semitendinosus samples removed from the left side. The muscle samplse were immediately frozen in Whirl-pac bags in dry ice-isopentane. Muscles were powdered according to the method of Mulvaney et al. (1985) and stored frozen at -7Ø C. The carcasses were properly disposed of by the MSU Physical Plant. RNA and DNA content was determined by the method of Munro and Fleck (1969) as modified by Mostafavi (1978).

I. WHOLE BODY PROTEIN TURNOVER MEASUREMENT

A. Plasma Alpha N-15 Lysine Analysis

1. Sample Preparation

Plasma samples obtained on day 7 during infusion of alpha N-15 lysine, were analyzed for atom percent excess of the stable isotope. One milliliter of plasma was placed in an ultrafiltration cone (BioRad, Richmond, CA) and centrifuged at 1,5ØØ x g for 6Ø minutes. 2ØØ ul of the ultrafiltrate was added to a tube containing the internal standard, L-lysine, vortexed and the resulting pH was adjusted to 2.Ø to 2.5. The plasma sample was then layered onto a bed of Dowex 5ØWx8 H+ resin (75Ø mg). After the plasma had entered the resin, 1Ø ml of distilled water was passed through the column to remove negatively charged

compounds, neutral compounds and ions. The plasma sample was then eluted from the column into a 12 mm x 100 mm culture tube with 3 ml of 2 N NH4OH followed by 1 ml of distilled water. The sample was evaporated to dryness under a gentle stream of nitrogen gas. After apparent dryness of the sample, .5 ml of methylene chloride was added to azeotropically remove the last traces of water.

2. Sample Derivatization

N-trifluoracetyl-n-butyl (TAB) esters of the plasma amino acids were prepared according to the method of Gehrke et al. (1987) with modifications. 2 ml of 3 N HCl in butanol was added to each sample tube, vortexed and heated at 100 C for 30 minutes with vortexing at 10 minute intervals. The butylated amino acids were then evaporated to dryness under a stream of nitrogen gas and azeotroped as before. 400 ul of trifluoroacetic anhydride in methylene chloride were added to the dry sample, vortexed and heated at 150 C for 5 minutes. The TAB derivitives were stored dessicated at 0 C until analysis.

3. Sample Enrichment Determination

Three microliters of the TAB derivatized sample was injected into a Hewlett Packard 5985 gas chromatograph-mass spectrometer. Gas chromatographic separation was performed by use of a 1.8 m 1% OV1 packed column with temperature programming from 180 to 250 C at 2 degrees/minute. Electron impact mass spectrometry was performed at 70 eV with the source temperature maintained at 200 C and the pressure at 5 x 10-6 torr. Selected ion monitoring of ion current at m/z 320, 321, 394, and 395 was performed with the electron multiplier set at 2800 volts.

Atom percent excess was calculated based on the peak areas of peaks at m/z 32Ø, 321, 394 and 395 according to the methods of Wolfe (1985).

B. Urinary (N-15)2 Urea Analysis

N-15 enrichment in urinary urea was determined following aeration of 1 ml of basic urine (pH 14.Ø) to remove the NH3. The urine pH was then adjusted to 6.5 and incubated with 4Ø units of urease. The sample pH was raised to 14.Ø and aerated with the liberated NH3 being trapped in 1% sulfuric acid. The sulfuric acid sample was sent to Isotope Services Inc., Los Alamos, NM for determination of N-15 enrichment with an isotope ratio-mass spectrometer.

Whole body protein turnover and lysine flux calculations were determined using the plasma and urine N-15 enrichment values at plateau according to the formulas of Wolfe (1985) (Appendix B).

II. FRACTIONAL PROTEIN TURNOVER MEASUREMENT

The determination of fractional rates involves measuring the specific activity of the precursor pool and protein bound pool. The methods of Garlick et al. (1976) and Bergen et al. (1987) were used to calculate specific activities and fractional rates (Appendix C).

A. Protein Fractionation

One gram of tissue homogenate from each longissimus and semitendinosus sample was separated into the myofibrillar and sarcoplasmic protein fractions by the method of Helander (1957) as modified by Mostafavi (1978). Phosphate buffer (.Ø5 M) was used, however, to extract the sarcoplasmic proteins rather than

water. Each protein fraction was precipitated with sulfosalycylic acid (SSA) and the resulting pellet was washed and hydrolyzed in 10 ml of 6 N HCl at 100 C for 20 hours. One milliliter of the hydrolysate was neutralized, diluted and tyrosine content determined by ion exchange chromatography. The remaining 9 ml was evaporated under vacuum and resuspended in 1.5 ml of distilled water. One milliliter of the concentrated hydrolysate was placed in a scintillation vial with 10 ml of aqueous scintillation cocktail. Vials were placed in a Searle 6872 liquid scintillation counter for the determination of beta counts (C-14). Another volume of the concentrated hydrolysate was used to determine the percentage of beta counts present as C-14 tyrosine by thin layer chromatography. A 200 ul aliquot of the hydrolysate was spotted on a silica plate. The plate was developed in a 4:1:1 solution of butanol, acetic acid and water. The tyrosine band was then removed from the plate and counted in a liquid scintillation counter. The percent beta counts in the tyrosine band was used to correct the total disintegrations per minute (dpm) in each protein fraction. This correction factor, based on previous results, was taken to be 80% (Appendix C).

B. Tissue Free Pool Analysis

One gram of powdered tissue was homogenized in 10% SSA, allowed to stand on ice for 2 hours and centrifuged. The supernatant was decanted and the pellet washed twice with 5% SSA. The washes were combined with the original supernatant and passed through a Dowex 50Wx8 H+ resin bed (1.5 g). The column was washed with 20 ml of distilled water and the amino acids eluted

with 1Ø ml of NH4OH followed by 5 ml of distilled water. The collected fraction was then evaporated under vacuum and resuspended in 2 ml of .Ø1 N HCl. One ml of the concentrated sample was placed in a vial and analyzed for tyrosine content via ion exchange chromatography. Following determination of tyrosine content, 2ØØ ul from the vial was spotted on silica plates in the same manner described previously for an estimation of C-14 tyrosine counts. The remaining milliliter was counted in a liquid scintillation counter. The total counts obtained were multiplied by 7Ø% to correct each free pool sample for the percentage of the counts present as tyrosine (Appendix C). Based on preliminary results using thin-layer chromatography, 3Ø% of the total counts were found in compounds other than tyrosine.

FEEDING TRIAL GROUP

Eight barrows (68 + kg) were randomly alotted to either a 16% crude protein control diet (n=4) or the same diet supplemented with 2Ø ppm Ractopamine (n=4). The barrows were weighed after consuming the appropriate diets for 2 weeks and slaughtered. The left side longissimus and semitendinosus muscles were removed, trimmed of subcutaneous fat, weighed and subsampled. The subsamples were frozen in dry ice and isopentane, powdered by the method of Mulvaney et al. (1985) and stored at -7Ø C. Moisture content and ether extractable fat content was determined by AOAC methods (1975). Nitrogen determination was performed by a micro-Kjeldahl method as outlined by the American Instrument Company (1961). RNA and DNA content was determined by the method of Mostafavi (1978). Muscle weights were used in the estimation of

fractional accretion rates according to Bergen et al. (1987). (Appendix C).

RESULTS

Moisture, fat and protein contents of the longissimus and semitendinosus muscles following two weeks of feeding the control or Ractopamine diet are presented in Table 1. No significant differences in moisture, fat or protein content were found between muscles of the control or treated barrows.

Barrows receiving the 20 ppm Ractopamine supplemented diet for one week exhibited lower DNA levels in the longissimus and semitendinosus than the control barrows (Table 2). This decline indicates that Ractopamine has stimulated an increase in muscle accretion as evident by the dilution of DNA content with muscle protein. No differences in RNA content were observed (Table 2).

Fractional synthesis, degradation and accretion rates of the longissimus and semitendinosus muscles are presented in Table 3. No changes in protein synthesis rates of the two muscles were found after one week.

The effects of Ractopamine supplementation for one week on myofibrillar and sarcoplasmic protein turnover are presented in Table 4. It appears that the later-maturing longissimus muscle is less responsive to the early effects of Ractopamine than the earlier-maturing semitendinosus. An increase in the fractional synthesis rate of the semitendinosus myofibrillar proteins was observed.

In the longissimus muscle, no effect of Ractopamine on

myofibrillar protein synthesis was observed. However, an increase in thee sarcoplasmic protein synthesis rate of the treated barrows is evident.

For the calculation of whole body protein turnover, N-15 lysine kinetic data in the blood and urine pools was determined. Figure 2. demonstrates that the N-15 lysine atom percent excess plateau in the plasma was obtained during the six hour infusion. Urinary urea N-15 atom percent excess is also presented in Figure 3. For the calculation of lysine flux, only the mean plasma and urine plateau enrichment values over the last four hours of the infusion were used. The resulting lysine flux values did not differ significantly between the control and Ractopamine treated barrows (Table 5).

Similar to the fractional protein turnover results, whole body protein turnover rates were also unaffected after one week of Ractopamine supplementation (Table 5). The Ractopamine treated barrows synthesized 12.7 grams of protein per kilogram of body weight per day which translates to 941 grams of protein per day. This was not found to differ significantly from the control barrows which synthesized 939 grams of protein per day. Whole body protein degradation rates also did not differ between the two groups. Values for whole body protein degradation rates in the control group averaged 769 grams of protein per day while the Ractopamine treated group degraded 746 grams of protein per day.

TABLE 1. EFFECT OF TWO WEEKS OF RACTOPAMINE FEEDING ON MOISTURE, FAT AND PROTEIN CONTENT OF THE LONGISSIMUS AND SEMITENDINOSUS MUSCLES

	LONGISSIMUS			SEMITENDINOSUS		
	MOISTURE	FAT	PROTEIN	MOISTURE	FAT	PROTEIN
	%			%		
CONTROL	73.4	5.6	21.4	74.2	6.6	19.6
RACTOPAMINE	73.9	4.7	21.8	75.3	4.6	20.0
SE	.9	1.1	.5	1.0	1.2	.4

TABLE 2. RNA AND DNA CONTENT (MG/G TISSUE) OF LONGISSIMUS AND SEMITENDINOSUS MUSCLES AFTER ONE WEEK OF RACTOPAMINE TREATMENT

	RNA		DNA	
	LD	ST	LD	ST
CONTROL	1.7	1.4	.25a	.27a
RACTOPAMINE	1.7	1.3	.21b	.23b
SE	.2	.1	.18	.15

a,b Means within a column without a common superscript differ (P<.Ø5).

TABLE 3. FRACTIONAL ACCRETION RATES, SYNTHESIS RATES AND BREAKDOWN RATES (%/DAY) FOR LONGISSIMUS AND SEMITENDINOSUS MUSCLES FOLLOWING ONE WEEK OF RACTOPAMINE TREATMENT

	LONGISSIMUS			SEMITENDINOSUS		
	FAR	FSR	FBR	FAR	FSR	FBR
CONTROL	1.2	5.6	4.4	1.Ø	6.3	5.3
RACTOPAMINE	1.2	5.Ø	3.8	1.1	6.7	5.6
SE		1.1Ø			.9Ø	

TABLE 4. MEAN FRACTIONAL MYOFIBRILLAR AND SARCOPLASMIC SYNTHESIS RATES (%/DAY) FOR THE LONGISSIMUS AND SEMITENDINOSUS MUSCLES FOR BARROWS CONSUMING THE CONTROL OR RACTOPAMINE SUPPLEMENTED DIET FOR ONE WEEK

	MYOFIBRILLAR		SARCOPLASMIC	
	LD	ST	LD	ST
CONTROL	4.Ø	3.4a	3.8a	5.6
RACTOPAMINE	3.4	5.8b	5.2b	5.4
SE	.7Ø	.8Ø	1.Ø	1.1

a,b Means within a column without a common superscript differ (P<.Ø5)

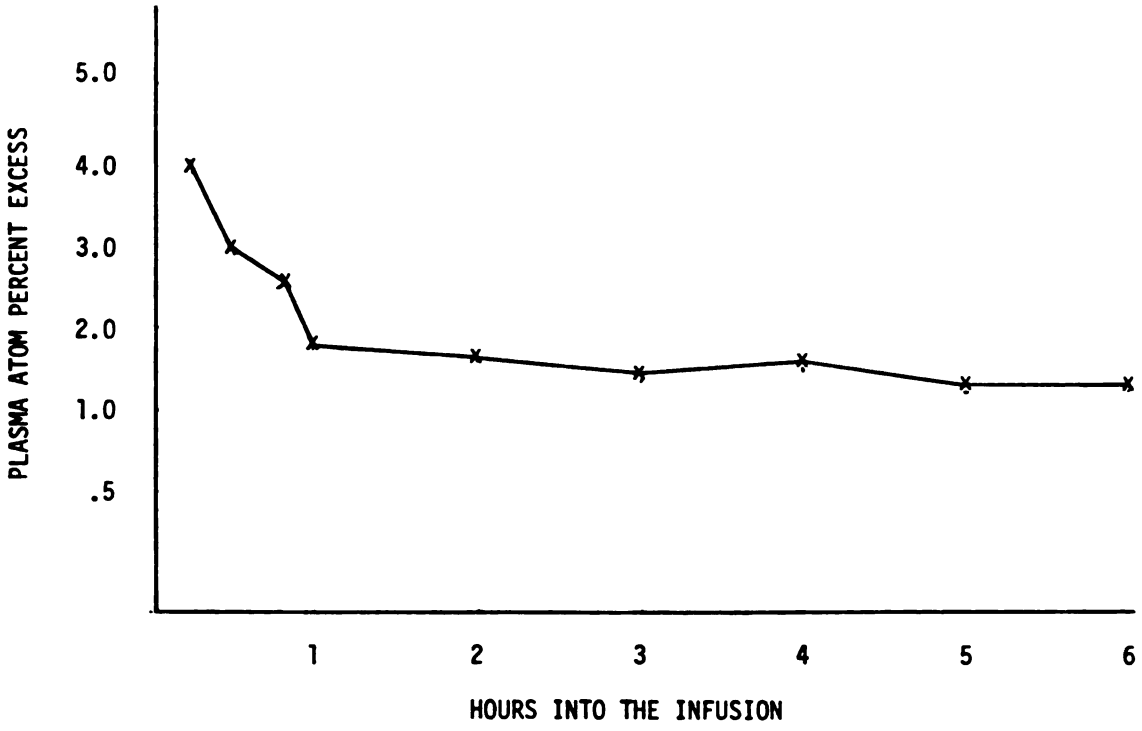


FIGURE 2. PLASMA LYSINE ENRICHMENT FOLLOWING A PRIMED-CONTINUOUS INFUSION OF N-15 LYSINE

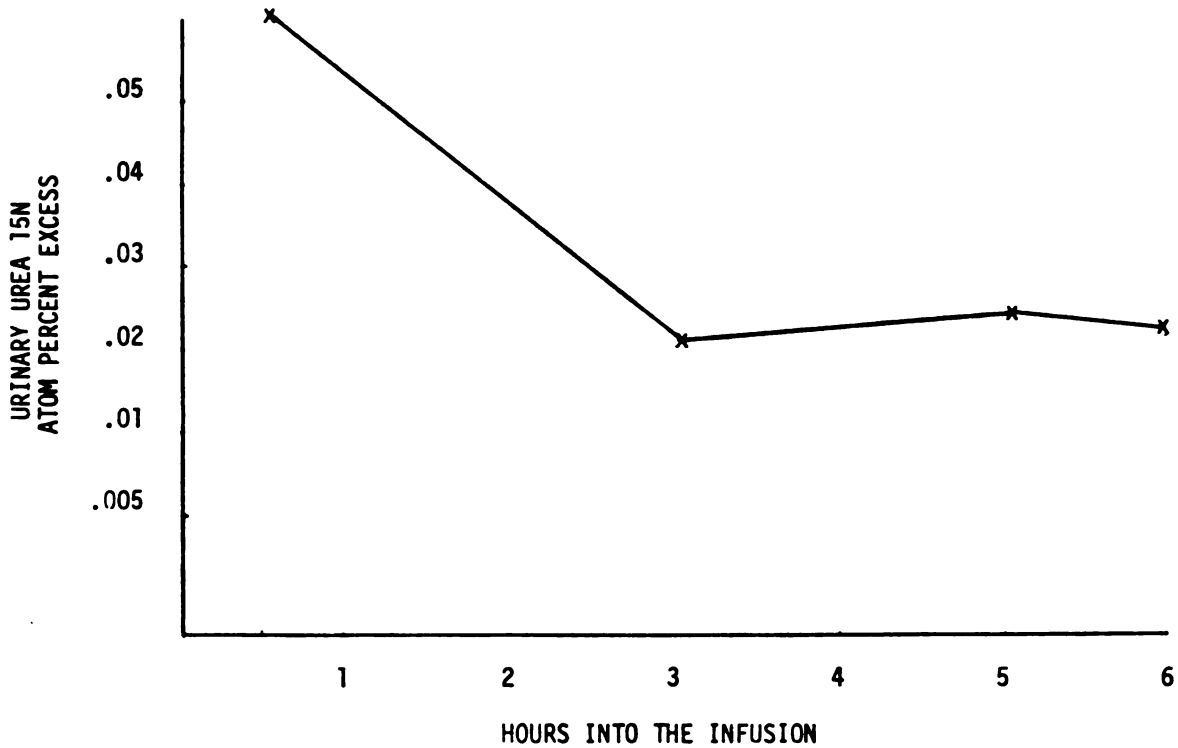


FIGURE 3. ENRICHMENT OF N FROM URINARY UREA FOLLOWING A PRIMED-CONTINUOUS INFUSION OF LYSINE IN WHICH THE UREA POOL WAS PRIMED WITH (N-15)2 UREA

TABLE 5. EFFECT OF ONE WEEK OF RACTOPAMINE FEEDING ON PLASMA LYSINE FLUX, WHOLE BODY PROTEIN SYNTHESIS AND WHOLE BODY PROTEIN DEGRADATION

	LYSINE FLUX mmol/kg/h	WBPS g/kg/d	WBPD g/kg/d
CONTROL	4.8	12.8	10.4
RACTOPAMINE	4.8	12.7	10.1
SE	.08	.21	.40

DISCUSSION

Ractopamine has been shown to promote muscle accretion in growing and finishing swine (Anderson et al., 1987; Veenhuizen et al., 1987). Concurrent with an increase in muscle mass, a decrease in fat deposition is also observed. The optimal dose required to achieve these effects is 20 ppm fed daily for three or more weeks (Anderson et al. 1987). The purpose of this study was to determine if 20 ppm Ractopamine could enhance the rate of muscle protein deposition after one week of feeding in a 16% crude protein diet.

The early reports of Veenhuizen et al. (1987) and Anderson et al. (1987) found that carcass characteristics in finishing swine fed 20 ppm Ractopamine were improved after approximately 50 days. However, Ractopamine supplementation for one or two weeks did not alter carcass composition. Failure to demonstrate an increase in protein content per gram of tissue or a decrease in fat content per gram of tissue indicates that an improvement in carcass composition is not yet apparent after two weeks of Ractopamine treatment.

DNA content per gram of protein is an indicator of skeletal muscle hypertrophy. As the muscle cell increases in size, protein accummulates and, therefore, dilutes the DNA content. This implies that muscles with a more rapid protein accretion rate would also have a higher protein/DNA ratio. Ractopamine

administration for one week effectively lowers the DNA concentration. This indicates a stimulation of protein accretion which was not reflected by an increase in either total muscle protein content or muscle weight.

Fractional protein turnover rates are a better indicator of protein metabolism than the protein/DNA ratio. Bergen et al. (1987) reported an enhanced fractional synthesis rate in barrows receiving Ractopamine for 3 weeks. A similar finding was not observed in barrows fed the same level of Ractopamine for one week in the present study. Perhaps more than one week of dietary supplementation of Ractopamine is necessary to promote an increase in the rates of protein synthesis.

A decline in protein degradation rates was not evident after one week of feeding. A plausible explanation is that one week of Ractopamine feeding is of insufficient duration to illicit an effect on rates of protein degradation. Fractional breakdown rates are commonly calculated as the difference between fractional synthesis rate and fractional accretion rate. Because fractional accretion rates are determined as the difference between two populations, it is important that these two groups be as homogeneous as possible in order to minimize error. A precise estimate of fractional synthesis rate is also necessary. This is difficult due to the inability of the continuous infusion procedure to detect slight changes in fractional synthesis rates. Accurate determinations of fractional accretion rate and fractional synthesis rate are, therefore, necessary in order to obtain accurate fractional breakdown rates. The small numbers of

animals used in this study may have biased or masked the effects of Ractopamine on fractional degradation and accretion rates.

Because failure to detect changes in the overall fractional synthesis rates in longissimus and semitendinosus muscles may have been due to the fact that early fractional synthesis rate differences for the total muscle protein fraction may be no larger than the detection limit of the continuous infusion procedure, myofibrillar and sarcoplasmic proteins were extracted to determine whether differences in isotope incorporation rates could be found. For the longissimus muscle, the Ractopamine barrows demonstrated higher rates of sarcoplasmic protein synthesis with no change in the rate of myofibrillar protein synthesis. The sarcoplasmic protein fraction contains the cytosolic enzymes responsible for maintaining the integrity of the muscle cell. It is plausible that an increase in their rate of synthesis would in turn lead to more enzymes and synthetic machinery necessary to support an enhanced rate of myofibrillar protein synthesis. For the semitendinosus muscle, myofibrillar protein synthesis was stimulated following one week of Ractopamine administration while the rate of sarcoplasmic protein synthesis remained unchanged. The myofibrillar proteins constitute 40 to 50% of the total muscle proteins. Because these proteins comprise the bulk of the total muscle protein, a slight increase in their rate of synthesis ccould mediate larger increases in protein accretion assuming rates of protein degradation remain unchanged.

The overall effect of one week of Ractopamine supplementation

on fractional protein turnover appears to be a stimulation of protein synthesis. However, this stimulation could only be detected in certain protein fractions. These data lend limited support to the findings of Bergen et al. (1987) who reported elevated fractional protein synthesis rates in the longissimus and semitendinosus muscles of barrows receiving Ractopamine for 3 weeks. Other beta agonists also demonstrate an effect of protein degradation. Zeman et al. (1986) and Reeds et al. (1986) reported that the mode of action of Clenbuterol is through inhibition of rates of fractional protein degradation with no effect on rates of skeletal muscle protein synthesis. Ractopamine appears unique with respect to its ability to affect protein synthesis and degradation.

Whole body protein turnover encompasses protein synthesis and degradation not only in skeletal muscle but in the entire body. Unpublished results of Bergen et al. (1987) demonstrate an increase in liver weights after 2 weeks of Ractopamine supplementation. In order to attain a larger liver mass, liver protein synthesis and degradation rates would have to be altered. It is possible that an alteration in these rates would be evident in whole body protein turnover rates. Since no differences in whole body protein synthesis or degradation rates were apparent after one week, it can be speculated that the protein turover rates of the internal organs have not been affected. No other studies involving beta agonists have reported their effects on whole body protein turnover.

Another objective of this study was to determine the efficacy

of stable isotopes in the determination of protein turnover in large animals. Krishnamurti and Schaefer (1987) studied whole body protein metabolism in pregnant ewes using N-15 leucine. They were able to obtain results which coincided with earlier data obtained using radioisotopes. In this study, assuming no treatment effect, the results agree well with those of Garlick et al. (1976). Garlick et al. (1976) infused C-14 tyrosine and C-14 lysine into 75 kg pigs. Their values for whole body protein synthesis were 684 g of protein per day. While our results for whole body protein synthesis are slightly higher, this may be attributable to the choice of isotope. Garlick et al. (1976) used C-14 tyrosine as a tracer of protein carbon, while in the present study N-15 lysine was used as a tracer of protein nitrogen. When a carbon isotope is used, a larger source of error is introduced in comparison to nitrogen isotopes. In general, carbon is reutilized to a larger extent than nitrogen. It is also more difficult to trap labeled CO2 from expired breath than to collect labeled nitrogen in the urine. These sources of error combined with the fact that the pigs used by Garlick et al. (1976) were probably not growing as rapidly as the pigs in this study, place the whole body protein turnover results in close agreement with the values obtained by Garlick et al. (1976).

Cost of analysis is the major disadvantage when using stable isotopes in large animals. Sample preparation and purification for gas chromatography-mass spectrometry analysis is costly but the largest expense is the actual mass spectrometry analysis.

Therefore, assuming animal costs remain constant, the use of radioisotopes would still prove to be more cost effective than stable isotopes. The only time where stable isotopes would be considered more practical than radioisotopes, would be in the determination of protein metabolism in expensive animals. Another case where stable isotopes would prove beneficial, would be when the animal is used as its own control with repeated measurements over time. In summary, stable isotopes are not cost effective for today's animal scientist interested in metabolic regulation due to high costs and often a lack of readily available equipment.

CONCLUSIONS

1. 2Ø ppm Ractopamine supplementation for one week is of insufficient duration to stimulate whole body protein synthesis.

2. 2Ø ppm Ractopamine supplementation for one week is of insufficient duration to stimulate fractional protein synthesis in the longissimus and semitendinosus muscles.

3. One week of Ractopamine supplementation stimulates fractional myofibrillar protein synthesis in the semitendinosus but not in the longissimus muscle.

4. One week of Ractopamine supplementation stimulates sarcoplasmic protein synthesis in the longissimus but not in the semitendinosus muscle.

5. Direct evidence in support of Ractopamine stimulation of protein accretion is not apparent after one week of supplementation, however, an indirect stimulation of protein accretion is evident by a decrease in DNA concentration (ug/g tissue).

6. Ractopamine supplementation for one week has no effect on RNA content in the longissimus and semitendinosus muscles.

APPENDICES

APPENDIX A. CALCULATION OF PRIMING DOSE AND INFUSATE CONCENTRATION FOR THE MEASUREMENT OF WHOLE BODY PROTEIN SYNTHESIS AND DEGRADATION VIA A CONTINUOUS INFUSION

I. PRIMING DOSE-CONTINUOUS INFUSION RATIO

$P/F = C(V)/Ra$

Given:

P = priming dose
F = infusion rate
C = lysine pool concentration
V = volume of lysine body pool
Ra = rate of appearance of unlabeled amino acid

Assumptions:

Ra = 4.4 umole/kg/min
C = .7 umole/ml
V = 70% of body weight

II. DOSE CALCULATIONS FOR 2% ATOM PERCENT EXCESS ENRICHMENT LEVELS

Equilibrium atom percent excess = F/Ra

Given:

F = alpha N-15 lysine infusion rate
Ra = rate of appearance of unlabeled lysine

Assume:

Ra = 4.4 umole/kg/min

III. UREA PRIMING DOSE

Urea prime = F(C)(P/F)

Given: F = alpha N-15 lysine infusion rate
C = percent lysine flux catabolized
P/F = ratio of priming dose to infusion rate

Assume:

C = 10% of lysine flux catabolized

APPENDIX B. FORMULAS FOR THE CALCULATION OF LYSINE FLUX, WHOLE BODY PROTEIN SYNTHESIS AND WHOLE BODY PROTEIN CATABOLISM

Whole Body Protein Synthesis =

$$\frac{\text{lysine flux} - \text{lys breakdown(mMol/kg/h)}}{\text{3.4 mMol lys/ g N}} \times 6.25\text{(g protein/g N)} \times 24\ \text{h/d}$$

Where:

lysine flux (mMole/kg/h) =

$$\frac{\text{alpha N-15 lys infusion rate (mMol/kg/h)}}{\text{plasma lysine enrichment (APE)}} - \text{total lysine infusion rate (mMole/kg/h)}$$

lysine breakdown = lysine flux X % lysine flux deaminated

$$= \frac{\text{flux} \times \text{urea N excretion (mmole/kg/h)} \times \text{urea N APE}}{\text{isotope infusion rate (mmole/kg/h)}}$$

Whole Body Protein Catabolism =

$$\frac{\text{flux}}{\text{3.4 mmole lys/g N}} \times 6.25\ \text{(g protein/g N)} \times 24\ \text{h/d}$$

APPENDIX C. CALCULATION OF SPECIFIC ACTIVITIES AND FRACTIONAL RATES

$$\frac{Sb}{Si} = \frac{R}{R-1(1-e-Rkst)} \frac{(1-e-kst)}{} - \frac{1}{R-1}$$

Given:

ks = fractional synthesis rate
t = .25 day
R = 400 = ration of protein bound to free tyrosine
Sb = specific activity of bound protein pool
Si = specific activity of tissue free pool

$$Sb = \frac{\text{total dpm X \% tyr recovery}}{\text{total tyr content (nmole)}}$$

$$Si = \frac{\text{total dpm X \% tyr recovery}}{\text{total tyr content (nmole)}}$$

$$\text{\% tyrosine recovery} = \frac{\text{dpm in tyr band}}{\text{total dpm}}$$

$$\text{Fractional accretion rate} = \frac{\text{daily accretion rate}}{\text{(muscle protein pools of the initial group + final slaughter group)/2}}$$

$$\text{Fractional breakdown rate} = \text{Fractional synthesis rate} - \text{Fractional accretion rate}$$

APPENDIX D. RAW DATA: LONGISSIMUS FRACTIONAL SYNTHESIS RATES (%/D)

		TOTAL		MYOFIBRILLAR		SARCOPLASMIC	
SAMPLE	SAi*	SAb/SAi	FSR	SAb/SAi	FSR	SAb/SAi	FSR
CONTROL							
148-11	6.34	.00834	5.16	.00705	3.74	.00720	3.81
	6.34	.00896	5.49	.00709	3.75	.00612	3.33
259-11	5.00	.00721	4.57	.00733	3.85	.01900	8.67
	5.00	.01149	6.78	.00693	3.71	.01851	8.45
237-17	6.88	.00730	4.63	.00712	3.77	.00624	3.39
	6.88	.00853	5.27	.00847	4.35	.00649	3.50
2-10	5.25	.01068	6.35	.00870	4.45	.00879	4.48
	5.25	.01085	6.45	.00916	4.64	.00869	4.44
TREATED							
2-8	6.12	.00825	5.12	.00624	3.39	.00853	4.37
	6.12	.00830	5.14			.00900	4.57
259-13	6.22	.00959	5.81	.00490	2.79	.01272	6.11
	6.22	.01111	6.59	.00547	3.04	.01248	6.02
237-14	4.39	.00637	4.13	.00826	4.26	.00555	3.08
	4.39	.00603	3.95	.00876	4.47		
140-16	6.59	.00704	4.49	.00559	3.09	.01043	5.17
	6.59	.00746	4.71				

* SA=DPM/nMOLE

APPENDIX E. RAW DATA: PLASMA N-15 LYSINE ISOTOPE RATIO AND ATOM PERCENT EXCESS

SAMPLE	HOURS INTO INFUSION 3*	4	5	6
CONTROL				
259-11	.125Ø(1.91)	.1237(1.78)	.1238(1.79)	.1235(1.76)
2-1Ø	.1544(1.71)	.1557(1.84)	.155Ø(1.77)	.1558(1.78)
148-12	.125Ø(1.91)	.1238(1.79)	.1236(1.8Ø)	.123Ø(1.71)
237-17	.1559(1.86)	.1565(1.92)	.1562(1.89)	.1559(1.86)
TREATED				
259-13	.1235(1.76)	.1226(1.67)	.1228(1.69)	.125Ø(1.91)
2-8	.1233(1.74)	.1226(1.67)	.1237(1.78)	.1238(1.79)
237-14	.124Ø(1.81)	.123Ø(1.71)	.1228(1.69)	.1235(1.76)
14Ø-16	.1196(1.37)	.12ØØ(1.41)	.12Ø5(1.46)	.12ØØ(1.41)

* ISOTOPE RATIO (ATOM PERCENT EXCESS)

APPENDIX F. RAW DATA: WHOLE BODY PROTEIN SYNTHESIS

SAMPLE	LYSINE FLUX uMOL/KG/H	LYSINE INTAKE MG/KG/H	WBPS G/KG/D	WBPD G/KG/D
CONTROL				
259-11	286.8	8.7	12.6	1Ø.5
2-1Ø	292.2	1Ø.2	12.7	1Ø.4
148-12	289.2	8.7	12.8	1Ø.7
237-17	274.2	11.4	12.1	9.4
TREATED				
259-13	295.2	14.Ø	13.Ø	9.6
2-8	296.4	7.6	13.1	11.3
237-14	293.4	12.4	12.9	1Ø.Ø
14Ø-16	368.4	12.Ø	16.3	13.4

APPENDIX G. RAW DATA: MUSCLE WEIGHTS, RNA AND DNA

	LONGISSIMUS			SEMITENDINOSUS		
SAMPLE	WT G	RNA MG/G	DNA MG/G	WT G	RNA MG/G	DNA MG/G
INITIAL SLAUGHTER						
207-11	1637	1.22	.32	290	1.20	.26
259-12	1497	1.49	.23	278	1.20	.28
215-12	1506	1.84	.21	293	1.07	.27
Y29-10	1380	1.32	.21	291	1.00	.25
258-13	1404	1.21	.22	224	1.02	.28
151-12	1380	2.22	.23	283	.91	.24
FINAL SLAUGHTER						
CONTROL						
151-11	1636	1.16	.20	351	1.08	.28
146-12	1868	1.24	.23	403	1.15	.30
155-16	1864	1.20	.25	337	.85	.20
148-13	1646	1.30	.26	263	.82	.28
TREATED						
243-14	1553	1.01	.21	326	.95	.20
147-17	1669	.95	.18	374	.94	.21
146-13	1793	1.46	.20	344	1.40	.23
255-11	1680	1.02	.19	335	1.09	.23

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