# EFFECT OF RECOMBINANT PORCINE SOMATOTROPIN (rpST) AND DIETARY CRUDE PROTEIN CONCENTRATION ON LIPID METABOLISM AND CARCASS COMPOSITION OF FINISHING PIGS

By

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# ABSTRACT

## EFFECT OF RECOMBINANT PORCINE SOMATOTROPIN (rpST) AND DIETARY CRUDE PROTEIN CONCENTRATION ON LIPID METABOLISM AND CARCASS COMPOSITION OF FINISHING PIGS

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Thirty-two pigs (avg. 74 kg) were used to determine the effects of rpST and dietary crude protein (CP) concentration on lipid metabolism and carcass composition. Pigs were injected intramuscularly with either rpST (50 ug/kg BW/day; n=16) or vehicle (n=16) at 0900 hours for 24 days. A 14 % CP (n=8) or 20 % CP (n=8) corn/soy diet was fed within each injection group. The right side of the carcass was graded, fat thickness and muscle measurements taken and quality scored. The left side was physically dissected into separable skin, soft tissue and bone, and tissue samples obtained for enzyme assays, compositional analysis, fatty acid profiles and determination of nucleic acid content. rpST and CP individually decreased percent carcass fat by decreasing lipogenic capacity. Lipolysis was not affected. Fatty acid profiles were

slightly altered by rpST while alterations in lipid and protein metabolism resulted in changes in composition. rpST increased percent carcass muscle and weights of specific organs. Total bone weight and individual long bone length and weight were not affected by rpST or CP.

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effects of beta adrenergic agonists, especially ractopamine (Lilly Research Labs, Greenfield, IN), on lipid and protein metabolism in swine, however, less work has been done with somatotropin (ST). The objective of this experiment was to examine the effect of rpST, delivered at 50 ug/kg/day, and dietary crude protein concentration, either 14 or 20% crude protein, on lipid metabolism, as assessed by key lipogenic enzyme activities, fatty acid synthesis and measures of lipolysis, and carcass composition in finishing pigs.

# INTRODUCTION

Over the past 35 years there has been a shift in animal production systems from producing animals that are relatively fat to producing animals with a greater percent lean. This shift is the result of a more health conscious society demanding leaner food products and a recognition by producers that fat animals are less efficient. The industry has responded by investigating strategies to decrease adipose tissue accretion and increase lean tissue gain. Among the strategies is the experimental administration of recombinant porcine somatotropin (rpST).

Administration of rpST to pigs alters their body composition toward that of a leaner animal and improves the performance of finishing pigs when fed a diet of adequate nutrient content. rpST has been shown to increase average daily gain, improve feed efficiency, increase protein deposition, and decrease both total adipose tissue mass and lipid accretion.

To date, extensive research has been reported by the Muscle and Growth Biology group at Michigan State University on the

# LITERATURE REVIEW

## SOMATOTROPIN

Somatotropin (ST), commonly known as growth hormone, is an essential hormone for proper animal growth. The effects of ST have been known for approximately 60 years since the initial discovery (Evans et al. 1922, 1931) that a substance in the anterior pituitary increased growth of rats. Rats, administered an alkaline preparation of bovine pituitary extract, exhibited increased growth along with changes in total body composition manifested as a higher proportion of muscle relative to fat (Lee and Shaffer, 1934). Progress was slow in characterizing production responses and identifying the mechanisims whereby ST acts due to the limited supply and variable purity pituitary derived somatotropin. A method was developed for the isolation of porcine ST (pST) from fresh porcine pituitaries (Raben and Weatermyer, 1951). Later, a procedure was developed for the preparation of pST from frozen pig pituitaries (Papkoff et al., 1952). By 1970 the molecuar weight of pST was determined to

be 22 kilodaltons (kd) (Chen et al., 1970). It was not until 1983 that the entire amino acid sequence of pST was established in conjunction with the sequencing a cloned cDNA for pST (Mills et al., 1970). The three dimensional structure of pST has been reported to be a small 22 kd globular protein with two disulfide bonds and composed of 191 amino acids in a single chain (Abdel-Mequid et al., 1987).

On the basis of structural homologies, pST may be included as a member of the somatomammotropin family of hormones: growth hormone, prolactin, and chorionic somatomammotropin (Daughaday, 1974). ST from pigs, cattle, sheep, and man are all similar in structure and in mode of action (Santome et al., 1965). pST shares a high degree of homology with bovine somatotropin (bST), differing only in 18 amino acids. Human somatotropin (hST) and pST differ in sequence by 56 amino acids (Etherton, 1989). ST from a mammalian species lower on the phylogenetic tree will not elicit a typical ST response in mammalian species higher on the phylogenetic tree. However, ST from higher mammalian species will elicit the ST response in lower mammalian species (Kostyo, 1974) suggesting an evolutionary

hierarchy in terms of ST action.

## SOMATOTROPIN SECRETION

ST is a polypeptide hormone secreted by cells known as the somatotropes (acidophils) of the anterior pituitary in mammals. Secretion from the anterior pituitary is controlled by hypothalamic releasing and inhibitory hormones which are secreted in the hypothalamus and transported to the anterior pituitary via the hypothalamic-hypophysial portal vessels (Guyton, 1986). Somatocrinin or growth hormone releasing factor (GRF), stimulates release of ST from the anterior pituitary, while somatostatin (SRIF) inhibits release. ST is secreted from the pituitary in episodic pulses with a diurnal rhythm. ST is secreted as a response to 1.) starvation, 2.) hypoglycemia (Machlin et al., 1968a; Swiatek et al.,1968) 3.) low concentration of fatty acids (Blackard et al.,1971), 4.) exercise 5.) excitement 6.) trauma (Reichlin, 1973; Machlin et al., 1968 a,b) or 7.) deep, slow wave sleep (Tanner, 1972; Takahashi, 1968; Honda, 1967; Parker, 1967) and is partially controlled by negative feedback from high serum levels of ST or insulin-like growth factor-1 (Phillips, 1986).

## INSULIN-LIKE GROWTH FACTORS

According to the somatomedin hypothesis, (Daughaday, 1982; Isaksson, 1986) a portion of the ST effects are mediated by production and/or release of the somatomedins, insulin-like growth factors-1 and -2 (IGF-1 and IGF-2 respectively), from the liver and other tissues which act to stimulate chondrogenesis and satellite cell proliferation (Isaksson, 1987; Allen, 1988). IGF-1 is a 70 amino acid, single chain, polypeptide with a molecular mass of 7.649 Kd while IGF-2 is a 68 amino acid, single chain, polypeptide with a molecular mass of 7.471 Kd (Rinderknecht and Humbel, 1987 a,b; Etherton,1989). IGF-1 secretion is ST dependent and has a marked effect on postnatal growth (Moses et al.,1980; Rudman et al., 1981; Adams et al., 1983) whereas IGF-2 is less responsive to ST (Froesch et al., 1985) and has an important role in regulating fetal growth (Daughaday et al., 1982; Gluckman and Butler, 1983; Rotwein et al., 1987). Both IGF-1 and IGF-2 increase with exogenous ST administration; however, the IGF-2 response is not as pronounced as the IGF-1 response (Etherton, 1989). IGF concentrations remain elevated for up to 24

hours after serum pST concentrations return to normal in pigs administered pST which implies that pST acts by inducing de novo synthesis of IGF-1 rather than by causing its release from storage pools (Sillence and Etherton, 1987). Grant et al. (1991) reported that administration of 50 ug rpST/kg BW/day increased liver IGF-1 mRNA and IGF-1 serum concentrations suggesting an endocrine role of IGF-1 in mediating ST induced muscle hypertrophy in swine.

Green and coworkers (1985) have proposed a dual effector theory involving ST and IGF-1. The dual effector theory implies that ST may have direct and indirect effects on tissue growth; these concepts will be elaborated on in the sections that follow.

## SOMATOTROPIN SERUM CONCENTRATIONS

A number of studies have been conducted to determine the concentration of circulating pST in pigs and are summarized in Table 1. Several factors influence the concentration of circulating pST in the pig; these factors include gender, age, body weight, growth rate, heredity, and other hormones.

**TABLE 1 SERUM CONCENTRATIONS OF PORCINE ST AT DIFFERENT AGES AND WEIGHTS**

| | |
|---|---|
| Birth to 3 weeks<br>.5 kg to 8 kg | 17 - 20 ng/ml |
| 3 weeks to 8 weeks<br>8 kg to 18 kg | 5.41 ng/ml* |
| 8 weeks to 12 weeks<br>18 kg to 50 kg | 2.51 - 10.7 ng/ml |
| 12 weeks to 18 weeks<br>50 kg to 85 kg | 1.6 - 8.0 ng/ml |
| 18 weeks to market<br>85 kg to 110 kg | 2.47 - 3.10 ng/ml |

Data represents a summary of data from several studies.
Machlin et al. 1968
Swiatek et al. 1968
Siers and Hazel 1970
Bidner et al. 1973
Topel et al. 1973
Sillence and Etherton 1987
Dubriel et al. 1988
* only one value from the studies cited falls into this category

## SOMATOMEDIN SERUM CONCENTRATIONS

IGF-1 is the predominant somatomedin involved in postnatal growth and has been the focus of much of the insulin-like growth factor research. Lee et al. (1991) report a preponderance of IGF-2 over IGF-1 in fetal serum and postnatal increases of both IGFs (Figure 1) indicating that IGF-2 may also play a role as a postnatal growth factor, whereas IGF-1 may be primarily a postnatal growth factor. Data indicate that barrows between 30 and 80 kg body weight have serum concentrations of IGF-1 ranging from 53 ng/ml to 500 ng/ml; however, the majority of the data indicates levels of 100 ng/ml to 200 ng/ml (Buonomo et al., 1987; Campbell et al., 1990; Chang et al., 1990; Etherton et al., 1987; Sillence and Etherton, 1987; Smith and Kasson, 1990; Wilson and Hintz, 1982). Very little, if any, free IGF has been reported in the serum of swine (Evock et al. 1990) and has been shown to be associated with IGF binding proteins, the significance of has yet to be determined.

## RECEPTORS

Several investigators have shown that responsiveness to ST

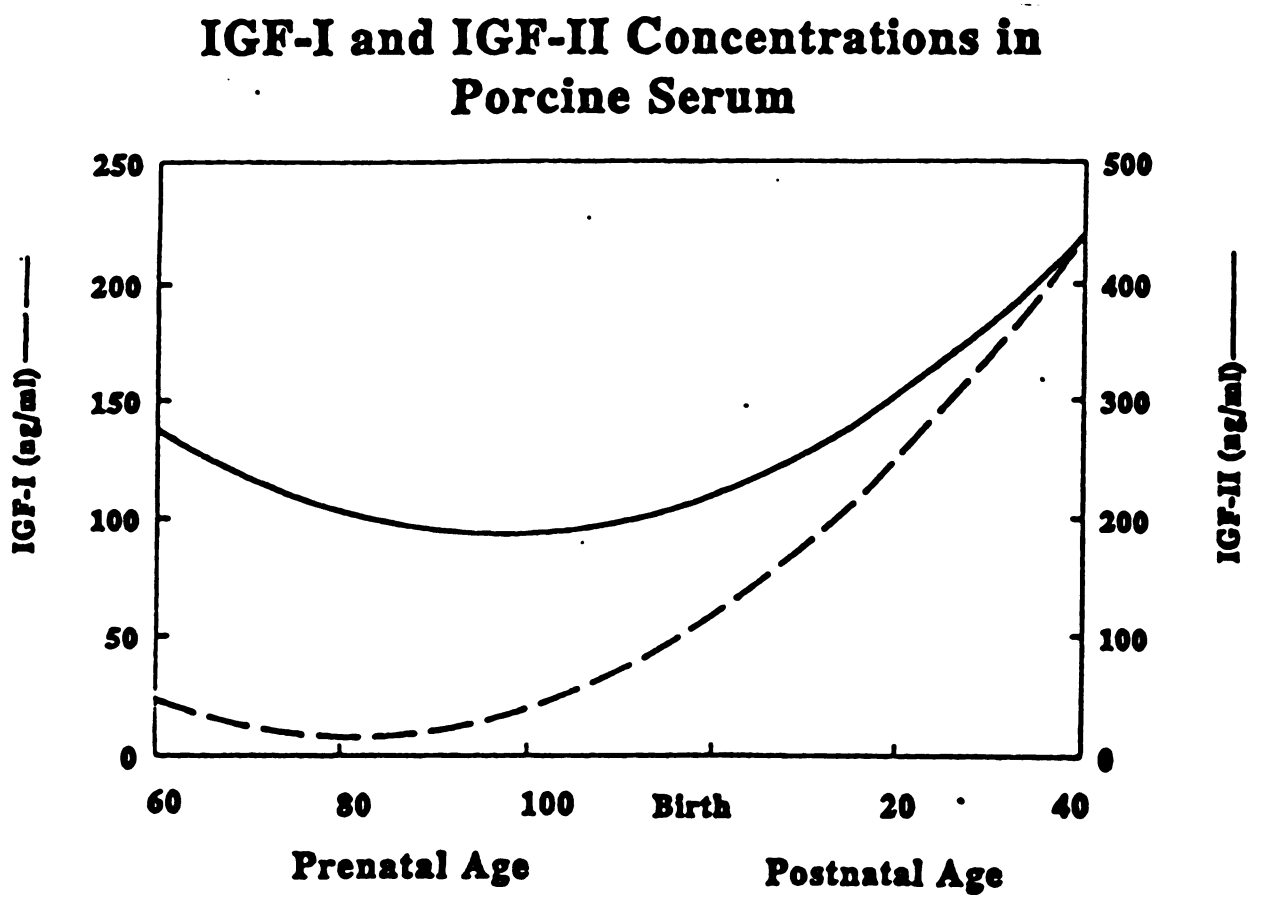


**FIGURE 1 IGF-1 AND IGF-2 SERUM CONCENTRATIONS IN PIGS (Lee et al. 1991)**

is a function of age for various animal species (Goodman and Coiro, 1981; Gluckman et al., 1983; Maes et al., 1983). Growth during the fetal and early postnatal period appears to be largely independent of pituitary ST whereas, a progressive increase in responsiveness begins to occur during the later neonate period (Gluckman et al., 1983; Freemark et al., 1986; Maes et al., 1983). Tissues identified as having ST receptors include adipocytes and preadipocyte cell lines, liver membranes and hepatocytes, chondrocytes, various skeletal muscles and cultured lymphocytes (Boyd et al., 1989).

There are three types of cell surface receptors which bind the insulin like growth factors and/or insulin; however, the affinities differ. The relative affinities for the individual receptors are as follows: (Nissley and Rechler, 1984; Rechler and Nissley, 1985)

| Insulin receptor: | IGF type 1 receptor: |
|---|---|
| insulin > IGF-2 > IGF-1 | IGF-1 > IGF-2 > insulin |

The IGF type 2 receptor has a greater affinity for IGF-2 and does

not bind IGF-1 or insulin (Rosenfield et al., 1987).

## DOSE RESPONSE RELATIONSHIP

Pig responses to pST are dose dependant (Boyd et al., 1986; Etherton et al., 1987; Ender et al., 1989a,b; Zimmerman 1989).

Administration of 0, 30, 60, 120, or 200 ug pST/kg/day resulted in the maximal rate of gain at 60 ug/kg/day, carcass lean was maximized between 120-200 ug/kg/day, fat accretion was minimal at 200 ug/kg/day and above, while feed intake decreased linearly with each dose, (Figure 2) (Boyd et al., 1988; Etherton et al., 1987). Dry matter intake and fat depth decreased with increasing pST dose while loin eye area was increased and feed efficiency improved with higher doses (Zimmerman, 1989). To date, the literature does not furnish enough information to establish an ideal dose of pST to be administered to pigs. It is clear from the data reported that different measurements are altered with changing doses. The "ideal" dose may well be one that does not maximize responses but does take into account other variables which may affect the response to pST such as gender, genotype, diet and economics.

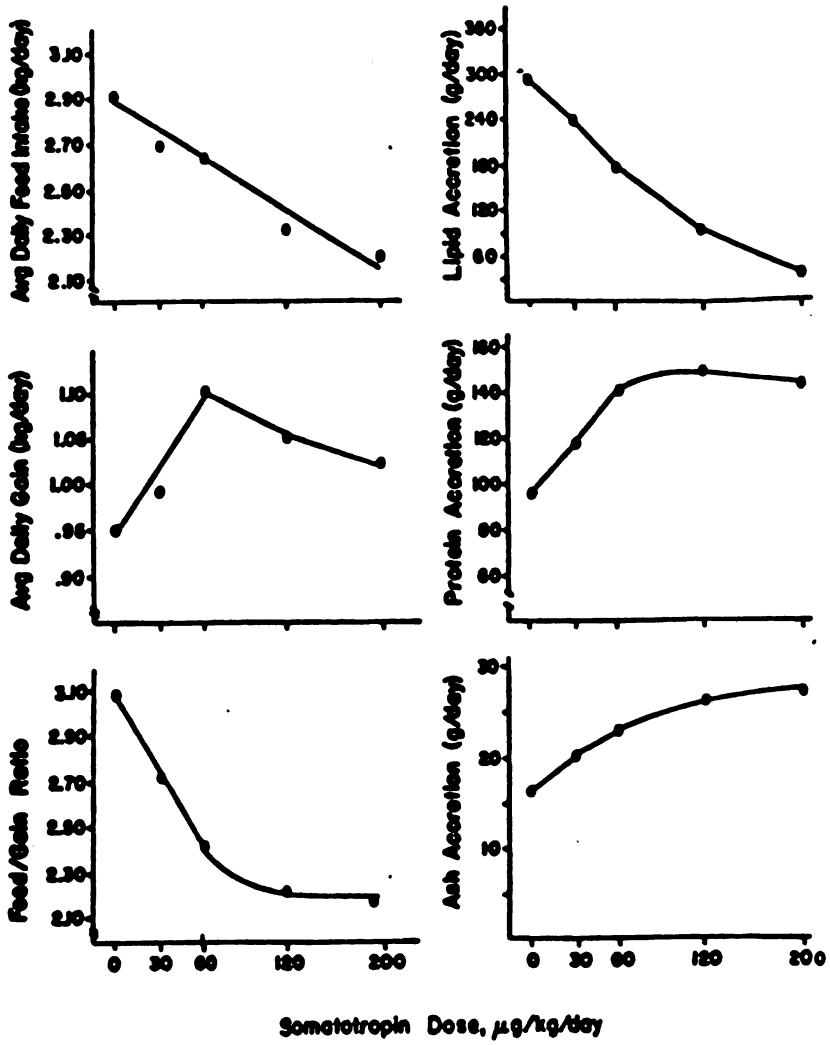


**FIGURE 2** **DOSE RESPONSE RELATIONSHIP BETWEEN ST AND SEVERAL MEASUREMENTS IN SWINE. (Boyd et al. 1989)**

## NUTRITIONAL EFFECTS

The National Research Council (NRC 1988) dietary nutrient requirements for finishing swine (50-110 kg) are based on the typical corn/soybean meal diet as presented in Table 2.

Treatment of finishing pigs with pST alters the ratio of lean/fat, feed intake (Boyd, 1986; McLaren, 1987), and rate of gain; this may indicate that the pST treated pig has altered nutrient requirements. The diet of ST treated pigs must be sufficient in energy density to support the accelerated protein accretion. Marked increases in protein accretion associated with pST administration result in a 12-16% higher energy requirement than control pigs (Campbell et al., 1988). Considering the facts that 1.) ST treated pigs have a higher maintenance cost and 2.) ST decreases voluntary feed intake in the pig (Campbell et al., 1988, 1989; Etherton et al., 1987; Evock et al., 1988), manipulation of the diet is essential if the pigs are to consume appropriate amounts of nutrients to support the increased protein accretion. Finishing swine diet formulations typically contain 3,300 kcal digestible energy/kg. In order to increase the energy density of the diet, carbohydrate may be replaced by higher energy source, such as dietary lipid. The minor dietary

**TABLE 2 NRC MINIMAL DIETARY REQUIREMENTS OF FINISHING SWINE**

| | |
|---|---|
| energy concentration (kcal ME/kg diet) | 3,275.00 |
| % crude protein | 13.00 |
| % lysine | 0.60 |
| % vitamins and minerals | 3.00 |

---

NRC Nutrient Requirements of Swine 1988 p. 51.

adjustment would ensure that dietary energy density is within constraints of appetite and appropriate for the increased protein deposition potential of pST treated pigs. Adding lipid to the diet will lead to greater gains by the pigs, however, that increased gain is also a greater proportion of fat relative to muscle.

The initiation and maintenance of the increased levels of protein accretion noted for pST treated pigs requires a higher level of essential amino acids. There have been a number of studies to determine the optimum level of amino acids, especially the first limiting amino acid of a corn/soy diet, lysine, for pST treated pigs. The NRC estimate, 18.7 g lys./day, reflects a composite of values which probably represent phenotypes accruing 100 -110 g of protein per day. Results indicate that growth and carcass traits of ST treated finishing pigs are optimized with dietary lysine in the range of 1.0-1.2 % which represents a two-fold increase over the NRC requirement at .6% lys. (Goodband, 1988,1989). Today it is generally accepted by researchers that the dietary lysine level for pST treated finishing pigs is between 1.0 and 1.4%. Results indicate that the lysine requirement is at least 1 % of the diet and if all the lysine is supplied by native proteins in a corn/soy diet an 18 % crude protein diet is

required. Current consensus is that a diet of greater than 14 % crude protein (Smith et al., 1989; Zimmerman et al., 1989; Newcomb et al., 1988) and greater than 1 % lysine is at best a minimum requirement for pST treated finishing pigs. Research to date indicates that the amino acid requirements vary depending on the pigs potential for growth, genotype, sex, and on the level of pST administered.

## SOMATOTROPIN ACTION

The awareness that the pituitary produces substances that are involved in the growth process prompted animal scientists to administer pituitary preparations of ST to pigs with the intention of improving the efficiency of growth (Etherton, 1989) and carcass composition. Early studies (Giles, 1942; Turman and Andrews, 1955; Henricson and Ullberg, 1960) were important; however, the data were inconclusive due to the purity of the preparation used. Machlin (1972) reported that pigs treated with a pituitary preparation of pST had increased weight gain and improved feed efficiency. Although the potential beneficial effects of treating pigs with ST were known, the

commercialization of a pST product was not practical because of the limited availability of pig pituitaries and the high cost of purification (Etherton, 1989). With the advent of recombinant DNA techonology, it became possible to produce large quantities of biologically pure, active peptides; especially rpST. Today, a majority of the researchers studying the effects of ST utilize the recombinant analog of ST since it has the same amino acid sequence and has been shown to stimulate pig growth performance in a similar manner (Evock et al., 1988) to pST and is both cheaper and easier to acquire.

## ALTERATIONS IN RESPONSE TO HOMEOSTATIC SIGNALS

Homeostasis is defined as the maintenance of a relatively constant internal physiological environment while homeorhesis pertains to the long term adjustments by the organism in response to certain situations. The shift in lipid and protein metabolism that occurs upon ST treatment involves a coordinated response primarily to homeorhetic signals and secondarily homeostatic regulation (Boyd and Bauman, 1989). Results

indicate that treatment with ST results in a reduced response of adipose tissue to homeorhetic signals that stimulate lipogenesis and an enhanced repsonse to homeorhetic signals that affect lipolysis and protein synthesis. The treated animal is still reponsive to homeostatic signals but there may be a larger change in the magnitude of the response. The extent to which lipid accretion is depressed and protein accretion increased is dependent upon the magnitude of the reduction in synthesis and degradation and is undoubtedly related to energy balance (Bauman and McCutcheon, 1986). A growing animal in positive energy balance administered ST has reduced rates of lipid synthesis (Boyd and Bauman, 1989) and increased rates of protein synthesis. On the other hand, when the animal is in negative energy balance the major effect of ST is on lipid mobilization (Machlin, 1972; Roupas et al., 1991). The energy status of the animal must be maintained at a relatively constant level and therefore serves as the homeorhetic signal which maintains the long term energy status of the animal not restricting the growth of the animal; small fluctuations represent homeostatic signals which remain secondary to the homeorhetic signals.

## PROTEIN SYNTHESIS

The primary effect of ST is to increase protein accretion and growth in a variety of tissues and organs. Campbell et al. (1989) reported that pST stimulates overall protein metabolism and that the positive effect of pST on protein deposition occurs in pigs because protein synthesis exceeds protein breakdown. ST enhances the transport of amino acids through the cell membrane (Kostyo, 1968, 1973, 1977), enhances protein synthesis by the ribosomes (Kostyo, 1973; Garren, 1962), increases the transcription of DNA to RNA (Kostyo, 1977), and has no effect on protein degradation (Goldberg, 1969, 1980). Tissues of hypophysectomized animals have a reduced ability to synthesize both protein and RNA (Korner 1967) while administration of ST to hypophysectomized animals stimulates RNA and protein synthesis in skeletal muscle (Kostyo, 1973; Knobil, 1966; Florini, 1966), heart, liver (Kostyo, 1973), adipose tissue and cartilage and increases the amount of DNA in skeletal muscle (Beach and Kostyo, 1968) and liver (Cheek et al. 1969) of hypophysectomized rats.

## EFFECT OF ST ON MUSCLE

Muscle tissue growth is the result of hyperplasia, an increase in cell number, and hypertrophy, an increase in cell size. Hyperplasia predominates prenatally and continues to a predetermined point at which further growth by cell divison stops. Further muscle tissue growth occurs through hypertrophy. Hypophysectomized rats administered ST have an increased DNA content of muscle (Beach and Kostyo, 1968; Goldspink and Goldberg, 1975) without an increase in the ratio of total DNA content of muscle to cyoplasmic mass (Beach and Kostyo, 1968). The source of the new nuclei in the postnatal animal is satellite cells (Allen et al., 1979; Campion, 1984). ST has no direct effect on satellite cell proliferation or muscle protein synthesis in satellite cell derived myotubes (Allen et al., 1983, 1986) however, several myoblast cell lines and satellite cells demonstrate a mitogenic response to IGF-1 (Allen et al., 1984; Florini, 1985, 1987; Dobson et al., 1985). Evidence suggests that the dual effector theory applies to muscle also; ST has no direct effect on muscle cell proliferation and that the effects of ST are

indirectly mediated by somatomedins produced in other tissues transported to the muscle by the circulatory system (Allen, 1983,1986; Daughaday, 1981).

## LIPID METABOLISM

### LIPOGENESIS OVERVIEW

In swine, an appreciable amount of lipid is deposited daily in adipose tissue; approximately 180 to 280 g/day for pigs between 50 and 90 kg (Etherton, 1989). During this phase of growth, fat accretion predominates while muscle and bone accretion tend to plateau. It is during this phase of growth of the animal that an exogenous agent such as ST should be administered in order to improve carcass composition and animal performance. The administration of pST to pigs results in decreased carcass fat. The decrease in carcass fat in pST-treated pigs is the function of several biochemical changes in adipose tissue. These biochemical changes are evidenced by decreased enzyme activities, decreased tissue sensitivity to insulin, decreased glucose transport, and an interaction with guanine nucleotide binding proteins (G proteins).

## DECREASED ENZYME ACTIVITIES

Administration of ST to rats decreases the activities of several enzymes involved in lipogenesis including: acetyl CoA carboxylase, ATP citrate-lyase, malic enzyme, and fatty acid synthase (Magri et al 1990; Goodman, 1963; Bornstein, 1983; Schaffer et al., 1985). Data are sparse pertaining to lipogenic enzyme activity in adipose tissue of pigs, however there are much data on bovine and avian domestic species which utilize different substrates than those of the pig. There is evidence that several of the enzymes associated with lipogenesis had shown markedly decreased activities in pigs treated with somatotropin (Magri,1987; Harris, 1990; Schaffer, 1985) (Table 3). Data indicate that administration of ST to pigs negatively influences lipogenesis by decreasing activities of key enzymes.

## DECREASED TISSUE SENSITIVITY TO INSULIN

ST blunts the ability of insulin to maintain lipogenesis in a dose dependent manner in porcine, ovine and bovine adipose tissue (Vernon,1982; Etherton, 1987; Etherton et al.,1986,1987, Walton, 1986). ST has no effect on insulin binding, receptor number,

**TABLE 3 SELECTED ENZYME ACTIVITIES IN ADIPOSE TISSUE OF PIGS**

| ENZYME | ACTIVITY |
|---|---|
| fatty acid synthase[a] | decreased |
| acetyl CoA carboxylase[a] | decreased |
| malic enzyme[a,b] | decreased |
| glucose 6 phosphate dehydrogenase[a,b] | decreased |
| 6 phosphogluconate dehydrogenase[a,b] | decreased |

a= D. Harris (1990)
b= K. Magri (1987)

affinity for the receptor, or receptor tyrosine kinase activity (Magri, 1990).

## DECREASED GLUCOSE TRANSPORT

When ST is administered in greater than physiological amounts to pigs, metabolic disturbances are produced which include an elevated plasma insulin concentration, increased hepatic glucose production, decreased sensitivity of peripheral tissues to insulin and hyperglycemia (Kostyo, 1987). Basal rates of glucose transport decrease in a dose dependant manner in adipose tissue of hypophysectomized rats administered ST (Schoenle et al., 1983). Growing pigs administered rpST show a chronic elevation of both basal and fasting concentrations of glucose (Wray-Cahen, 1987a,b) and show a decline in glucose concentrations upon insulin challenge, however the concentration remains higher than controls. Reduced rates of glucose clearance in response to insulin or glucose challenges have also been observed in adult human males and in sheep (Rosenfeld et al., 1982; Hart et al., 1984). Physiological concentrations (1, 10

ng/ml) of pituitary porcine ST markedly supress the stimulatory effects of insulin on lipogenesis in swine adipose tissue in vitro (Walton et al., 1986). The shift in carbohydrate metabolism seems to develop over several days of treatment (Wray-Cahen, 1987a).

## LIPOLYSIS

It is frequently stated that ST stimulates lipolysis based on a number of early studies which reported increased plasma concentrations of non-esterified fatty acids or glycerol upon ST administration (Raben and Hollenberg, 1959; Goodman et al.,1959; Engel et al., 1958; Wilgram et al., 1959; Goodman and Coiro, 1981; Goodman and Grichting, 1983; Grichting et al., 1983; Goodman, 1984). Administration of ST increases plasma free fatty acid concentrations of pigs (Machlin, 1968; Chung et al., 1985). The results of the ST effect on lipolysis were difficult to interpret due to different procedures applied in different laboratories and species differences. It appears that ST may not be lipolytic but rather may potentiate the responsiveness of adipose tissue to other lipolytic stimuli (Frigeri, 1980). It has been hypothesized

that ST acts as a modulator or amplifier of physiological stimuli for lipid mobilization rather than as an acute stimulator of lipolysis (Goodman, 1974). ST may influence lipolysis through interference of an adipose cell membrane associated guanine nucleotide binding protein, G protein (Roupas et al. 1991) (Figure 3). ST may inhibit the G inhibitory protein, $G_i$, which inhibits the beta adrenergic receptor catecholamine enhanced G stimulatory protein, $G_S$, and result in a subsequent increase in cylcic adenosine monophosphate (cAMP) production (Roupas et al.1991). cAMP has an important role in the regulation of lipogenesis and lipolysis at the cytoplasmic level by affecting the phosphorylation state of specific enzymes, kinetic properties of enzymes and availability of substrates. Inhibition of the $G_i$ protein results in the deletion of the negative feedback on cAMP synthesis thereby allowing catecholamine stimulation of the $G_S$ protein which increases the level of cAMP and results in stimulation of cAMP-dependent events; lipolysis.

## FATTY ACID PROFILE OF PORCINE ADIPOSE TISSUE

It has long been appreciated that the fatty acid profile of

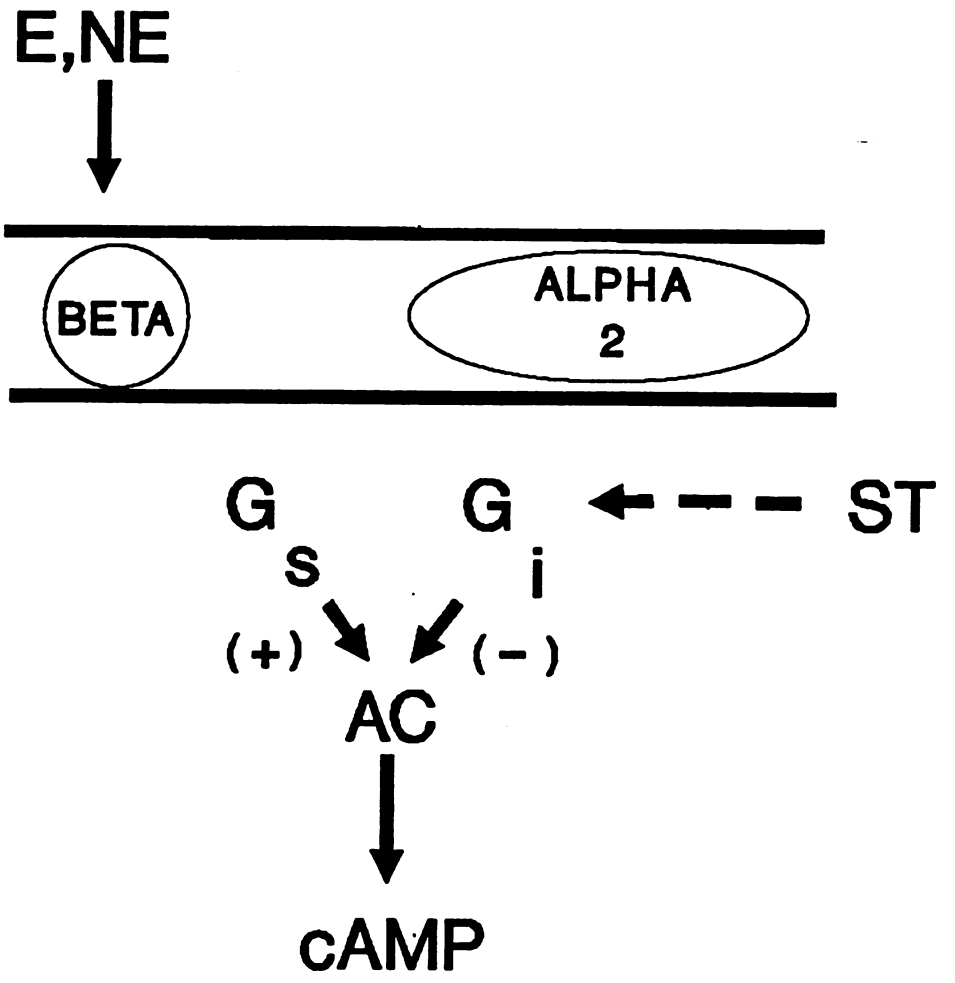


**FIGURE 3** **SCHEMATIC OF SOMATOTROPIN ACTION AT THE G-INHIBITORY PROTEIN IN ADIPOSE TISSUE. (E=epinephrine, NE=norepinephrine, ST=somatotropin, $G_s$=G stimulatory protein, $G_i$=G inhibitory protein, AC=adenylate cyclase, cAMP=cyclic adenosine monophosphate)**

porcine adipose tissue may be influenced to a greater or lesser degree by the fatty acid composition of the dietary fat (Di Giorgio, 1962). The fatty acid profile of the diet is reflected in the adipose tissue of man (Johnson, 1957), eggs (Fisher, 1957), pigs (Beadle, 1948; Villegas et al., 1973; Wahlstrom et al., 1971; Koch et al., 1968), rats (Dam, 1958; Johnson 1958), chicks (Dam, 1958), hens (Hegsted, 1960) and fish (Kelly, 1958). The changes in the fatty acid profile are dependant upon both the composition of the dietary fat and the amount fed (Hegsted, 1960). The fatty acid profile of the adipose tissue is not identical to that of the diet because a portion of the dietary fatty acids may be metabolized before reaching the adipose tissue (Kirschner and Harris, 1961; Bloom et al., 1951). Certain fatty acids may be less readily incorporated into adipose tissue or more readily mobilized (Di Giorgio, 1962) than others. In addition, fatty acids in various tissues of an animal may be derived from endogenous synthesis as well as from dietary sources. The polyunsaturated fatty acid content of porcine adipose tissue increased slightly in response to pST while saturated fatty acid content decreased slightly and are very similar to values of non-treated pigs (Prusa,

1988a,c,1989; Skelley et al., 1975; Malmfors et al., 1978; Marchello et al., 1983). Finishing pigs administered pST had significantly less palmitic, stearic, and oleic while no change was noted in linoleic acid (Wander et al. 1991).

## BONE AND MINERAL METABOLISM

Although not a major component of the observed increase in average daily gain in rpST treated pigs, bone growth is increased. Early studies reported no direct effects of ST on in vitro cartilage growth, however, a response was elicited with serum from ST treated animals. The ability of serum from ST treated animals to stimulate cartilage growth lead to the the hypothesis that ST- dependant plasma factors were responsible for the increased growth response. One such ST dependant plasma factor is insulin-like growth factor-1 (IGF-1). IGF-1 infused into hypophysectomized rats increased tibial epiphyseal growth. The hypothesis that ST has no direct effect on bone growth was not totally accepted and was shown to be at least partially influenced by a direct ST effect. Work to date had lead Green and

coworkers to propose the dual effector theory for ST on growth. ST has been postulated to stimulate differentiation of prechondrocytes to chondrocytes which proliferate dependent upon the somatomedins which are produced locally in response to ST (Green et al., 1985; Isakkson et al., 1986). Cells at the proximal part of the growth plate (chondrocyte progenitor cells) have ST receptors (Isakkson, 1985), and IGF-1 produced locally in response to ST acts as either an autocrine or paracine promoter of chondrocyte proliferation (Schlechter et al., 1986b; Boyd and Bauman, 1989). The result of ST administration is increased longitudinal bone growth via the somatomedins (Etherton, 1989).

## PERFORMANCE AND CARCASS DATA

The administration of rpST to finishing pigs improves performance and alters carcass composition. rpST increases average daily gain (ADG), improves feed efficiency (gain/feed) and depresses feed intake. Carcass composition of pigs administered rpST is altered toward that of a leaner animal and is partially observed in decreased backfat thickness and increased loin eye area (LEA) while total carcass muscle is

increased and total carcass adipose tissue decreased. The data summarized in Table 4 represents a summary of 18 studies involving finishing barrows with an initial weight of no less than 40 kg, fed a diet of greater than 14% crude protein, and receiving various doses of pST. The numbers in parentheses represent the number of studies the data include.

## TABLE 4 PERCENT IMPROVEMENT IN CARCASS AND PERFORMANCE VALUES WITH rpST

**PERCENT IMPROVEMENT**- EXPRESSED AS A PERCENT OF CONTROL

| | MIN. | MAX. |
|---|---|---|
| AVERAGE DAILY GAIN, kg (17) | +105 | +122 |
| FEED/GAIN (16) | -3 | -42 |
| FEED INTAKE, kg (9) | -5 | -23 |
| LOIN EYE AREA, cm (13) | +106 | +132 |
| BACKFAT, cm (12) | -4 | -47 |
| % LIPID (4) | -18 | -68 |
| % PROTEIN (6) | +108 | +135 |
| ADIPOSE TISSUE, kg (5) | -8 | -66 |
| MUSCLE, kg (4) | +106 | +126 |

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## METHODS AND MATERIALS

A 2 x 2 factorial design was utilized with thirty-two crossbred barrows (Hampshire, Landrace, Yorkshire) to determine effects of rpST and dietary crude protein concentration on lipid metabolism in finishing pigs from November 17, 1989 - December 13, 1989. Initially thirty-six barrows with an average weight of 70 kg were fed a 14% crude protein corn/soy bean meal diet balanced to NRC requirements for vitamins and minerals for an eleven day period at the Michigan State University Swine Facilities. During this initial eleven day period general performance was evaluated. On day twelve, thirty-two barrows that exhibited good health were selected and allocated to eight pens of four pigs each according to the guidelines of Professor J. Gill, Department of Animal Science Michigan State University (Statistical aspects of design and analysis of experiments with animals in pens). Two blocks of four pens each were formed so that pigs within a given block were relatively uniform by body weight establishing a heavy and a light block. The average initial body weight for the heavy and light blocks

were 81.7 and 66.8 kg, respectively. Pigs in each block were assigned to pens to minimize differences among pens not within pens. Barrows from block one were randomly assigned to pens # 1-4, while barrows from block two were randomly assigned to pens # 5-8.

Four treatments were randomly assigned to the four pens of each block and are presented in Table 5. Pigs were housed in a pole barn type building in pens. Pens were constructed of wooden sides and a concrete floor. Straw was used as bedding. Pens were cleaned daily and clean straw laid down. Daily temperature was recorded at time of morning injections. The building also had a supplemental heat source. Building temperature ranged from $12^0$ to $18^0$C throughout the experimental period.

Pigs were fed a typical corn/soy diet (Table 6) ad libitum from an upright two bowl self feeder. Each pen was equipped with one nipple waterer. Feed level and waterers were checked twice daily to ensure adequate availability of nutrients. Feed offered and orts were recorded daily and intake and feed efficiencies calculated weekly.

Pigs were weighed weekly. New animal weights were used to

**TABLE 5 Treatments**

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0 ug/kg/d rpST, 14 % crude protein, 8 barrows

50 ug/kg/d rpST, 14 % crude protein, 8 barrows

0 ug/kg/d rpST, 20 % crude protein, 8 barrows

50 ug/kg/d rpST, 20 % crude protein, 8 barrows

**TABLE 6 Composition of Diets**

| Ingredients | 14% CP | 20% CP |
|---|---|---|
| | (% of dry matter) | |
| Corn | 80.75 | 63.75 |
| Soybean meal (44% CP) | 16.00 | 33.00 |
| Mono-di-calcium phosphate | 1.00 | 1.00 |
| Calcium carbonate | 1.00 | 1.00 |
| NaCl | 0.25 | 0.25 |
| Vitamin/trace mineral premix | 1.00 | 1.00 |
| **Calculated Analysis** | | |
| Crude protein | 14.00 | 20.00 |
| L-lysine | 0.65 | 1.10 |
| ME (kcal/kg) | 3175.00 | 3157.00 |

Premix provided per kg of diet: Vitamin A, 3,300 IU; Vitamin D3, 600 IU; Vitamin E, 15 IU; Riboflavin, 3.3 mg; Nicotinic acid, 17.6 mg; d-Pantothenic acid, 13.2 mg; Choline, 110 mg; Vitamin B12, 19.8 ug; Zn, 74.8 mg; Fe, 9.4 mg; Mn, 37.4 mg; Cu, 9.9 mg; I, 0.5 mg; Se, 0.1 mg.

adjust rpST dose administered for the following week. rpST injection solution was prepared according to the manufacturer (The Upjohn Company, Kalamazoo, MI) as follows: rpST was reconstituted daily in .03 $NaHCO_3$ + .15 M NaCl at pH 9.5. Pigs were injected intramuscularly on the right side of the neck with either 50 ug/kg body weight rpST or 2 ml vehicle daily at 0900 hours for 24 days. In order to remain within the slaughter/handling capacity of the Michigan State University meat laboratory, treatments were initiated over a four day period so that eight pigs could be slaughtered per day over a four day period. The four pigs in each pen were numbered with a livestock marker from #1-4 for identification of treatment initiation. One pig from each pen was slaughtered per day. Before slaughter, pigs were numbered 1-32 in order of treatment initiation, so that on day one pigs 1-8 were slaughtered and pigs 9-16 on day two etc. for all subsequent identification.

On day 25, 24 hours after the last injection, animals were weighed and transported to the Michigan State University Meat Laboratory and slaughtered. Animals were stunned with an electric current and exsanguinated. At the time of slaughter tissue samples were collected. The left side of the carcass was physically

dissected into separable soft tissues and bone. Several specific muscles and fat depots were weighed and subsampled for proximate analysis. Several bones and internal organs were weighed and length of the bones was also recorded. Twenty-four hours postmortem the right side of the carcass was graded and measurements of fat thickness, loin eye area taken, and muscle quality scored.

Adipose tissue was obtained from the middle subcutaneous (SC) layer over the left shoulder at slaughter from each pig for lipogenic and lipolytic assays and determination of composition; moisture, fat, protein, and fatty acid profile. The SC adipose tissue sample was immediately placed in Krebs-Ringer buffer, $37^0$ C, until determination of enzyme activity. Fatty acid synthesis was measured utilizing a tritiated water procedure of Mulvaney et al. (1984) (appendix C) while malic enzyme activity was measured according to the procedure of Ochoa (1955) (appendix A). Lipoprotein lipase activity was determined by a stable radioactive substrate emulsion according to Nilsson-Ehle and Schotz (1976) (appendix B) and glycerol release determined through the use of a Boehringer Mannheim Glycerol Test Kit.

Additional samples of subcutaneous and intermuscular adipose tissue were taken from the left side and frozen immediately in liquid nitrogen after sampling and stored at -80$^0$ C until determination of percent moisture, fat and protein and compilation of a fatty acid profile of the subcutaneous adipose tissue. Intramuscular adipose tissue was sampled from two pigs of each treatment and frozen immediately in liquid nitrogen after sampling and stored at -80$^0$ C until determination of percent moisture, fat and protein. Before analysis, samples were powdered with $CO_{2(s)}$. Percent protein was determined by the Kjeldahl method while percent fat was determined by petroleum ether extraction of neutral lipids using the Soxhlet method. Fatty acid profiles were compiled according to the dry column class separation of lipids procedure of Marmer and Maxwell (1979) (appendix E) and gas liquid chromatography. Weights of the dissected subcutaneous and leaf fat was taken for each animal. Backfat measurements were also recorded for each animal at the first rib, last rib, and last lumbar vertebra and average backfat calculated. Backfat thickness was also measured at the tenth and last rib.

Weights of specific muscles include the longissimus dorsi and

semitendinosus muscles. The longissimus dorsi muscle was assayed for DNA and RNA content according to the procedure of Munro and Fleck (1969) modified in our lab (Bates, Gillett, Barao, and Bergen, 1985) (appendix D). The fatty acid profile was also determined for the intramuscular adipose tissue of the longissimus muscle (Marmer and Maxwell, 1983) (appendix E).

## STATISTICAL ANALYSIS

All data were statistically analyzed by the General Linear Models Procedure of SAS (1987). A randomized complete block design was used in the experimental analysis. The effects of block, dietary crude protein concentration, rpST, and the interactions were tested using variation among pens (block x protein x growth hormone) as the error term. Variation among pens was pooled with variation among pigs used as the error term in cases in which the F statistic for variation among pens was less than 2 x $F_{alpha0.05}$ due to the low number of pens per treatment and degrees of freedom for the error term according to Gill (1989). There is, however, the potential for bias from pooling of sum of squares and corresponding degrees of freedom for pens and animals. In cases where it was

necessary to contrast means, Bonferonni t-tests were used for the analysis as outlined by Gill (1978).

# RESULTS and DISCUSSION

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON LIPID ACCRETION

Administration of 50 ug/kg/day rpST reduced percent carcass fat ($P<.01$), dissectible subcutaneous adipose tissue ($P<.01$) and intrafasicular adipose tissue ($P<.05$) but did not affect either leaf fat or intermuscular fat (Table 7). In growing pigs, an appreciable amount of lipid is deposited daily in adipose tissue (approximately 180 to 280 g/d for pigs between 50 and 90 kg) (Etherton, 1989). The administration of rpST to pigs decreases rates of lipid accretion and adipose tissue mass by as much as 80% (Boyd, R.D., 1988). Administration of rpST decreases backfat thickness (Campbell et al. 1988, 1989); however, in this study average backfat thickness, first rib, and last lumbar backfat were unaffected by administration of 50 ug rpST/kg BW, while tenth rib and last rib back fat were decreased ($P<.01$) by rpST (Table 8). Administration of rpST to pigs affects the repartitioning of nutrients influencing composition of growth. rpST alters nutrient partitioning in support of increased protein accretion

TABLE 7 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON WEIGHTS OF SPECIFIC ADIPOSE TISSUE DEPOTS IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| dissectible SC AT (kg) | 8.82 | 6.81[d] | 8.37 | 6.31[d] |
| | (.43) | (.40) | (.73) | (.62) |
| intrafasicular AT (kg) | 2.40 | 1.37[c] | 1.64 | 1.38[c] |
| | (.33) | (.24) | (.17) | (.24) |
| intermuscular AT (kg) | 2.46 | 2.24 | 3.62 | 1.78 |
| | (.64) | (.51) | (.48) | (.46) |
| perirenal AT (kg) | 1.20 | 1.04 | 1.27 | .96 |
| | (95.71) | (98.43) | (163.24) | (121.36) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.05 compared with 14 and 20% crude protein controls.
d= P<.01 compared with 14 and 20% crude protein controls.

**TABLE 8 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON SPECIFIC MEASUREMENTS OF BACKFAT THICKNESS IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| average backfat (cm) | 1.20 | 1.06 | 1.14 | 1.08 |
| | (.06) | (.07) | (.07) | (.06) |
| tenth rib backfat (cm) | 1.19 | .82[c] | 1.15 | .83[c] |
| | (.11) | (.04) | (.13) | (.06) |
| last rib backfat (cm) | .95 | .66 | .84 | .61 |
| | (.12) | (.09) | (.08) | (.07) |
| first rib backfat (cm) | .69 | .58 | .69 | .61 |
| | (.04) | (.06) | (.07) | (.06) |
| last lumbar backfat (cm) | 1.27 | 1.24[c] | 1.24 | 1.11[c] |
| | (.07) | (.10) | (.12) | (.10) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 compared to 14 and 20% crude protein controls.

and decreased lipid accretion.50 ug rpST/kg BW/day, altered the composition of adipose tissue depots and decreased percent ether extractable lipid in intramuscular, subcutaneous, and intramuscular adipose tissue depots ($P<.01$) and increased percent protein ($P<.05$) and water ($P<.01$) (Table 9,10,11).

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON LIPOGENESIS AND LIPOLYSIS

No interactions of rpST and dietary CP concentration were observed for either measurement of lipogenesis. Both malic enzyme activity and fatty acid synthesis were depressed by the high protein diet ($P<.05$) (Table 12). rpST decreased lipid accretion by decreasing activity of key lipogenic enzymes. Consistent with previous results shown in Table 1 (Magri et al. 1985, Harris et al. 1990), administration of rpST depressed malic enzyme activity ($P<.01$). Although a lipolytic response to ST has been documented (Roupas et al. 1991), it has become clear that in domestic animals in positive energy balance, the lipolytic effects of ST are minor and do not account for the decrease observed in lipid accretion. This is in agreement with my results which indicate that lipolysis was not

TABLE 9 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON THE PERCENTAGE OF MOISTURE, ETHER EXTRACT, AND PROTEIN FOR INTRAFASICULAR ADIPOSE TISSUE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| intrafasicular AT % moisture | 44.57 (1.42) | 53.16[c] (.85) | 47.69 (.23) | 55.99[c] (.80) |
| intrafasicular AT % ether extractable lipid | 33.83 (.87) | 30.42[c] (.40) | 36.32 (.58) | 23.00[c] (1.25) |
| intrafasicular AT % protein | 13.71 (.93) | 16.84[d] (.12) | 16.68 (.12) | 18.03[d] (.28) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 compared to 14 and 20% crude protein controls.
d= P<.05 compared to 14 and 20% crude protein controls.

TABLE 10 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON THE PERCENTAGE OF MOISTURE, ETHER EXTRACT, AND PROTEIN FOR SUBCUTANEOUS ADIPOSE TISSUE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| SC AT % moisture | 13.41 (1.21) | 15.65[c] (.73) | 12.07 (.76) | 16.56[c] (1.31) |
| SC AT % ether extractable lipid | 83.40 (1.96) | 81.86[c] (.86) | 85.30 (.82) | 79.93[c] (1.37) |
| SC AT % protein | 2.37 (.11) | 2.55[d] (.29) | 2.12 (.13) | 2.86[d] (.29) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 compared to 14 and 20% crude protein controls.
d= P<.05 compared to 14 and 20% crude protein controls.

TABLE 11 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON THE PERCENTAGE OF MOISTURE, ETHER EXTRACT, AND PROTEIN FOR INTERMUSCULAR ADIPOSE TISSUE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| intermuscular AT % moisture | 19.96 (.82) | 23.10[c] (1.05) | 19.24 (1.27) | 28.20[c] (1.97) |
| intermuscular AT % ether extractable lipid | 76.28 (1.04) | 72.73[c] (1.33) | 76.32 (1.57) | 66.60[c] (2.41) |
| intermuscular AT % protein | 3.68 (.24) | 3.86[d] (.22) | 4.01 (.54) | 4.85[d] (.44) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 compared to 14 and 20% crude protein controls.
d= P<.05 compared to 14 and 20% crude protein controls.

**TABLE 12 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON MEASURES OF LIPOGENESIS and LIPOLYSIS FOR THE MIDDLE SUBCUTANEOUS ADIPOSE TISSUE OF FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| **fatty acid synthesis** (nmole $^3H_20$ incorporated/min/g) | 1.03 (.17) | .89 (.12) | .85[c] (.10) | .52[c] (.09) |
| **malic enzyme** (nmole malic acid oxidized/mg protein/min) | 405.85 (68.69) | 206.24[d] (37.65) | 317.25[c] (57.75) | 113.65[c,d] (25.98) |
| **lipoprotein lipase** (nmole FA liberated per mg protein/hr) | 686.13 (132.53) | 784.38 (110.33) | 755.63 (114.78) | 606.25 (139.72) |
| **basal glycerol release** (mg glycerol released /mg protein) | 16.65 (1.54) | 20.38 (1.42) | 17.90 (1.80) | 15.75 (1.07) |
| **epinephrine stimulated glycerol release** (mg glycerol released/mg protein) | 91.39 (13.05) | 103.81 (11.75) | 97.97 (11.88) | 89.62 (10.46) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.05 compared to control and rpST 14% crude protein.
d= P<.01 compared with 14 and 20% crude protein controls.

affected by rpST which may be due, in part, to the fact that the pigs were allowed ad lib access to feed during the twenyty-four rpST withdrawl period.

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON FATTY ACID PROFILES

No treatment effects were observed for subcutaneous (SC) adipose tissue neutral lipid (NL) fraction (table 13). However, C20:0 decreased with increasing dietary crude protein concentration ($P<.01$) plus rpST ($P<.05$) and C22:5 w3 increased with rpST ($P<.05$) (Table 14) in the SC adipose tissue phospholipid (PL) fraction. C16:0 and C18:0 decreased ($P<.05$) in the LD NL while C22:5 w3 increased with rpST (Table 15). C16:0 was decreased ($P<.05$) in the LD PL fraction by rpST, while C22:4 was increased in the high protein treatments (Table 16). A slight increase in percent unsaturated fatty acids was observed for LD intramuscular adipose tissue. It appears that SC adipose tissue may be less responsive to the modifying effects of rpST than LD intramuscular adipose tissue in altering the fatty acid profile as intramuscular adipose tissue is later maturing and may be influenced to a greater degree than

**TABLE 13 PORCINE SUBCUTANEOUS ADIPOSE TISSUE NEUTRAL LIPID FATTY ACID PROFILES[a,b]**

| FATTY ACID | CO14 | rpST14 | CO20 | rpST20 |
|---|---|---|---|---|
| 14:0 | 1.30+/-.08 | 1.26+/-.16 | 1.47+/-.08 | 1.26+/-.31 |
| 16:0 | 25.87+/-.39 | 25.36+/-.07 | 25.38+/-.02 | 25.34+/-.1 |
| 16:1 | 3.03+/-.01 | 2.77+/-.06 | 3.11+/-.48 | 2.80+/-.11 |
| 18:0 | 12.24+/-.40 | 13.61+/-.93 | 11.97+/-.69 | 13.2+/-.01 |
| 18:1 | 43.47+/-.04 | 42.68+/-.01 | 42.53+/-.07 | 43.1+/-.44 |
| 18:2 | 10.97+/-.16 | 10.99+/-.61 | 10.60+/-1.0 | 10.4+/-.14 |
| 18:3 ω6 | .28+/-.02 | .29+/-.07 | .36+/-.01 | .29+/-.03 |
| 18:3 ω3 | 1.55+/-.22 | 1.35+/-.11 | 1.51+/-.23 | 1.57+/-.77 |
| 20:4 | .22+/-.66 | .24+/-.02 | .22+/-.04 | .23+/-.02 |
| >20:4 | .20+/-.07 | .14+/-.03 | .28+/-.13 | .23+/-.07 |

a= treatment means followed by standard errors
b= data are presented as percent distribution

**TABLE 14 PORCINE SUBCUTANEOUS ADIPOSE TISSUE PHOSPHOLIPID FATTY ACID PROFILES[a,b]**

| FATTY ACID | CO14 | rpST14 | CO20 | rpST20 |
|---|---|---|---|---|
| 14:0 | 0+/-0 | .44+/-1.1 | .38+/-.13 | .09+/-.54 |
| 16:0 | 22.07+/-.2 | 19.09+/-1.4 | 20.04+/-0 | 19.1+/-.14 |
| 16:0 | 2.22+/-.10 | 1.86+/-.13 | 2.41+/-1.71 | 2.60+/-.21 |
| 18:0 | 12.47+/-.98 | 12.78+/-1.33 | 12.31+/-.95 | 12+/-.02 |
| 18:1 | 40.12+/-.90 | 38.96+/-.23 | 33.73+/-.16 | 38.4+/-.95 |
| 18:2 | 12.57+/-.30 | 11.03+/-.55 | 8.64+/-4.6 | 9.95+/-1.2 |
| 18:3 ω 6 | 0+/0 | 0+/-0 | .02+/-.13 | .09+/-.06 |
| 18:3 ω 3 | 3.09+/-1.66 | 2.00+/-.33 | 3.48+/-.61 | 2.65+/-2.2 |
| 20:4 | .86+/-.90 | .87+/-05 | .56+/-.27 | .67+/-.04 |
| >20:4 | 1.18+/-.24 | .98+/-.43 | 1.32+/-.30 | 1.63+/-.43 |

a= treatment means followed by standard errors.
b= data presented as percent distribution.

TABLE 15 PORCINE LONGISSIMUS DORSI MUSCLE NEUTRAL LIPID FATTY ACID PROFILES[a,c]

| FATTY ACID | CO14 | rpST14 | CO20 | rpST20 |
|---|---|---|---|---|
| 14:0 | 1.35+/-.07 | 2.30+/-.35 | 1.70+/-.07 | 1.54+/-.53 |
| 16:0 | 25.67+/-.51 | 25.24+/-.51[b] | 29.05+/-.03 | 24.93+/-.09[b] |
| 16:1 | 4.04+/-.06 | 4.33+/-.67 | 5.17+/-.18 | 4.06+/-.05 |
| 18:0 | 12.73+/-.13 | 11.55+/-.71[b] | 13.13+/-.19 | 11.49+/-.09[b] |
| 18:1 | 47.20+/-.71 | 48.18+/-.02 | 41.70+/-.13 | 45.75+/-1.2 |
| 18:2 | 3.77+/-.42 | 4.49+/-.25 | 4.15+/- 2.14 | 4.95+/-.22 |
| 18:3 $\omega$ 6 | .14+/-.03 | .36+/-.14 | .14+/-.06 | .23+/-.02 |
| 18:3 $\omega$ 3 | 1.16+/-.18 | 2.34+/-.12 | 1.28+/-.40 | 1.46+/-1.09 |
| 20:4 | .29+/-.20 | .37+/-.07 | .23+/-.08 | .22+/-.03 |
| >20:4 | .40+/-.11 | .58+/-.26 | .36+/-.12 | .58+/-.24 |

a= treatment means followed by standard errors.
b= P<.05 as compared to 14 and 20% crude protein controls.
c= data are presented as percent distribution.

**TABLE 16 PORCINE LONGISSIMUS DORSI MUSCLE PHOSPHOLIPID FATTY ACID PROFILES[a,c]**

| FATTY ACID | CO 14 | rpST14 | CO20 | rpST20 |
|---|---|---|---|---|
| 14:0. | 48+/-.16 | .28+/-.85 | .36+/-.36 | .76+/-.65 |
| 16:0 | 16.67+/-3.5 | 12.66+/-4.4[b] | 17.71+/-.06 | 13.32+/-.2[b] |
| 16:1 | 2.70+/-1.06 | 1.80+/-.14 | 2.60+/-.29 | 2.94+/-.27 |
| 18:0 | 8.81+/-.68 | 7.05+/-2.86 | 8.46+/.96 | 7.88+/-.03 |
| 18:1 | 27.81+/-.26 | 27.03+/-.55 | 24.85+/-.17 | 23.1+/-1.1 |
| 18:2 | 21.24+/-.35 | 21.40+/-.46 | 26.50+/-3.0 | 18.1+/-3.1 |
| 18:3 $\omega$6 | .15+/-.10 | .23+/-.08 | .10+/-.77 | .40+/-.34 |
| 18:3 $\omega$3 | 2.01+/-1.12 | 1.51+/-.54 | 1.37+/-.57 | 1.54+/-1.8 |
| 20:4 | 8.18+/-1.3 | 19.39+/-.06 | 7.76+/-.53 | 11.2+/-.53 |
| >20:4 | 1.38+/-.37 | 2.07+/-.54 | 1.09+/-.28 | 2.28+/-.48 |

a= treatment means followed by standard errors.
b= P<.05 as compared to 14 and 20% crude protein controls.
c= data are presented as percent distribution.

earlier maturing depots. The effect of rpST and level of dietary crude protein on percentage saturated and unsaturated fatty acids in subcutaneous adipose tissue and intramuscular adipose tissue of the longissimus dorsi muscle is reported in Table17. Previous research indicates that administration of rpST to pigs slightly alters the fatty acid profile of adipose tissue (Prusa et al., 1989; Wander et al., 1991). Modification of the fatty acid profile may be occurring because lipogenesis is depressed in ST treated animals as evidenced by decreases in key lipogenic enzymes and that any incorporation of fatty acids into the adipose at that time will be due to the direct esterification of the dietary fatty acids into the adipose tissue. Under normal circumstances, a typical swine corn/soybean meal diet has a relatively low fat content (approximately 3% of the calories) however the mere 3% may not be sufficient to observe a significant alteration in the fatty acid profile of the adipose tissue. It would be interesting to test the effect of rpST and the addition of a lipid source on the fatty acid profile of pig adipose tissue. It is possible that enrichment of the adipose tissue with dietary fatty acids may occur to a greater extent when pigs are fed a diet of a greater

TABLE 17 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON PERCENTAGE SATURATED AND UNSATURATED FATTY ACIDS IN SUBCUTANEOUS ADIPOSE TISSUE AND INTRAMUSCULAR ADIPOSE TISSUE OF THE LONGISSIMUS DORSI MUSCLE IN FINISHING PIGS[a]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| SC NL % SATURATED FATTY ACIDS | 13.14 | 13.41 | 12.94 | 13.27 |
| SC NL % UNSATURATED FATTY ACIDS | 8.53 | 8.35 | 8.37 | 8.37 |
| SC PL % SATURATED FATTY ACIDS | 11.51 | 10.77 | 10.91 | 10.39 |
| SC PL % UNSATURATED FATTY ACIDS | 8.58 | 10.42 | 7.17 | 8.00 |
| LD NL % SATURATED FATTY ACIDS | 13.25 | 13.03 | 14.63 | 12.65 |
| LD NL % UNSATURATED FATTY ACIDS | 8.14 | 8.66 | 7.58 | 8.18 |
| LD PL % SATURATED FATTY ACIDS | 8.65 | 6.66 | 8.84 | 7.32 |
| LD PL % UNSATURATED FATTY ACIDS | 9.07 | 9.06 | 9.18 | 8.51 |

a= data represent treatment means

proportion of lipid and administered rpST. Appendix F is a list of names and numerical equivalents of common fatty acids.

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON NUCLEIC ACIDS

pST increases transcription and synthesis of RNA, and total tissue DNA in responsive tissues (Evock et al. 1991, Kostyo 1973, Beach and Kostyo 1967, and Cheek et al. 1969). In the present study, rpST and the high protein diet increased LD RNA concentration ($P<.01$) (Table 18) but had no effect on LD DNA concentration. The LD RNA:DNA ratio was unaffected by dietary crude protein concentration and rpST indicating no differences in the protein synthetic capacity of the LD. Protein:RNA ratio (Table 18) is lower in pigs administered rpST which may indicate a lower efficiency of protein synthesis in the LD. It may well be that the effect of rpST on metabolism in the LD is manifested in the form of increased transcription followed by increased translation in studies of a longer treatment period (C. Rehfeldt and K. Ender, personal communication). Along similar lines total DNA in the LD was unaffected while rpST increased total RNA of the LD at 24 days. The high protein diet increased the LD

TABLE 18 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON NUCLEIC ACIDS AND RESPECTIVE RATIOS OF THE LONGISSIMUS DORSI (LD) MUSCLE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| LD WEIGHT (kg) | 1.90 | 1.91 | 1.86 | 1.92 |
| | (.09) | (.06) | (.08) | (.08) |
| DNA (mg/g) | .36 | .40 | .33 | .30 |
| | (.02) | (.04) | (.04) | (.04) |
| RNA (mg/g) | 1.50 | 1.69[c] | 1.29[d] | 1.52[c,d] |
| | (.04) | (.09) | (.06) | (.04) |
| DNA/LD (mg/tissue) | 674.77 | 765.75 | 617.49 | 573.68 |
| | (49.36) | (76.92) | (74.31) | (72.16) |
| RNA/LD (g/tissue) | 2.83 | 3.24[e] | 2.45 | 2.94[e] |
| | (1.1) | (2.3) | (1.5) | (1.9) |
| RNA:DNA | 4.31 | 4.48[c] | 4.34[d] | 5.50[c,d] |
| | (.28) | (.45) | (.51) | (.60) |
| PROTEIN:RNA | 282.29 | 241.39[c] | 333.19[d] | 278.74[c,d] |
| | (19.52) | (12.49) | (15.24) | (10.46) |
| PROTEIN:DNA | 1218.28 | 1080.94 | 1455.86[f] | 1534.43[f] |
| | (120.56) | (124.66) | (202.61) | (177.96) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.
d= P<.01 as compared to control and rpST 14% crude protein.
e= P<.05 as compared to 14 and 20% crude protein controls.
f= P<.05 as compared to control and rpST 14% crude protein.

protein:DNA ratio (P<.05) (Table 18). Administration of rpST and the high protein diet elevated liver DNA (P<.03, P<.01) (Table 19) while rpST alone increased liver RNA (P<.01) (Table 19). The liver RNA:DNA ratio increased with the high protein diet (P<.01) (Table 19). Unlike the LD, liver grows mainly as a result of hyperplasia, although hypertrophic growth also occurs, and is indicated by a greater DNA concentration per liver (P<.05). rpST increased liver total DNA (P<.01) and RNA concentration (P<.01) indicating a greater cell number and greater protein synthetic capacity.

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON MEAT QUALITY

The decrease in percent carcass adipose tissue was accompanied by a decrease in intramuscular adipose tissue of the LD muscle (Table 7). A decrease in intramuscular adipose tissue may influence meat quality. A minimum intramuscular adipose tissue concentration may be required for acceptable meat quality (Beermann, 1990). Muscle tissue quality was evaluated using the longissimus dorsi muscle. Quality, as determined by an evaluation of marbling, color, and wateriness/firmness was not affected by

**TABLE 19 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON NUCLEIC ACIDS AND RESPECTIVE RATIOS OF THE LIVER IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| LIVER WEIGHT (kg) | 1.58 | 1.91[c] | 1.76 | 1.99[c] |
| | (.06) | (.08) | (.06) | (1.12) |
| DNA (mg/g) | 1.56 | 1.77[e] | 1.29[d] | 1.45[d,e] |
| | (.10) | (.13) | (.03) | (.10) |
| RNA (mg/g) | 8.07 | 8.53[c] | 7.33 | 8.95[c] |
| | (.16) | (.30) | (.31) | (.41) |
| DNA/LIVER (g/tissue) | 2.41 | 3.32[c] | 2.21 | 2.89[c] |
| | (.16) | (.21) | (.09) | (.26) |
| RNA/LIVER (g/tissue) | 12.56 | 16.10[c] | 12.87 | 17.78[c] |
| | (.73) | (.57) | (.63) | (1.2) |
| RNA:DNA | 5.33 | 4.97 | 5.87[d] | 6.27[d] |
| | (.41) | (.33) | (.34) | (.34) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.
d= P<.01 as compared to control and rpST 14% crude protein.
e= P<.05 as compared to 14 and 20% crude protein controls.

rpST or by dietary protein concentration (Table 20). Few studies have addressed the color of rpST treated muscle compared to control muscles. Kanis et al. (1988) report that the LD muscle of ST treated pigs was lighter in color while Prusa (1988e) reports no difference in the color of broiled or roasted semimembranosus muscle. Consistent with results of color, the waterines/firmness score has been reported to show few differences between ST treated animals versus control animals (Prusa 1988e, Beermann 1988, Novakofski 1987, and Evok 1988).

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON SPECIFIC ORGANS

The modifying effects of rpST are not restricted to skeletal muscle and adipose tissue but are involved in whole body metabolism. Increases in specific organ weights have been reported for pigs administered pST (Evock et al. 1991, Chung et al. 1985, and Kraft et al. 1985). In agreement with previous data, heart, liver, and kidney weights were heavier in rpST treated animals ($P<.01$) (Table 21). Increasing dietary CP concentration increased kidney weight ($P<.01$) (Table 21) which may be partly due to the handling by the

TABLE 20 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON MEAT QUALITY [a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| MARBLING | 5.38 | 3.88 | 6.00 | 5.88 |
| | (1.12) | (.69) | (1.19) | (1.09) |
| COLOR | 6.13 | 7.50 | 7.63 | 6.25 |
| | (.95) | (.46) | (.50) | (1.05) |
| FIRMNESS/WATERINESS | 6.25 | 6.50 | 7.50 | 7.00 |
| | (.96) | (1.15) | (.76) | (.90) |

a= data represent treatment means.
b= standard errors are in parentheses.

TABLE 21 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON WEIGHTS OF SPECIFIC ORGANS IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| heart weight (g) | 380.51 | 382.21[c] | 338.86 | 428.73[c] |
| | (25.65) | (25.46) | (12.50) | (23.19) |
| liver weight (kg) | 1.58 | 1.91[c] | 1.76 | 1.99[c] |
| | (.06) | (.08) | (.06) | (1.12) |
| kidney weight (g) | 330.25 | 403.31[c] | 390.9[d] | 465.33[c,d] |
| | (19.49) | (13.61) | (13.73) | (15.59) |
| total skin weight (kg) | 9.99 | 9.75 | 9.45 | 10.36 |
| | (3.32) | (5.20) | (3.33) | (3.40) |
| total bone weight (kg) | 3.62 | 3.74 | 3.52 | 3.67 |
| | (.06) | (1.39) | (1.44) | (1.70) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 compared to 14 and 20% crude protein controls.
d= P<.01 compared to control and rpST 14% crude protein.

kidney of excess nitrogenous waste or a response by the kidney to the administration of rpST and increasing dietary crude protein concentration. Unlike other organs examined, skin weight was not affected. Previous experiments reported increased growth of specific long bones in response to ST, however, 50 ug rpST/kg BW/day or high protein diet for 24 days had not affected total bone weight nor lengths or weights of individual long bones (Table 22,23).

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON MUSCLE

Much of the increased protein accretion observed upon the administration of rpST is represented by muscle tissue accretion. Percent carcass muscle was increased ($P<.01$) (Table 24) upon administration of rpST however, weight of fat-free muscle was not affected (Table 24). The discrepancy between percent carcass muscle and weight of fat-free muscle is due to the fact that the value reported for the fat-free muscle does not take into account the ether extractable lipid content. In concert with the increased muscle protein accretion weights of specific muscles have been

**TABLE 22 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON WEIGHTS OF SPECIFIC LONG BONES IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| femur weight (g) | 336.54 | 331.14 | 323.01 | 333.15 |
| | (6.49) | (10.16) | (10.70) | (11.59) |
| tibia weight (g) | 252.15 | 254.91 | 232.64 | 239.49 |
| | (6.92) | (9.20) | (6.38) | (9.89) |
| humerus weight (g) | 311.25 | 298.39 | 287.11 | 304.93 |
| | (7.13) | (10.18) | (10.19) | (12.66) |
| radius weight (g) | 234.14 | 231.50 | 224.45 | 234.45 |
| | (5.86) | (8.21) | (7.10) | (9.01) |

a= data represent treatment means.
b= standard errors are in parentheses.

**TABLE 23 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON LENGTH OF SPECIFIC LONG BONES IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| femur length (cm) | 19.81 | 19.56 | 19.64 | 19.46 |
| | (.18) | (.34) | (.27) | (.28) |
| tibia length (cm) | 18.73 | 18.21 | 17.96 | 18.10 |
| | (.28) | (.29) | (.27) | (.25) |
| humerus length (cm) | 17.19 | 17.21 | 16.89 | 17.19 |
| | (.25) | (.19) | (.40) | (.26) |
| radius length (cm) | 19.63 | 19.50 | 19.21 | 18.99 |
| | (.18) | (.29) | (.29) | (.26) |

a= data represent treatment means.
b= standard errors are in parentheses.

**TABLE 24 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON SELECTED CARCASS MEASURES IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| % CARCASS MUSCLE | 52.48 | 56.39[c] | 52.59 | 57.18[c] |
| | (.92) | (.57) | (1.25) | (1.68) |
| % CARCASS AT | 29.02 | 23.56[c] | 29.31 | 20.73[c] |
| | (1.07) | (1.29) | (1.49) | (1.48) |
| LOIN EYE AREA ($cm^2$) | 28.13 | 29.23 | 27.48 | 30.97 |
| | (1.29) | (.97) | (1.35) | (1.16) |
| FAT FREE MUSCLE (kg) | 41.92 | 40.46 | 40.92 | 46.72 |
| | (1.29) | (3.19) | (.96) | (1.72) |
| TOTAL SOFT TISSUE WEIGHT (kg) | 27.39 | 25.90 | 27.16 | 26.55 |
| | (1.17) | (.69) | (.91) | (1.06) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.

reported to increase as well (Boyd et al. 1986). Unlike previous reports, neither the semitendinosus nor the longissimus dorsi muscle weights were affected nor was loin eye area (Table 24,25). Consistent with the reported nutrient partitioning capabilities of rpST (Prusa, 1989), compositional analysis indicates that rpST reduced percent ether extractable lipid in the muscle composite ($P<.05$) and soft tissue ($P<.01$) samples while percent moisture and protein were increased, respectively, in the muscle composite ($P<.01$, $P<.05$), LD muscle ($P<.01$), and soft tissue ($P<.01$) (Table 26,27,28). The high protein diet elevated percent protein in the muscle composite ($P<.01$), LD ($P<.01$) and soft tissue ($P<.05$) samples (Table 26,27,28).

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON PERFORMANCE

50 ug rpST/kg BW/day increased average daily gain and improved feed efficiency (Table 29) in agreement with previous studies (Evock et al. 1991; Zimmerman, 1989). Average daily gain and feed efficiency are improved and are limited by a decreased feed intake at higher doses of rpST which hampers the ability of the pig

**TABLE 25 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON WEIGHT OF SPECIFIC MUSCLES IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| LONGISSIMUS DORSI WEIGHT (kg) | 1.90 (.09) | 1.91 (.06) | 1.86 (.08) | 1.92 (.08) |
| SEMITENDINOSUS WEIGHT (g) | 397.95 (16) | 392.53 (17.61) | 363.41 (13.78) | 418.68 (24.38) |

a= data represent treatment means.
b= standard errors are in parentheses.

TABLE 26 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON PERCENTAGE MOISTURE, ETHER EXTRACT, AND PROTEIN OF THE LONGISSIMUS DORSI MUSCLE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| LONGISSIMUS DORSI MUSCLE | | | | |
| % MOISTURE | 72.79 | 74.20[c] | 72.74 | 74.15[c] |
| | (.18) | (.28) | (.30) | (.18) |
| LONGISSIMUS DORSI MUSCLE | | | | |
| % ETHER EXTRACTABLE LIPID | | | | |
| | 3.44 | 2.65 | 3.25 | 2.19 |
| | (.52) | (.47) | (.52) | (.37) |
| LONGISSIMUS DORSI MUSCLE | | | | |
| % PROTEIN | 22.08 | 20.95[c] | 22.33[d] | 22.07[c,d] |
| | (.26) | (.27) | (.30) | (.27) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.
d= P<.01 as compared to control and rpST 14% crude protein.

TABLE 27 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON PERCENTAGE MOISTURE, ETHER EXTRACT, AND PROTEIN OF THE MUSCLE COMPOSITE SAMPLE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| **MUSCLE COMPOSITE** | | | | |
| **% MOISTURE** | 73.90 | 75.18[c] | 73.92 | 74.93[c] |
| | (.30) | (.23) | (.30) | (.30) |
| **MUSCLE COMPOSITE** | | | | |
| **% ETHER EXTRACTABLE LIPID** | | | | |
| | 3.38 | 2.39[e] | 2.60 | 2.17[e] |
| | (.49) | (.34) | (.24) | (.37) |
| **MUSCLE COMPOSITE** | | | | |
| **% PROTEIN** | 20.85 | 19.86[e] | 21.25[d] | 20.92[d,e] |
| | (.26) | (.30) | (.23) | (.25) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.
d= P<.01 as compared to control and rpST 14 % crude protein.
e= P<.05 as compared to 14 and 20% crude protein controls.

TABLE 28 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON PERCENTAGE MOISTURE, ETHER EXTRACT, AND PROTEIN OF SOFT TISSUE IN FINISHING PIGS[a,b]

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| SOFT TISSUE % MOISTURE | 54.45 | 59.63[c] | 53.84 | 61.89[c] |
| | (1.21) | (.73) | (.76) | (1.31) |
| SOFT TISSUE % ETHER EXTRACTABLE LIPID | 29.98 | 24.10[c] | 30.38 | 20.24[c] |
| | (1.96) | (.86) | (.82) | (1.37) |
| SOFT TISSUE % PROTEIN | 14.34 | 15.37[c] | 14.55[d] | 16.66[c,d] |
| | (.11) | (.29) | (.13) | (.29) |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.01 as compared to 14 and 20% crude protein controls.
d= P<.05 as compared to control and rpST 14% crude protein.

**TABLE 29 THE EFFECT of rpST and LEVEL OF DIETARY CRUDE PROTEIN ON PERFORMANCE IN FINISHING PIGS[a]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| GAIN/FEED RATIO | .21 | .31 | .20 | .32 |
| AVERAGE DAILY GAIN (kg/day) | .83 | .91 | .76 | .85 |
| FEED INTAKE (kg/group/24 days) | 750.38 | 562.25 | 740.50 | 502.63 |

a= data represent treatment means.

to grow at an accelerated rate depressing the response seen at lower levels of rpST where feed intake is not as much of a limiting factor. According to data reported by Boyd et al. (1989) average daily gain starts to decline at doses of rpST over 60 ug/kg/day with a concurrent steady decrease in feed intake and a levelling of feed efficiency.

## EFFECT OF rpST AND LEVEL OF DIETARY CRUDE PROTEIN ON SERUM LEVELS OF ST AND IGF-1

Consistent with reports of Etherton et al. (1987) and Owens et al. (1990), administration of rpST to pigs increased the serum concentrations of both somatotropin and IGF-1 ($P<.001$) (Table 30). The obvious rise in ST after exogenous administration of rpST has been hypothesized to be stimulating the release of the mediator of ST action, IGF-1, which has been reported to be synthesized primarily by the liver (Phillips et al. 1980, Herington et al. 1983). Long term administration of rpST to pigs has been reported to decrease the endogenous secretion of pST most likely due to the negative feedback on the hypothalamus by the exogenous ST and/or other factors (IGF-1).

**TABLE 30 THE EFFECT OF rpST and LEVEL OF DIETARY CRUDE PROTEIN ON SERUM LEVELS OF SOMATOTROPIN AND INSULIN LIKE GROWTH FACTOR-1 IN FINISHING PIGS[a,b]**

| | CO 14 | rpST 14 | CO 20 | rpST 20 |
|---|---|---|---|---|
| serum pST (ng/ml) | 4.38 | 64.75[c] | 3.83 | 74.93[c] |
| serum IGF-1 (ng/ml) | 198.6 | 308.9[c] | 209.8 | 418.9[c] |

a= data represent treatment means.
b= standard errors are in parentheses.
c= P<.001 compared to 14 and 20% crude protein controls.

# SUMMARY

The experimental administration of rpST to finishing pigs was found to be an effective tool in improving swine growth and carcass compositon when fed a diet of adequate nutrient concentration. Administration of 50 ug rpST/kg BW/day to finishing pigs fed a diet containing either 14 or 20% crude protein resulted in marked changes in swine growth and carcass composition.

The effect of rpST on lipid metabolism represents a substantial portion of the actions of rpST. Lipogenic capacity was depressed due to decreased activity in synthesis pathways and reductions in key lipogenic enzyme activities. Lipolysis was not affected. This finding may be a consequence of time period, dosage, or by the fact that animals were allowed ad lib access to feed during the twenty-four hour rpST withdrawl period before slaughter. Fatty acid profiles were altered slightly resulting in a greater percentage of unsaturated fatty acids relative to saturated fatty acids. Carcass composition was altered resulting in decreased % lipid, and greater % protein and water in tissues examined. Concurrent with the

decreased lipogenic capacity and change in carcass composition was a decreased % carcass fat.

A portion of the effect of rpST on metabolism was observed as a greater percent carcass muscle, however, individual weights of the longissimus dorsi and semitendinosus muscles were not affected. At this stage of development, an increase in muscle would be due to the recruitment of new satellite cells and subsequent hypertrophy of the muscle which has been shown to be the action of IGF-1.

The effects of rpST are not limited to fat and muscle but may also affect other tissues as well. rpST increased weights of heart, liver and kidney, however, skin weight was not affected. Of the tissues examined, an anabolic response to rpST was observed in the liver as evidenced by measures of DNA, RNA, and protein. Total bone weight and individual long bone weights and lengths were not affected by rpST or dietary crude protein concentration.

Although a response to most measurements was observed, the response was not as great as it could have been at a higher dosage. 50 ug rpST/kg BW/day is a dose reported by the manufacturer (The UpJohn Company, Kalamazoo, MI) to be one in which typical

performance values were optimized. Unlike previous results, average daily gain did not appear to be affected while feed efficiency appeared to be improved. A higher dose of rpST administered to finishing pigs, perhaps 80 ug rpST/kg BW/day, may be more effective in increasing lean while decreasing fat.

In conclusion, although the benefits of administering rpST to finishing pigs are known, use as a commercial product may lie years from now. A particular limitation is the fact that the product must be injected and not fed due to the nature of the hormone which would be quite a task on large swine production operations. In addition, the optimum dose administered and nutrient requirements of pigs administered rpST are not fully known. It appears that the optimal dosage is influenced by age, weight, and genotype of which the possible combinations are quite numerous. In terms of nutrition, altered patterns of tissue growth in pigs administered rpST may require different levels of specific nutrients in the diet to meet increased metabolic demands. To date, much work has been reported on dietary crude protein concentration and energy level both of which are necessities in the type of growth associated with the administration of rpST, however, very little work has been reported

in terms of vitamin and mineral requirements. It is clear that rpST increases carcass protein and decreases carcass lipid both of which represent the direction of change within the swine industry.

# APPENDIX A

## Appendix A

### Malic Enzyme Analysis
(Ochoa, S., 1955)

This procedure is for adipose tissue.

Procedure:

1. One gram of adipose tissue is homogenized in 10 ml of .15 M KCL containing 5 mM mercaptoethanol.
   - For best results, dice the adipose tissue.
   - Small particles may be present in the homogenate; do not worry about them because it is probably connective tissue.

2. Place the sample in a cetrifuge tube and centrifuge for 20 minutes at 20,000 x g (zero degrees).
   - The resulting supernatant is the clear layer just below the fat cake.
   - This fraction is used for assaying the enzyme activity and total protein.
   - Decant this fraction into the appropriate labelled tube.

   *** NOTE: KEEP THE SAMPLE ON ICE !**

3. Add the following reagents to a 3 ml cuvette:
   a. .5 ml .05 M $MnCl_2$
   b. .5 ml .25 M Glycylglycine buffer
   c. .5 ml .0005 M NADP
   d. and either .5 ml water
      .2 ml water and .3 ml supernatant.
      .3 ml water and .2 ml supernatant.
      .4 ml water and .1 ml supernatant.

4. Blank the machine with the samples.

5. Add 1.0 ml .0011 M L-Malic acid to the cuvette. This starts the reaction.
   * The final volume of the solutions is 3 ml.

   The reaction contains:
   125 umoles glycylglycine buffer
   0.25 umoles NADP
   25 umoles $MnCL_2$
   1.1 umoles Malic acid

6. Using a recovering spectrometer at 340 nm and temperature at 25 degrees Celsius.

7. Read the change in absorbance over 3 to 5 minutes.

SOLUTIONS

1. .25 M glycylglycine buffer
   Dissolve 1.65 g of glycylglycine in 30 ml of water.

   The solution (pH 5.3) is adjusted to 7.4 by dropwise addition of 2 N NaOH and volume is made up with water to 50 ml.

2. .05 M $MnCL_2$

3. .0005 M NADP

4. .0011 M L-MALIC ACID

5. .15 M KCL containing 5 mM Mercaptoethanol

Using the above solutions add .5 ml of each solution to the cuvette.

REACTION:

NADP--------->NADPH

Absorbance is set at 340 nm

*Lowry's are run on the supernatant to determine the protein concentration and in order to put the data on a per mg of protein basis.

REAGENTS:

1. Glycylglycine buffer (.25 M) at pH 7.4
   -the molecular weight is 132
   -33 g/l

   The buffer is prepared by dissolving 1.65 g (33 g in 600 ml) in 30 ml of water.

   The solution (pH 5.3) is adjusted to ph 7.4 by dropwise addition of 2.0 N NaOH (about 1.15 ml) and is brought to volume with water.

   The final volume is 50 ml (1,000 ml).

2. $MnCL_2$ (.05 M)
   -the molecular weight is 197.91 ($MnCL_2$ $H_2O$)
   -9.9 g/l

3. NADP (.0005 M)
   -the molecular weight is 758.40684
   -.38 g/l

   Actually, 9.5 mg/25 ml or 3.8 mg/10 ml

4. L-Malic Acid (.0011 M)
   -the molecular weight is 156.1
   -172 g/l

5. Mercaptoethanol (.005 M)
   -the molecular weight is 78.13352
   -.39 g/l
   -density = 1.1 thus 1/1.1 = x/.39 x = .35 ml

6. KCL
   -the molecular weight is 74.555
   -11.25 g/l

FOR ENZYME PREP**

0.35 ml Mercaptoethanol
11.25 ml KCL
1,000 ml $H_2O$

**CALCULATION:**

$$\text{umoles/ml} = \frac{E \times V}{10^{-6} \times e \times d \times v}$$

E = measured optical density change
V = volume of the solution in the cuvette
v = volume of the sample taken for determination
e = extinction coefficient of light absorbing substrate (NADPH)
d = light path of the cuvette

**the final basis: nmoles of substrate used/minute/mg protein

# APPENDIX B

## Appendix B

### Lipoprotein Lipase Assay
(Nilsson, P. and Schotz, M.C., 1976)

1. Isolate adipose tissue sample.
2. Place sample in ice cold 0.9% saline solution.
3. Cut the sample into .3 mm slices.
4. Homogenize the individual slices in 3 ml of cold homogenization buffer using a Brinkman polytron (3-20 second bursts/minute) at a setting of 7.
5. Centrifuge the homogenate at 10,000 g for 30 minutes at $O^0C$.
6. Aspirate the resulting infranatant and strain through a single layer of cheesecloth.
7. Keep the infranatant on ice; this is the enzyme source.

The **routine assay system** for LPL activity involves the following components in a total volume of 1 ml:

- -0.2 mmole of Tris buffer (pH 8.5)
- -1 mmole NaCl
- -10 umoles $CaCl_2$
- -20 mg Bovine Serum Albumin (fatty acid free)
- -0.1 ml normal pig serum
- -2.5 mg gum arabic
- -13.45 umoles of glyceryl tre ($^{14}C$) oleate (.02 uCi/umole of triolein)

*Substrate is added to the reaction as a gum arabic-triolein emulsion prepared by sonication

8. Gas with nitrogen the mixture of $^{14}C$ triolein and appropriate amounts of cold triolein for 30 minutes after the smell of solvent is gone.
9. Add gum arabic and mega pure water to the above mixture and sonicate at the maximum energy level for

60 seconds.

10. Preincubate substrate, assay buffer, and pig serum at $30^0$ C with shaking (30 strokes/minute) for 20 minutes before the initiation of the assay.

11. Continue incubating at $30^0$ C with shaking (30 strokes/minute) using 50 ml culture tubes to provide a larger surface area for enzyme-substrate interaction.

12. Terminate the reaction with the addition of 6 ml of Doles extraction solution.

13. Vortex vigorously for 30 seconds.

14. Let stand for 5 minutes.

15. Add 5 ml of heptane (containing 0.4 umoles triolein) and 0.5 ml water.

16. Vortex for 30 seconds.

17. Allow the phases to separate overnight (12-15 hours).

## RESIN PREPARATION

1. Fatty acids are isolated from a 3-4 ml aliquot of the hepatane phase by a resin method.

2. Prepare an ion exchange resin (Sigma, Amberlite, IRA 400, 20-50 mesh, hydroxyl charged form) by suspending resin in 2.5 M NaOH (45:1, w/v).

3. Let equilibriate for 10 hours with occasional stirring.

4. Wash the resin with deionized water until the water tests neutral with pH paper.

5. Resuspend in isopropyl alcohol (0.7 g/ml).

6. After 24 hours, replace the isopropanol with isopropylalcohol:water (9:1) mixture (0.7 g/ml), let the resin equilibriate for 6 hours with occasional stirring.

7. Repeat twice more before the resin was washed several times with n-heptane until the odor of isopropanol is no longer detected.

8. Store the resin in a tinted glass jar at $4^0$C until use.

## PREPARATION OF SAMPLE FOR ASSAY

*Purify resin by washing with 2 ml of heptane containing triolein (40 mg/ml)

1. Add the heptane aliquot from the reaction tube to 20 ml scintillation vials with 1 g of wet resin.

2. Vortex for 1 minute.

3. Aspirate excess solvent from the vials and wash the resin 3 times with 5 ml of heptane.

4. Add 1 ml of NCS stabilizer (Amersham) to each vial.

5. Incubate at $60^0$C for 20 minutes to displace the fatty acids from the resin.

## ANALYSIS

1. Determine the **fatty acid radioactivity** on a liquid scintillation counter with 10 ml of 0.4% Omniflour (New England Nuclear)-Toluene scintillation mixture.

2. Determine **recoveries** by using a standard solution of $^{14}C$-toluene added to vials containing resin.

3. Determine recoveries by using a standard solution of $^{14}C$ oleic acid bound to albumin included in the same assay mixture as the samples except emulsions of unlabeled triolein are used.

4. Determine the recoveries by calculating the ratio of the $^{14}C$-oleic acid counts obtained with the standard solution included in the assay and those counts obtained with the same amount of standard solution added directly to the counting vials containing resin which had been previously washed with heptane and solubilizer as in the assay.

# APPENDIX C

## Appendix C

**Lipogenesis Assay-** a tritiated water procedure (Mulvaney, D.R., 1984)

*Solutions:*

1. KREBS RINGER BICARBONATE BUFFER (pH 7)

| Stock Solutions: | | Volume of stock solution to make a 5x strength buffer |
|---|---|---|
| NaCl | 4.5 g/100 ml | 25.0 ml |
| KCl | 5.75 g/100 ml | 1.0 ml |
| $CaCl_2$ | 6.10 g/100 ml | 0.75 ml |
| $KH_2PO_4$ | 10.55 g/100 ml | 0.25 ml |
| $MgSO_4$ | 9.25 g/100 ml | 0.25 ml |
| $NaHCO_3$ | 65.00 g/100 ml | 5.25 ml |
| | | Total...32.50 ml |

-gas with 5:95 ($CO_2$:$O_2$) for 1 hour
-on day of use, dilute the 32.5 ml with (32.5 x 4)-->130 ml of $H_2O$, gas with 5:95 for 15 minutes before using. The total is162.5 ml.

2. INSULIN

-10 mg of porcine insulin in 24 ml at 24 units/mg is

approximately10 ug/ml
-Freeze in 1 ml aliquots
-**Note**: Take up 10 mg in 5 ml water.
Dissolve by **slowly** adding .1 N NaOH
Adjust pH back to appoximately pH 7 with .1 N HCL.
Bring up to 24 ml.

3. .1 N NaOH

2 N = 80 g/l or 20 g/250 ml
.1 N = 5 ml of 2N/100 ml

4. .1 N HCL

Concentrated HCl = 12 N

1 ml in 120 ml water is approximately .1

5. .9% NaCL

9 g/l water

6. KOH/ETHANOL

30% KOH (150 g/500 ml)

95% EtOH

-Ratio of 3:7 (KOH/EtOH)

-Need 10 ml of this sample.

ON THE DAY OF INCUBATION:

-Make this fresh daily.

| | 100 ml | 150 ml | 50 ml |
|---|---|---|---|
| INSULIN (10 u/ml) | 1.0 | 1.5 | .5 |
| GLUCOSE (MW = 180.16) 10 mM = .01 M | .18g | .27g | .09g |
| TRITIATED WATER ($^3H_2O$) Final Concentration 200 uCi/ml | 1.0 ml | 1.5 ml | .5 ml |
| KREBS RINGER BICARBONATE BUFFER | | | |

Make up to 100, 150, or 50 ml etc.
-Bubble gas (95:5) $O_2$:$CO_2$ through the buffer for approximately10 - 15 minutes.
-Test the pH and adjust to pH 7.4 if necessary with .1 N HCL/.1 N NaOH.

The incubation buffer has the label:

.09 g glucose

.5 ml insulin

.5 ml $^3H_2O$

49 ml Krebs Ringer Buffer

pH = 7.4

- use 3 ml per flask

## LIPOGENESIS ASSAY

1. Obtain adipose tissue.

2. Place in oxygenated Krebs Ringer Buffer at 38 degrees Celsius.

3. Slice tissue on Stadie Riggs microtome.

4. Tissue slice should weigh from 70 - 100 mg.

5. Transfer slice to 25 ml Erlenmyer flask which contains 3 ml buffer (containing the labelled water).

6. Gas with 95:5 for 15 seconds before replacing the rubber stopper.

7. Be sure to record time the flask was put into the water bath.

8. Incubate with shaking for 2 hours at approximately 70 strokes per minute.

9. At 2 hours, remove the flask from the incubator.

10. Remove tissue slice from flask with forceps.

11. Rinse slice in .9% saline three to four times.

12. Blot slice on paper towel.

13. Weigh the slice and record weight.

14. Trasfer slice to a 50 ml culture tube (saponification tube) which have 10 ml of KOH/EtOH (3:7).

**Note**: The saponification tubes can be stored at this point.

15. Heat the tubes in a water bath at 50 - 60 degrees Celsius for 2 hours or until the tissue has dissolved and then for about 30 more minutes to be sure. Make sure there are no globules.

16. Extract twice (each tube) with 5 ml Petroleum Ether.

17. Be sure to vortex each time
    for 1 minute for the first extract.
    for 30 seconds for the second extract.

18. Discard the petroleum ether by aspiration or syringe
    The layer which is being discarded contains the nonsaponifiable lipids and cholesterol.
    *The remaining solution will be cloudy.

19. Add one full disposable pipette of 12 N HCL to bring the pH to approximately 2.0.
    The addition of the 12 N HCL converts the soaps to fatty acids.

20. Extract three time as above with petroleum ether and vortex
    for 1 minute the first extract.
    for 30 seconds the second extract.
    for 30 seconds the third extract.

21. Transfer the petroleum ether layer to scintillation vials.

    -Use pasteur pipettes.

    -Use separate pasteur pipettes for each treatment group.

22. Dry the scintillation vials under a stream of air.

23. Add 10 ml of scintillation fluid.
    Scintillation fluid:
    4 g Omniflour
    230 ml Ethanol
    770 ml Toluene

**OTHER NOTES:**

Total cpm should be checked on the buffer.
ie. 100 ul of incubation mix into vial

Bring to 10 ml with water (1:99).

Mix.

Take 100 ul of this to count and then correct back for original activity.

*This eliminates error in pipetting 10 ul.

**NOTE**: should have at least three samplings

EXAMPLE OF HOW TO GET EFFICIENCY OF $^{3}H$ STANDARDS

| NO. | CPM | EXAMPLE RATIO WITH EXTERNAL STANDARD | DPM | Efficiency |
|---|---|---|---|---|
| 1 | 146,000 | .758 | 276,602 | 50% |
| 2 | | .717 | | |
| 3 | | .632 | | |
| 4 | | .520 | | X |

**CALCULATIONS:**

a. DPM

Total CPM/ul of a 400,000 (ie)

Mean channel ratio with external standard of .520 (ie) therefore the efficiency from the graph is approximately 30%.

CPM / EFFICIENCY = DPM OR $\frac{1{,}333{,}333}{1.333 \times 10^{6}/uL}$

DPM / FLASK = $1.333 \times 10^{6} \times 10^{3}(ul/ml) \times 3$ ml

= $3.999 \times 10^9$ DPM /FLASK

b. Calculation of nmole $^3H_2O$ converted to fatty acid/min/g of tissue

the media contains
55 moles/liter
55 mM/ml
165 mM/flask .....of $^3H_2O$

THEREFORE:

The specific activity =
$3.999 \times 10^9$/165 mM (or $1.65 \times 10^8$ nmole)
=24.24 DPM/nmole

To determine nmole $^3H_2O$ converted to fatty acid/minute/g

= CPM x 1/efficiency x 1/minutes x 1,000/mg x 1/specific activity

# APPENDIX D

## Appendix D

### Determination of RNA and DNA concentration

A modification of the method of Munro and Fleck (1969) was used to determine RNA and DNA

1. Approximately .2 g frozen moist tissue was weighed in duplicate Corex tubes.
2. 2 ml of cold deionized water was added to each tube.
3. The sample was homogenized with a polytron.
4. After each individual sample .5 ml deionized water was added to a second tube to rinse the polytron of any residue and was added to the corresponding Corex tube (the Corex tube now contains the homogenized sample and 2.5% perchloric acid (PCA).
5. Tubes were stoppered.
6. Tubes were vortexed.
7. Tubes were placed in an ice bath for at least 10 minutes.
8. At this point it would be wise to turn on your water bath to 37°C so as not to waste any time in the extraction.
9. Tubes were centrifuged at 34,800 x g for 15 minutes.
10. The supernatant was discarded.
11. The pellet was broken up with an applicator stick (2 work best), be sure to get any excess off the applicator sticks.
12. 5ml of 1% percholoric acid were added to each tube.
13. Tubes were stoppered.
14. Tubes were vortexed.
15. Tubes were centrifuged at 34, 800 x g for 15 minutes.
16. The supernatant was discarded.
17. The pellet was broken up.
18. 4 ml of .3N potassium hydroxide were added to each tube.
19. The tubes were stoppered.
20. The tubes were sealed with tape to prevent popping.
21. The tubes were incubated in a 37°C water bath for 2 hours.
22. In order to get your sample into solution by the end of the

2 hour incubation it is helpful to vortex the samples occasionally to break up any globs.

23. At the end of the 2 hour incubation the tubes were placed on ice for 5 minutes.
24. It would be wise to turn the water bath up to 70°C which will be needed for the DNA extraction later on.
25. 5 ml of 5% perchloric acid were added to each tube.
26. This is an acid / base reaction and can cause the stoppers to pop off; to avoid the popping, stopper the tubes and invert a couple of times by hand, releasing the pressure each time.
27. Then you may stopper the tubes and vortex without any popping tubes.
28. The tubes were placed on ice for 15 minutes.
29. The tubes were centrifuged at 34,800 x g for 10 minutes
30. the supernatant was decanted into 25 ml graduated tubes and saved.
31. The pellet was broken up.
32. 5 ml of 5% perchloric acid were again added to each tube.
33. The tubes were stoppered.
34. The tubes were vortexed.
35. The tubes were centrifuged at 34,800 x g for 10 minutes.
36. The supernatant was decanted into the corresponding 25 ml graduated tube.
37. The pellet was broken up.
38. 5 ml of 5% perchloric acid were again added to each tube.
39. The tubes were stoppered.
40. The tubes were vortexed.
41. The tubes were centrifuged at 34,800 x g for 10 minutes.
42. The supernatant was decanted into the corresponding 25 ml graduated tube and the total volume of the tube was brought up to 20 ml with 5% perchloric acid, the tubes were covered with parafilm and vortexed.

**-this fraction contained the RNA**

*** THE PELLET WAS SAVED FOR DNA EXTRACTION**

43. The pellet was broken up.
44. 5 ml of 10% perchloric acid were added to each tube.
45. The tubes were stoppered.
46. The tubes were vortexed.
47. Marbles were placed on the top of each tube to act as condensers.
48. The suspension was digested in a 70°C water bath for 25 minutes.
49. At the end of the digestion the tubes were placed on ice for 5 minutes.
50. The tubes were centrifuged at 34,800 x g for 10 minutes.
51. The supernatant was decanted into 15 ml graduated tubes and saved.
52. The pellets were broken up.
53. 4.75 ml of 10% perchloric acid were added to each tube.
54. The tubes were stoppered.
55. The tubes were vortexed.
56. The tubes were centrifuged at 34,800 x g for 10 minutes.
57. The supernatant was decanted into the corresponding graduated tubes and the total volume was brought up to 10 ml with 10% perchloric acid, the tubes were covered with parafilm and vortexed.

**- this fraction contained the DNA**

# DETERMINATION OF RNA CONCENTRATION

Orcinol was used in a colorimetric procedure.

1. Before pipetting get your boiling water bath started.
2. Liver samples require a 1--> 3 dilution of the extracted fraction for RNA but not DNA.
   ie. In a separate tube mix 2 ml RNA fraction and 4 ml of 5% PCA.
   Take your 2 ml from this tube to read.
3. 2 ml of the RNA fraction were pipetted into 16 mm diameter glass tubes in duplicate (helpful to use disposable tubes).
4. Set up a set of standards.

   blank 5% PCA ------2 ml
   12.5 ug RNA/ml-----2 ml
   25 ug RNA/ml-----2 ml
   37.5 ug RNA/ml-----2 ml
   50.0 ug RNA/ml-----2 ml

5. To all of the above tubes 2 ml of 1% fresh orcinol reagent were added.
6. Make the orcinol reagent under the hood and you may want to place a watch glass on top of the beaker to prevent evaporation; also make sure the solution is mixed well.
7. Each tube was covered with parafilm and vortexed to make sure the tubes are thoroughly mixed.
8. Marbles were placed on the top of each of the tubes to act as condensers.
9. The tubes were placed in a boiling water bath for 30 minutes.
10. After the 30 minutes had elapsed, the tubes were removed and cooled in running cold water for 5 minutes and then allowed to reach room temperature.
11. The tubes were read at 680 nm on a Beckman Model 24 spectrophotometer.
12. The tubes were vortexed before each reading.

## DETERMINATION OF DNA CONCENTRATION

Diphenylamine and acetaldehyde were used in a colorimetric procedure.

1. Before pipetting get your water bath set at 30°C.
2. 2 ml of the DNA fraction were pipetted into 16 mm diameter glass tubes in duplicate (it is helpful to use disposable tubes).
3. Set up a set of standards.

blank 10% PCA------2 ml
12.5 ug DNA/ml-----2 ml
25 ug DNA/ml-------2 ml
37.5 ug DNA/ml-----2 ml
50 ug DNA/ml-------2 ml

4. To all tubes 2 ml of 4% diphenlyamine in glacial acetic acid and .1 ml of acetaldehyde solution were added.
5. Tubes were stoppered.
6. Each tube was parafilmed and vortexed to make sure each tube is thoroughly mixed.
7. Marbles were placed on top of each test tube to act as condensers.
8. The tubes were incubated overnight (12 - 16 hours) at 30°C.
9. After incubation the tubes were cooled to room temperature.
10. The tubes were read at 595 nm on a Beckman Model 24 spectrophotometer.

* Before reading the samples flush the system with a 1:1 acetic acid water solution so as not to cause any precipitate blockage in the system as water and acetic acid. produce a white precipitate; you may also want to do the same at the very end for the very same purpose.

## PREPARATION OF RNA STANDARDS

a. Dissolve 12.5 mg RNA in 250 ml 5% PCA.
   * this solution contains **50 ug RNA/ml**

b. Add 12.5 ml 5% PCA to 37.5 ml of (a.).
   * this solution contains **37.5 ug RNA/ml**

c. Add 25 ml 5% PCA to 25 ml of (a.).
   * this solution contains **25 ug RNA/ml**

d. Add 37.5 ml 5% PCA to 12.5 ml of (a.).
   * this solution contains **12.5 ug RNA/ml**

* STORE ALL OF THE ABOVE SOLUTIONS AT 2 TO 3°C

## PREPARATION OF DNA STANDARDS

a. Dissolve 12.5 mg DNA in 250 ml of 10% PCA.
   * this solution contains **50 ug DNA/ml**

b. Add 12.5 ml 10% PCA to 37.5 ml of (a.).
   * this solution contains **37.5 ug DNA/ml**

c. Add 25 ml 10% PCA to 25 ml of (a.).
   * this solution contains **25 ug DNA/ml**

d. Add 37.5 ml 10% PCA to 12.5 ml of (a.).
   * this solution contains **12.5 ug DNA/ml**

* STORE ALL OF THE ABOVE SOLUTIONS AT 2 TO 3°C

## PREPARATION OF 1% ORCINOL REAGENT

a. Make a .1% $FeCl_3$ in concentrated HCl.
b. Take 2 g of $FeCl_3$ and add 20 ml concentrated HCl in a small beaker.
c. Stir until dissolved.
d. Place 20 ml $FeCl_3$ solution in a glass bottle.
e. Add 1980 ml concentrated HCl to solution.
   * THIS IS STOCK SOLUTION
f. Make 1% orcinol solution by adding 100 ml of (e.) to 1 g orcinol in a volumetric flask.
g. Stir vigorously with a magnetic bar for about 20 minutes.
   * THIS SOLUTION MUST BE MADE UP FRESH PRIOR TO USE

## PREPARATION OF 4% DIPHENYLAMINE REAGENT

Make 4% diphenylamine solution by adding 100 ml glacial acetic acid to 4 g of diphenylamine.

* STORE AT 2 TO 3°C

---

## PREPARATION OF ACETYLALDEHYDE SOLUTION

a. Add .4 ml of acetylaldehyde to a 250 ml volumetric flask.
b. Bring to 250 ml with deionized water.
   * STORE AT 2 TO 3°C

## CALCULATIONS

### RNA

approximately .2 g----------> 20 ml final volume

have value in ug/ml (from linear regression)

want final volume in mg/g frozen moist tissue

ug/ml x 20 ml x (1/1000) x (1/sample weight)= mg RNA/g

therefore: mg RNA/g sample= ug/ml x .02 x (1/sample weight)

= A 680 x slope x .02 x (1/sample weight)

*NOTE: When analyzing a liver sample with a 1-->3 dilution
the .02 in the calculation becomes a .06

### DNA

approximately .2 g----------> 10 ml final volume

ug/ml x 10 ml x (1/1000) x (1/sample weight)= mg DNA/g

therefore: mg DNA/g sample

= ug/ml x .01 x (1/sample weight)

= A 595 x slope x .01 x (1/sample weight)

## PERCHLORIC ACID FOR RNA/DNA ANALYSIS

Reagent PCA is 70% strength from the bottle

For **10% PCA** need a 1 to 7 dilution or 1 part plus 6 parts distilled water

-Suggest 300 ml reagent PCA plus 1800 ml water.
-Add the concentrated PCA cautiously to the water already in the glass bottle.
-Swirl to mix thoroughly.
-Suggest making 3 bottles of 10% PCA since it will also be used for lesser dilutions.

For **5% PCA**

-Add 1,000 ml water to glass bottle.

-Add 1,000 ml of 10% PCA and mix thoroughly.

-Suggest making 2 bottles.

For **2.5% PCA**

-Use 900 ml of 5% PCA to 900 ml water.

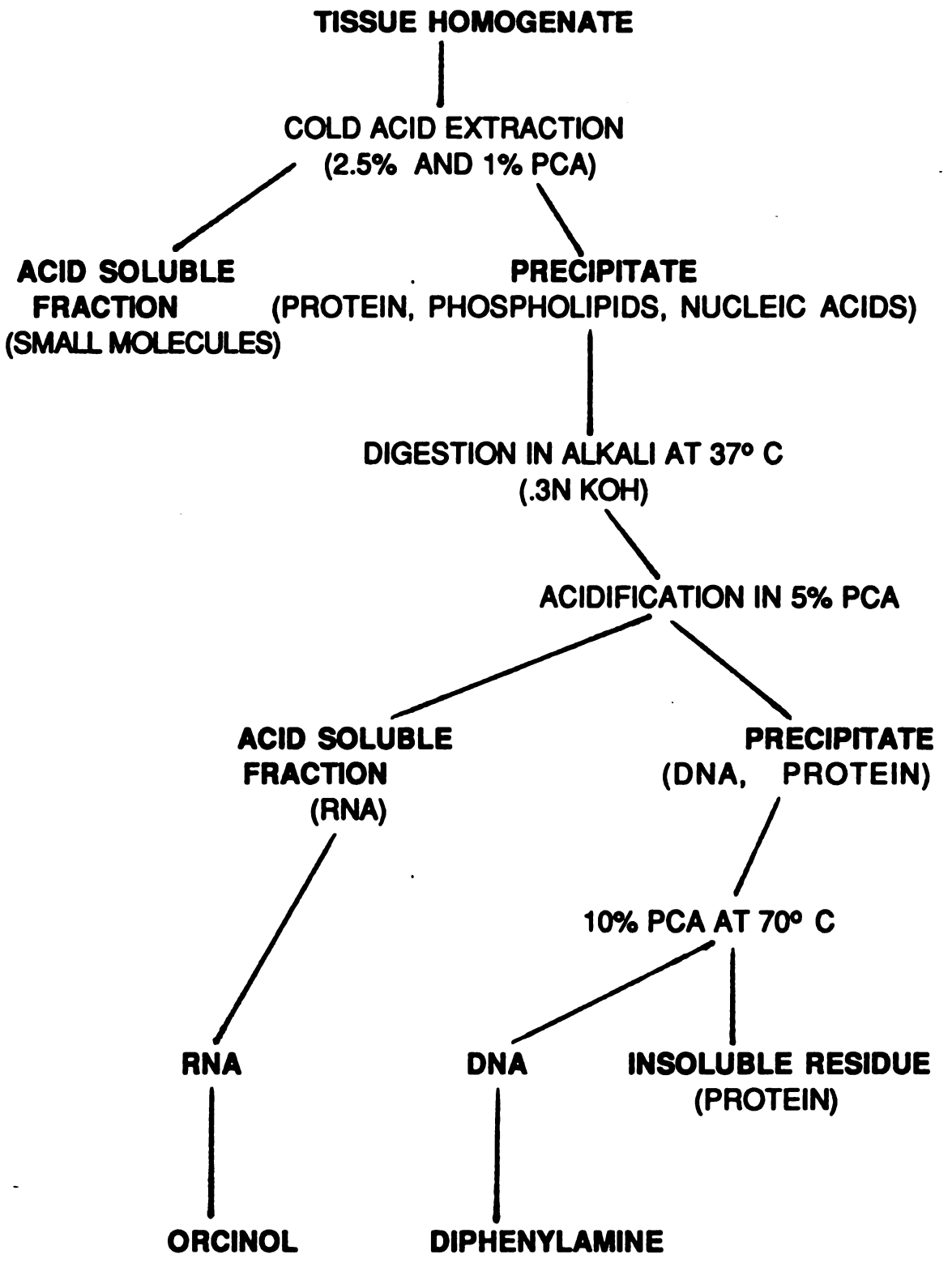
-Mix thoroughly.

For **1% PCA**

-Add 225 ml 5% PCA to 900 ml water.

-Mix thoroughly.

* STORE IN THE REFRIGERATOR.


TISSUE HOMOGENATE
COLD ACID EXTRACTION
(2.5% AND 1% PCA)
ACID SOLUBLE
FRACTION
(SMALL MOLECULES)
PRECIPITATE
(PROTEIN, PHOSPHOLIPIDS, NUCLEIC ACIDS)
DIGESTION IN ALKALI AT 37° C
(.3N KOH)
ACIDIFICATION IN 5% PCA
ACID SOLUBLE
FRACTION
(RNA)
PRECIPITATE
(DNA, PROTEIN)
10% PCA AT 70° C
RNA
DNA
INSOLUBLE RESIDUE
(PROTEIN)
ORCINOL
DIPHENYLAMINE

# APPENDIX E

## Appendix E

### Method for Dry Column Class Separation of Lipids (Marmer and Maxwell, 1983)

1. Use a 35 mm glass chromatography column.
2. Set up trap at bottom of column using a small amount of glass wool.
3. Pack column with 10 grams of a 1:9 $CaHPO_4$:Celite trap.

    *If using cured meats or samples with heavy pigments use 0.1 gram magnesium oxide.
    *Leave stopcock open while packing to let air escape.
    *Pack firmly but not excessively.

4. Weigh out 20-25 grams of $Na_2SO_4$ in a plastic weigh boat.

    *If a really moist sample use the full 25 grams.

5. Add 5 grams of ground meat on top of the $Na_2SO_4$ in the weigh boat so it doesn't stick.
6. Grind with a mortar and pestle until powdery.
7. Add 15 grams Celite and grind with mortar and pestle to a fine powder.
8. Moderately pack column with the mixture using a plastic funnel; packing half of the sample at a time to be sure of uniform packing.
9. Be sure that the collection flask is clearly labelled and under the column.
10. Get column wet before the collection is started by adding 50-60 ml $CH_2Cl_2$.
11. You may want to use a portion of the 50-60 ml to rinse the mortar and pestle, pouring the rinse onto the column.

**ELUTION OF NEUTRAL LIPIDS**

1. Elute neutral lipids with 150 ml of $CH_2Cl_2$.
2. Pour enough to fill the column without spilling over.
3. Let drain until solvent level reaches the top of the column.
4. Do not let the column get dry.

**CHANGE RECEIVING FLASKS**

**ELUTION OF PHOSPHOLIPIDS**

1. Elute phospholipids with 150 ml of 9:1 $CH_2Cl_2$:MeOH.
2. Rotovap flask contents to approximately a 25 ml volume.
3. If the volume after rotovaping is not 25 ml bring to volume with $CH_2Cl_2$ in a graduated flask.
4. Remove 2 ml of the solution from each flask and place in preweighed 1 dram glass vials.
5. Dry the 2 ml under $N_{2(g)}$.
6. Place in a drying oven overnight.
7. Take the weight of the glass vials the following day.
8. This will indicate the amount of sample needed to derivatize for GLC analysis of fatty acids.

   **sample + tube wt. - original tube wt.= mg fat/2 ml**

   *Want 10 - 16 mg fat in sample to be derivatized

   **mg fat/2 ml= 10-16 mg/x**

   *where x represents the amount to derivatize

9. Store the contents of each flask in a sealed glass graduated flask in the freezer.
10. It is best to derivatize as soon as you can since the samples are more stable in the derivatized form.
11. Once derivatized store in 1 dram vials sealed on the outside with parafilm in the freezer until time of GLC analysis.

## DERIVATIZATION OF SAMPLES FOR GLC ANALYSIS

1. After the the amount to derivatize has been determined.
2. Place the determined amount of sample into conical graduated test tubes.
3. Dry the samples on a nitrogen evaporator.
4. 1 ml of hexane is added to each tube along with 100 ul of 2 N KOH (in methanol).
5. Vortex the samples for 30 seconds.
6. Centrifuge the tubes in a clinical centrifuge at speed 4 for 5 minutes.
7. Remove the lower layer with a disposable pipette.
8. Add 0.5 ml saturated ammonium acetate.
9. Vortex the samples for 30 seconds and centrifuge.
10. Remove the lower layer as before.
11. Add 0.5 ml of deionized water.
12. Vortex and centrifuge as above and remove the lower layer.
13. Add a few crystals of sodium sulfate the remaining liquid.
14. Keep the tubes at room temperature for 30 minutes.
15. Either freeze or analyze by GLC.

# APPENDIX F

## Appendix F

### Common fatty acids

| Common Name | Number of Carbon Atoms (Number and Position of Double Bonds) | Series |
|---|---|---|
| formic acid | 1:0 | |
| acetic acid | 2:0 | |
| propionic acid | 3:0 | |
| butyric acid | 4:0 | |
| valeric acid | 5:0 | |
| caproic acid | 6:0 | |
| caprylic acid | 8:0 | |
| capric acid | 10:0 | |
| lauric acid | 12:0 | |
| myristic acid | 14:0 | |
| palmitic acid | 16:0 | |
| palmitoleic acid | 16:1, 9 | omega 7 |
| stearic acid | 18:0 | |
| oleic acid (cis) | 18:1, 9 | omega 9 |
| elaidic acid (trans) | 18:1, 9 | omega 9 |
| linoleic acid | 18:2, 9,12 | omega 6 |
| gamma linolenic | 18:3, 6,9,12 | omega 6 |
| alpha linolenic | 18:3, 9,12,15 | omega 3 |
| arachidic acid | 20:0 | |
| arachidonic acid | 20:4, 5,8,11,14 | omega 6 |
| timnodonic acid | 20:5, 5,8,11,14,17 | omega 3 |
| behenic acid | 22:0 | |
| erucic acid | 22:1,13 | omega 9 |
| clupanodonic acid | 22:5, 7,10,13,16,19 | omega 3 |
| cervonic acid | 22:6, 4,7,10,13,16,19 | omega 3 |
| lignoceric acid | 24:0 | |
| nervonic acid | 24:1, 15 | omega 9 |

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