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The effects of a high monosodium phosphate and alpha tocopherol supplemented milk replacer diet on veal muscle color, composition and cholesterol content

Agboola, Hammed Abiodun, Ph.D.
The Ohio State University, 1987

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THE EFFECTS OF A HIGH MONOSODIUM PHOSPHATE AND ALPHA TOCOPHEROL SUPPLEMENTED MILK REPLACER DIET ON VEAL MUSCLE COLOR, COMPOSITION AND CHOLESTEROL CONTENT

DISSERTATION

Presented in Partial Fulfillment of the Requirements for The Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

Hammed Abiodun Agboola, B.Sc., M.Sc.

* * * * *

The Ohio State University

1987

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Dedicated to my beloved Professor and a friend, Dr. R. F. Plimpton, who died after suffering from a heart attack just six days before the defense of this dissertation.
ACKNOWLEDGMENTS

The author wishes to express his appreciation to his advisor, Dr. Vern R. Cahill, for his guidance, love and assistance throughout my studies at The Ohio State University. Apart from Dr. Cahill's assistance in the preparation of this dissertation, I must not fail to thank him and Mrs. Cahill for their parental love towards me and my family by assisting us whenever such a need arose. I am greatly indebted to them all.

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Finally, my profound gratitude goes to my wife, Regina, the American woman who not only became a wife, but became a friend whom I will never forget for the rest of my life.
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CHAPTER I
INTRODUCTION

Henry and Bratzler (1960) have reported that fresh meat color has long been an important criterion to consumers in the selection of meat from the retail counter. This is particularly true with veal. The dilemma facing the veal industry on how to find a way to produce a more valuable, lighter color veal has been discussed (Kauffman, 1982). Dolly et al. (1978) and Thielske et al. (1978), reported that dark veal is not only downgraded on the average of about 17 cents per pound and at the extreme of 74 cents per pound, but that, in general, dark veal is very difficult to merchandise. Veal production in the United States increased for a total of 495 million pounds in 1984 and demand is increasing. With this increase in demand and the reduction in dairy cattle population and, considering the reduction in demand for beef, more beef calves may be profitably diverted toward veal production in the future. In addition, calves older and heavier than traditional veal will be used, confounding the color problem.

The relationship between iron intake, myoglobin concentration and dark color in veal had been reported (Bray et al., 1959; Niedermier et al., 1959; Swatland, 1985). A generous feeding of whole milk for raising calves is one way to produce lighter color veal, but milk is low in iron and subsequent development of anemia can occur in calves receiving
only milk. In addition to this problem, economic considerations cause the dairy industry to prefer selling fluid milk to the consumers rather than feeding it to calves.

At present, there has not been any work published which addresses the question of mechanisms which will permit control of myoglobin synthesis so as to enhance the desired development of light colored veal without the disadvantage of anemia development in the calf. This study was designed to address that problem.

Manipulation of the diet late in the feeding program may offer a potential solution for solving the problem. Phosphate compounds have been demonstrated as a sequestering agent for divalent metals such as iron (Hamm, 1960), and it is possible that such a chelator might be effective in tying up the iron in the blood or in the muscle, thus reducing the availability of it in myoglobin formation. Trout and Schmidt (1986) reported that the higher the chain length, the lower the ability of phosphate compounds to dissociate. This concept will make monosodium phosphate a compound of choice in the present study. Also, it has been reported (Lassister, 1967) that high dietary phosphorus inhibits iron absorption in lambs and in steers (Standish et al., 1971). Some compounds shown to inhibit iron uptake include phosphates, gastroferrin, endotoxins and oxalates (Saltman, 1965; Kroe et al., 1966, Cortell et al., 1967 and Crosby, 1968). This has not been demonstrated in veal calves. Alpha-tocopherol as an antioxidant (Menzel et al., 1972) could provide tissue protection from peroxide radical damage (Luzio 1972), and could also protect the calves from nutritional anemia (Church et al., 1972).
This study was undertaken to determine the effect of a high mono-
sodium phosphate and alpha tocopherol supplement in the diet of calves
late in the feeding program on:

1. Blood pigments, muscle pigments and muscle color.
2. Relationship between blood mineral composition and
   pH, muscle mineral composition and pH and subse-
   quent color of the product.
3. Nutritional characteristics of meat from calves
   (veal) involving mineral content, vitamin E con-
   tent and cholesterol.
4. Relationship between blood cholesterol composi-
   tion and muscle cholesterol and its derivatives.
CHAPTER II
LITERATURE REVIEW

Meat Color: General

The color of fresh meat has a major influence on consumers' decisions when meat is purchased at retail (Henry and Bratzler, 1960; and Forest et al, 1975). Most consumers have a concept of the proper appearance of meat from any given specie and any deviation from that color will be discriminated against (Forest et al., 1975). This is particularly true with veal where pale, light color is highly sought. A light pink color is desired because the consumers consider it to be a guarantee that the muscle has come from milk-fed veal calves (St. Laurent and Brisson, 1967, and Church et al., 1972). Lawrie (1950), Fox (1966), Forest et al. (1975), and Kauffman (1982), agreed that meat color is related to heme pigments, particularly myoglobin. A typical structure of myoglobin is depicted in Figure 1. It is a globular sarcoplastic protein consisting of a heme group surrounded by a globin moiety. There are two major portions, the heme prosthetic portion and the globin protein portion, which is made up of approximately 150 amino acid units. The heme portion of four pyrole rings is connected by methine bridges with a centrally located elemental iron usually in ferrous Fe^{2+} form. Kauffman (1982), Siedeman et al. (1984), and Ockerman (1983), reported that it is the chemical state of the iron
Figure 1. A schematic chemical structure of myoglobin (Adapted from Price and Schweigert, 1978).
which is responsible for the color of hemoglobin and myoglobin and, hence, the subsequent muscle color. Huffman (1980) noted that, even though there are a number of pigments present in the muscle, myoglobin is the only pigment present in large quantities to color meat.

Siedeman et al. (1984), reported that the color intensity of meat is determined by both antemortem factors such as stress, sex of the animals, age, species of the animals and postmortem factors such as ultimate pH and rate of pH decline. The authors noted that differences in myoglobin concentration cause differences in the color intensity of the meat between species. Lawrie (1950) also attributed factors such as anatomical differences within muscle and type of muscle fibers to affect myoglobin concentrations in muscle. Siedeman et al. (1984) reported that less active animals, such as those in a feed lot, will likely produce meat with lower myoglobin concentration and lighter in color than more active animals, such as wild game or grazing cattle on pasture. Lawrie (1950), Forest et al. (1975) and Siedeman et al. (1984), agreed that red muscle fibers tend to have greater myoglobin concentration than white muscle fibers because aerobic metabolism is more prevalent in red fibers than white fibers. White fibers are mainly anaerobic in nature.

Biosynthesis of Heme Compounds

Nair et al. (1972) outlined the steps involved in the synthesis of heme common to all aerobic cells (Figure 2). The initiation step involves the condensation of amino acid glycine and Succinyl-CoA inside the mitochondria. The enzyme involved in the condensation reaction
Figure 2. Outline of the biosynthesis of heme (Adapted from Nair et al., 1972).

\[ \text{GLYCINE} + \text{SUCCINYL-CoA} \]
\[ \text{(ALA SYNTHASE)} \]
\[ \text{delta-AMINOLEVULINIC ACID} \]
\[ \text{(ALA)} \]
\[ \text{(ALA DEHYDRATASE)} \]
\[ \text{PORPHOBILINOGEN} \]
\[ \text{PORPHYRINOGENS} \]
\[ \text{PORPHYRINS} \]
\[ \text{PROTOPORPHYRIN IX} \]
\[ + \text{Fe}^{++} \]
\[ \text{HEME} \]

\(^1\text{Rate determining enzymes for heme synthesis.}\)
is delta-aminolevulinate synthase. The condensation step is followed by extramitochondrial reaction which involves the condensation of two molecules of ALA to give porphobilinogen (PBG). Other intermediate steps lead to the formation of red protoporphyrin IX. The termination step in the pathway is completed inside the mitochondria by the introduction of ferrous iron (Fe\(^{2+}\)) into protoporphyrin IX by the enzyme ferrochelatase. This biosynthesis of heme alone further illustrates that iron is an important element of biological versatility.

**Redox Reactions of Myoglobin Iron and Meat Color**

Ockerman (1983), Siedeman et al. (1984) and Cahill et al. (1980) described the three major primary color states of myoglobin which is depicted in Figure 3. The early major forms when tissue is first exposed to the air are reduced myoglobin which is purple in color and where iron exhibits a positive valence of two (Fe\(^{2+}\)) and this is converted into oxymyoglobin which is bright cherry red and is the most desirable color and still has a valence of two. The formation of oxymyoglobin is due to the binding of an oxygen molecule to the free binding site of reduced myoglobin in a process called "oxygenation" (Huffman, 1980; and Seideman et al., 1984). Cahill et al. (1980). It has been noted that the process of oxygenation takes approximately 30 minutes when a cut meat surface is exposed to the air. The third form is metmyoglobin where iron is oxidized and exhibits a positive valence of three (Fe\(^{3+}\)). All of the color states named are reversible as indicated in Figure 3. Castro (1971) discussed the effect of some metal ions that can oxidize oxymyoglobin to produce undesirable brown metmyoglobin
Reduced Myoglobin
Color: purple
State of iron: Fe^{2+} (ferrous)

Oxy-myoglobin
Color: red
State of iron: Fe^{2+} (ferrous)

Metmyoglobin
Color: brown
State of iron: Fe^{3+} (ferric)

Choleglobin
Color: green
State of iron: Fe^{2+} or Fe^{3+}

Sulfmyoglobin
Color: green
State of iron: Fe^{3+} (ferric)

Cooked Meat
Color: dark brown
State of iron: Fe^{3+} (ferric)

Figure 3. The primary color forms of myoglobin (Adapted from Siedeman et al., 1984).
color. The process involves complete electron transfer from heme iron to bound oxygen and only occurs upon approach of a second metal ion or a proton. Suitable chelators, such as EDTA, chelate catalytic metal ions and make them less available for pigment oxidation.

In his extensive review, Siedeman et al. (1984) reported that the oxidation of reduced myoglobin to metmyoglobin is caused by those factors that reduce the oxygen tension to the meat surface and causes dissociation of the oxygen from oxymyoglobin. Such factors include low pH, salt, low oxygen atmospheres and presence of aerobic bacteria such as Pseudomonas species. High temperature can also cause the loss of the globin moiety's function of protecting heme leading to deoxygenation of oxymyoglobin to unstable reduced myoglobin. Walters (1975) attributed the actual oxidation of myoglobin to metmyoglobin to be caused by the activity of oxygen-utilizing enzymes inherent to muscle tissue. Lawrie (1974) reported that low storage temperature will suppress the residual activity of the oxygen-utilizing enzymes such as muscle succinic dehydrogenase.

Iron Nutrition

Historically, iron was believed by the ancients to be of heavenly origin. The pre-Greek, Greek and Roman cultures had utilized iron to cure a host of disorders and have ascribed to it many mythical properties and virtues. Some of these virtues have been associated with iron deficiency, now found in those parts of the world (Thomas, 1970). As has been pointed out in this review, the association of iron with the heme molecules is well established. Apart from its pigmentation
function, iron being a component of hemoglobin is responsible for oxygen transfer from the blood to the tissue. Myoglobin has more affinity for oxygen than hemoglobin; therefore, iron functions in myoglobin to receive the oxygen from hemoglobin which is subsequently used by the muscle for tissue metabolism: \( \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{Energy} \). The energy is trapped by adenosine triphosphate (ATP) and subsequently used for cell functions. The present review will henceforth focus on elemental iron utilization by livestock, its metabolism, its transportation and retention as they influence muscle color particularly in veal.

**Iron Intake**

Iron deficiency beyond the suckling stage of animal is a very rare occurrence because of the ubiquitous nature of iron (Conrad, 1984). Animals get sources of iron from feed, water, soil, and by chewing wire fences and nails. The nutritional requirement for iron varies with species of animal, growth rate and iron status of the animals (Formon, 1974). Most of the dietary iron given to the livestock is added to feed, given by injection or added to water in the form of ferrous sulfate and other available iron sales.

**Iron Absorption**

Iron absorption usually consists of three stages: (1) mucosal uptake, (2) mucosal transfer, and (3) mucosal storage (Thomas, 1970). Formon (1974) has indicated that iron absorption is influenced by animal age, adequacy of iron in the body, by the amount ingested, and by
the amounts and proportions of various other components of the diet, both organic and inorganic. Also, Bhargava and Gabbe (1984) reported that increased erythropoiesis and iron deficiency anemia are two factors that modulate iron absorption in rats, other than actual body iron stores. Most of the iron absorption takes place mainly in the duodenum and jejunum usually in the ferrous form (Wheby, 1966). However, Ammermer and Miller (1972) have reported that oral ferrous and ferric iron are equally absorbed by animals such as cattle, sheep and swine because natural reducing agent such as ascorbic acid could reduce ferric to ferrous iron in the gut. Goldberg and Martin (1964) have also indicated that both ferrous and ferric iron will be equally absorbed as long as the pH of the intestinal tract remains at approximately 2 to 3. Numerous researchers (Thomas, 1970, Benson and Rampone (1966), Cortell (1967), Crosby (1968) and Tucker et al., 1957) have identified compounds such as ascorbic acid, amino acids, synthetic chelating agents, alpha-tocopherol and alpha-ketoglutaric acid as having ability to increase iron uptake. On the other hand, phytates, phosphates, oxalates, gastroferrin and some alkalinizing agents have been shown to inhibit iron uptake. Weinbraub et al., 1964) indicated that most of the above compounds only influenced absorption of iron salts and have no effect on hemoglobin iron.

**Iron Transfer: Role of Mucosal Epithelium**

According to Levine et al. (1974) reviewed by Conrad (1984), the understanding of the stability of iron complexes is important for one to understand the mechanisms involved in iron transfer to and through the mucosal epithelium. The stability of iron complexes fall into two
classes based on their thermodynamic and kinetic stability. Levine et al. (1974) described the compounds of low effective stability as those that function mainly in giving up iron to the acceptor sites of the brush border of the cell. Example of the such compounds include amino acids and lactic acid. Other forms are iron complexes of high effective stability which are inert and move into the cell where iron is released by enzymatic degradation of the phorphyrin ring. An example of a complex of high effective stability is iron-hematoporphyrin.

Formon (1974) indicated that iron has to be released from iron-protein compounds and must be in a reduced (Fe^{2+}) state before it can be absorbed into the mucosa cell. However, Underwood (1971) indicated that iron in heme compounds is absorbed directly into mucosa cells of the intestine without the necessity of being released from its bound form. Figure 4a and 4b illustrate the mechanisms involved in regulation and control of iron absorption and transport. In Figure 4a (Thomas, 1970), the uptake of iron seems to be dependent on iron concentration and in some cases on utilization of ATP as an energy supplier. The transfer of iron from the mucosal cell to the blood is energy dependent at physiological concentrations. In Figure 4b (Forth and Rummel, 1973) indicated that the first major component involved in iron transfer is brush border or microvilli. Villi are finger-like organelles which are characteristically rich in blood supply and have a large surface area to volume ratio which facilitates absorption of iron in the duodenum. These microvilli serve as an iron receptor from the iron carriers such as labile iron or dissociation products of stable chelates (Figure 4b). Formon (1974) indicated that ferritin, a non-heme compound in which
Figure 4a. A diagramatic illustration of the energy dependent (ATP) mechanisms involved in the iron transport and retention to and through mucosal epithelium (Adapted from Thomas, 1970).
Figure 4b. An illustration of processes involved in iron transfer across duodenal and/or jejunal mucosa cell. (Adapted from Forth and Rummel, 1973).
in which iron is mainly stored accounts for approximately 8% of the movement of iron across the mucosal cell. Ferritin and hemosiderin are present in the greatest concentrations in liver, spleen and bone marrow. The same author reported that iron in plasma is completely bound to transferrin, a beta-globulin protein with a molecular weight of 86,000 and a compound with two separate binding sites. Each binding site has the capability of binding one atom of ferrous iron. The amount of iron bound to transferrin when all binding sites are saturated is referred to as the "total iron binding capacity" (TIBC). Transferrin responds to demand for iron according to body demand. Moore (1964 and 1973) described the multifunctionalism of transferrin: it is responsible for accepting iron that is being absorbed from the intestinal tract and for that released from sites of storage and from hemoglobin destruction. Secondly, transferrin-bound iron is delivered to the bone marrow for hemoglobin synthesis, to the placenta for fetal needs and to cells for iron containing enzymes.

Iron Excretion or Losses from the Animal Body

Losses of iron from the body usually occur from the gastrointestinal tract, primarily in the form of desquamated intestinal epithelial cells and also from the skin and urine (Formon, 1974). Conrad (1984) reported that excretion of iron is minimal. Cows may excrete iron into the digestive tract via saliva, blood, mucosa and bile. Weinbraub et al. (1964) found that young ruminants receiving minimum iron diets lose iron at the rate of about 0.4% of dietary iron per day but mature animals may conserve nearly all of their absorbed iron. It is important
to understand that the ultimate features of iron metabolism involve the acceptance of a minimum transfer of dietary iron into the body and to respond to demands for iron by the body and to be able to conserve iron for a complex nutritional requirements.

Iron in Veal Production

The major objective in rearing veal calves is to produce light pink meat to satisfy a growing consumer demand (Church et al., 1972 and Conrad, 1984). This poses a critical challenge for the animal nutritionists because the challenge is to find a way to regulate iron intake to prevent anemia, maximize calf feed efficiency and simultaneously produce pink meat desirable in veal. Chronologically, various researchers have approached veal production problems in different ways:

Bray et al. (1959) and Niedermier et al. (1959) in similar but separate studies worked with veal calves on a milk replacer diet supplemented with dietary iron and copper. They reported a significantly higher level of myoglobin content and dark color veal in the group of calves receiving 240 mg Fe and 7 mg of copper supplementation per day. The result further established the concern and interrelationship between iron intake and its consequence on color of veal.

In a separate study involving veal, St. Laurent and Brisson (1967) reported some effects of age, iron and desferrioxamine supplementation on changes in veal color and pigment content of skeletal muscles. In the experiment involving twenty-four Holstein male calves, ferrous sulfate was added to the milk to produce 50 mg elementary iron per calf daily for one group of calves. Desferrioxamine, proposed to act as a
chelator of iron and was added to milk at the daily rate of 500 mg for the group of calves receiving milk only and 1000 mg for calves receiving milk supplemented with iron. Examining the color and pigment content of vastuus lateralis muscle (thigh), iron supplementation caused a decrease in the brightness of the muscle but desferrioxamine had no effect on either the hemoglobin or myoglobin concentrations. The results of this study agreed with Bray et al. (1959) when only muscular tissue taken by biopsy technique was examined. This means that on the live animal, the brightness of the muscle is not exclusively a function of the myoglobin or hemoglobin concentration. The changes in the pigment concentration in this report (St. Laurent and Brisson, 1967) were generally influenced more by age of the animal than by the experimental treatments.

While studying the effect of dietary iron on the color and pigment content of veal muscle, MacDougall et al. (1973) found no consistent effect of copper on muscle color, but found that dietary iron intake did have some effects. In this tudy, the basal diet of the calves was supplemented with iron sulfate, iron citrate, and iron phytate to levels varying from 30 to 90 ugFe/g of feed on a dry matter basis. Results indicated that the concentration of myoglobin, myoglobin plus hemoglobin increased substantially as the level of dietary iron increased from 40 to 90 ug. The authors observed that as dietary iron increased from 40 to 90 ug. The authors observed that as dietary iron increased, blooming of the muscle color decreased. While evaluating muscles from different anatomical locations for pigment concentration,
the order in this study is biceps femoris > longissimus dorsi > semi-tendinosus.

Bremner and Dalgaro (1973a) studied the availability of different iron compounds on iron metabolism and the hemological status of veal calves. Iron was fed as iron sulfate, citrate, ferric-ethylenediamine tetraacetate and iron phytate. The main effect of availability of different iron compounds was found in the blood not in the tissue. However, iron from the phytate compound was the least available to the calves. This was because some of the calves treated with iron phytate became anemic at the end of the experiment. It was concluded that a dietary intake of 40 ugFe/g soluble iron is sufficient to prevent the development of other than a very mild anemia. Bernier et al. (1984) adjusting the iron content of milk replacer diet to 50 ppm prevented severe anemia without causing carcass to be downgraded for color using a Canadian grading standard. In their experiment involving thirty Holstein male calves receiving ferrous sulfate supplementation with and without dietary fiber, dietary iron interaction was found to have some significant effects (P<.001). Including dietary fibers in milk replacers had no effect on weight gain, but reduced incidence of diarrhea. Their finding suggests that certain dietary fibers may affect iron requirements because they interfere with iron utilization when blood hemoglobin is examined.

In summarizing his review on iron, Conrad (1984) stressed that iron obviously plays a central part in live processes. About 60% of the body iron is in hemoglobin, 10% in essential enzymes such as
cytochrome and 30% in storage such as in the liver, spleen and bone marrow. As has been discussed in this review, in rearing veal calves, the major concern is to produce calves with pale meat. Biochemically, this means raising calves with a minimum of myoglobin concentration in the muscle tissue. This could be approached by finding a way to regulate iron intake and retention without making calves anemic. The present research was designed (Figure 6) to achieve that goal.

Cholesterol: General View

There are two major classes of nonsaponifiable lipids: the terpenes and the steroids. Steroids are derivatives of the saturated tetracyclic hydrocarbon (Perhydrocyclopentannophenanthrene). All steroids originate from squalene which cyclizes readily. The first important steroid is lanosterol which in animal tissues is the precursor of cholesterol (Stryer, 1981). A schematic structure of cholesterol is depicted in Figure 5, while the pathway leading to cholesterol synthesis is shown in Figure 6. Cholesterol is the major form of sterol found in an animal (Ockerman, 1983). Cholesterol occurs in the plasma membranes of animal cells and in the lipoproteins of blood plasma. In general, sterols are not present in bacteria (Atlas, 1984). Cholesterol is the precursor of other steroids in animal tissues, which include, but are not limited to, bile acids, androgens or male sex hormones, estrogen or female sex hormones, the progestational hormone, and andrenaline hormone.
Figure 5. A schematic structure of cholesterol molecule (Adapted from Stryer, 1981).
Figure 6. A pathway leading to cholesterol synthesis from squalene (Adapted from Stryer, 1981).
Cholesterol In Human Nutrition

Measurement of plasma lipid profile, particularly cholesterol of individuals, has been a continuous scientific concern for the past 25 years. Goldsmit (1975) and Gibbons et al. (1982) reported that epidemiological and clinical investigations have identified and associated cholesterol and high blood pressure and with coronary heart disease. Goldsmit (1975) noted that the risk of developing coronary heart disease is positively correlated with the cholesterol concentration in plasma. This risk appears to be small at levels of less than 200 mg/100 ml blood. Dietary modifications, such as reduction of total fat intake, reduction in saturated fat rich food intake, and modification of plasma triglycerides structures can be used to control quantity of cholesterol in the plasma and in animal tissue.

Cholesterol Metabolism

Cholesterol in the animal system could be from exogenous or endogenous sources. The cholesterol of exogenous origin are from the diet while endogenous cholesterol are those synthesized by the liver. The exogenous cholesterol can be controlled by minimizing the amount of dietary fat intake. The endogenous source is genetically influenced which makes it very difficult to control. An individual who consumes low cholesterol diet can still have an abnormal level of plasma cholesterol if he is genetically prone to synthesize more of this sterol. Brown et al. (1981) noted that, due to the continuous cycling of cholesterol into and out of the blood, the plasma cholesterol concentration is not a single additive function of dietary cholesterol intake and...
endogenous synthesis, but it is usually a reflection of the rates of synthesis of the cholesterol carrying lipoproteins, coupled with the efficiency of the receptor mechanisms that determine their catabolism.

Gibbons et al. (1982) identified four main lipoprotein carriers in the plasma: (1) very low density lipoproteins (VLDL), (2) high density lipoprotein (HD), (3) low density lipoprotein (LDL) and intermediate density lipoprotein (IDL). Another molecule involved in cholesterol transport is chylomirons. The mechanism involved in cholesterol metabolism is well illustrated by Brown et al. (1981), Figure 7. As described in the model, both the exogenous and endogenous cycles of cholesterol transport systems begin with the secretion of triglycerides-rich particles called chylomicrons and VLDL. The chylomicrons are subsequently converted to cholesterol ester-rich particles or remnants, IDL and LDL. Brown et al. (1981) reported that the half time for the clearance of chylomicrons and their remnants from the plasma is approximately 5 minutes. This will suggest that the plasma cholesterol rises very little, if at all, after a single high cholesterol meal. The chylomicon remnants finally get into the liver where they are broken down into bile acids (Cook et al., 1955) or as unesterified cholesterol. Those that escaped reabsorptions are usually lost in the feces.

Some Related Studies Involving Cholesterol

Stromer et al. (1966) examined the cholesterol content of subcutaneous and intramuscular lipid depots from 72 carcasses of different maturity and marbling scores. They found that intramuscular lipid contained more cholesterol than subcutaneous fat from inner and external
Figure 7. An illustration of the pathways involved in the cholesterol metabolism from the exogenous and endogenous sources. (Adapted from Brown, et al., 1981).
layers. But, the cholesterol content of the l. dorsi muscle increased with a decrease marbling score when expressed on a lipid basis. The cholesterol content of the muscle did not change with maturity. The increase in cholesterol found in intramuscular lipid was attributed to those originated from membranes and intramuscular structures which are known to be high in cholesterol.

Jones et al. (1975) examined plasma cholesterol concentration in squirrel monkeys receiving a high cholesterol-butter fat diet. The dietary intake of the diet increased plasma cholesterol concentration from 250 mg/100 ml blood to 750 mg/100 ml. It was observed that hyperresponder (HP) monkeys absorbed higher percentages of ingested cholesterol than hyporesponders. These differences were attributed to some phenotypic effects. Dietary fat was found to be related to the amount of cholesterol absorbed and its concentration. Bile acids were found to be higher in the monkeys receiving a high cholesterol diet.

In a related study, Wrenn et al. (1980), while feeding 0.2% cholesterol and 7% tallow in liquid diets to veal calves, reported an elevation of plasma lipid and cholesterol of about 2 to 3 times in calves fed cholesterol. In addition, there were significant increases in the liver weight of calves receiving cholesterol supplemented diet. Liver fat was more elevated in this study when 0.2% cholesterol were fed than by doubling dietary fat.

Kunsman et al. (1981) reported the cholesterol content of beef bone marrow and mechanically deboned meat (MDM) from the steers placed on low energy (grass fed) and high energy (grain fed) rations. Marrow
from grass-fed animals average 119.6 mg/100 g and from grain-fed 150.6 mg/100 g. In terms of anatomical location, cervical cholesterol > lumbar > femur in order of 190.1, 124.1 and 91.0 mg/100 g marrow, respectively. The reason for increase in the concentration of cholesterol in MDM was due to an increase in the spinal cord material used. Perhaps the significant part of this study was the finding that % fat in marrow has little or no relationship to cholesterol content. The authors concluded that inclusion of MDM would have minute effect on total cholesterol content of processed meat. This last statement was based on the fact that if MDM were substituted for 20% of beef and pork, in weiners, the maximum increase in cholesterol content would be approximately 12 mg/100 g tissue. This would be an increase of only 5-7 mg of cholesterol consumption per frankfurter. This amount, according to the authors, is too small to be considered hypercholesteremia.

Davis et al. (1985a, 1985b), examined the rate of lipid (cholesterol) transport in preruminant calf by collecting bile to determine the flow rate and bile acid transport. Liquid diets used were formulated to contain 12.5% dried skim milk (SM) or 10.5% SM + 2% soybean oil (SBO), milk fat (MF), beef tallow (T) or one of these fats plus supplemented cholesterol was added. Results indicated that the average lipid transport in mesenteric lymph was found to be 8.94 (SM), 32.58 (SBO), 64.86 (MF) and 38.12 (T) diets all expressed in mg/h. kg body weight. At the same time, lipid and cholesterol transported averaged 1.09, 1.92, 2.41 and 2.70 mg/h. kg body weight in the order of dietary sources listed above. Source of fat or supplemental cholesterol had no
effect on amount of cholesterol or bile acid transported in bile; how­ever, calves fed SM did transport more cholesterol in bile than calves fed fat or fat plus cholesterol. Considering the lymph, lipid and cho­lesterol transported was less in SM calves than calves fed fat.

In a follow-up study (Davis et al., 1985b), it was noted that intesti­nal cholesterol synthesis was less when calves were fed SM or SM + 2% soybean oil than when fed beef tallow or milk fat. The authors agreed that liver performed an important role in cholesterol synthesis and degradation.

The effect of alpha tocopherol on cholesterol synthesis and degra­dation was studied (Chupukchanoen et al., 1985) using white rabbits. Accumulation of cholesterol was found in the plasma and muscle of vita­min E deficient rabbit. The increase in the cholesterol level was accounted for by an increase in plasma low density lipoprotein level and a decrease in cholesterol 7 alpha-hydroxylase, the major enzyme involved in degradation of cholesterol into bile acids for excretion.

**Cholesterol Regulations: General View**

So far in this review, it has been shown that cholesterol synthe­sis, degradation and distribution are accomplished by a very tightly controlled enzymatic mechanism. It would appear that many dietary nu­trients, such as alpha tocopherol (Chupukcharoen et al., 1985), sorbi­tol (Bauchart et al., 1985), protein type (Torre et al., 1980) and genetic potential of animal for cholesterol synthesis (Jones et al., 1975) would certainly influence the cholesterol metabolism in some
animal species. In other words, cholesterol metabolism is influenced by both extrinsic and intrinsic factors.

Gibbons et al. (1982) proposed a mechanism of phosphorylation in the regulation of cholesterol synthesis. The mechanism of phosphorylation and dephosphorylation are carried out in the liver by the kinase enzyme. Kinase enzyme are those enzyme capable of transferring phosphorus element in a metabolic process. Reduction kinase is capable of using phosphorus to inactivate (phosphorylate) hydroxymethylglutaryl-CoA reductase, the major enzyme involved in cholesterol synthesis in the liver. The dephosphorylated process activates the enzyme resulting into more synthesis of cholesterol by the liver.

To summarize the review on cholesterol metabolism, the present author thinks that even though genetics has its own role on cholesterol metabolism, nonetheless, cholesterol, as a lipid, can be regulated by dietary treatment. Numerous researchers have shown that dietary alterations can affect the composition of lipid in the plasma and animal tissue (Miller and Rice, 1967), Miller et al. (1967, 1981), Terrell et al. (1969), Field et al. (1978), Williams et al. (1983) and Agboola (1983).
CHAPTER III
MATERIALS AND METHODS

Experimental Design

The general design used for this study is depicted in Figure 8. The variables examined were divided into two parts: blood analyses and muscle analyses. It is a completely randomized design with one factor (treatment).

Thirty Holstein bull calves were penned individually in a well-ventilated barn, at 18.3°C (Cooling Acme Ventilation). They were fed a milk reconstituted diet (MRD) in individual feeding buckets. The nutritional composition of the MRD is shown in Figure 9. Each calf received 2.7 to 3.2 kg. MRD on a dry matter basis, 9 to 13 kg of water and 350 mg of chlorotetracycline per calf per day. All calves were on feed for a total of 16 weeks before being slaughtered at an average weight of 172 kg.

A blood sample was taken from the right jugular vein of each calf before first MRD feed at approximately 3 days of age. This blood was analyzed for packed cell volume (PCV), blood pigment, blood minerals, serum cholesterol and serum vitamin E to determine the physiological status of the calves. In the last two weeks of feeding, calves were randomly divided into two groups: the first group (N = 15), or the control group, continued to receive the milk replacer diet without
Thirty Holstein Bull Calves
Purchased 3 days old
Placed on Milk Replacer Diet (MRD)

Jugular vein blood analysed at
12th week of feeding for

<table>
<thead>
<tr>
<th>CHOLESTEROL</th>
<th>P</th>
<th>Mg</th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>PCV</th>
<th>Hemoglobin</th>
<th>Vitamin E</th>
</tr>
</thead>
</table>

After blood analyses, calves were randomly divided into two equal groups

Treated (N = 15)
MRD + 18 g monosodium phosphate
+ 100 mg Vitamin E
+ 350 mg chlorotetracycline
per calf per day

Control (N = 15)
MRD
No Phosphate & Vit E
Fed 350 mg chlorotetracycline per calf per day

Blood re-analysed as described a day prior to slaughter

All calves slaughtered at 16 weeks of age
Abomasum pH measured on the kill floor

Muscle analyses

<table>
<thead>
<tr>
<th>pH</th>
<th>P</th>
<th>Mg</th>
<th>Mn</th>
<th>Fe</th>
<th>Ca</th>
<th>Zn</th>
<th>Cu</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
</table>

Sensory Color Reflectance Pigments Cholesterol Proximate

Statistical

Figure 8. Experimental Design

High phosphorus fed as monosodium phosphate two weeks prior to slaughter. Vitamin E fed as coated alpha-tocopherol acetate. Each calf received 2.7 to 3.2 kg of milk replacer diet (MRD) on a dry matter basis and 13 kg of water daily.
PROFESSIONAL VEAL STARTER

MEDICATED
For aid in stimulating growth
and improving feed efficiency

ACTIVE DRUG INGREDIENT
Oxytetracycline .............. 50 grams per ton

► WARNING: Withdraw this feed 5 days before slaughter ◄

GUARANTEED ANALYSIS
Crude Protein, not less than ..................... 21.0%
Crude Fat, not less than ......................... 16.0%
Crude Fiber, not more than ............... 0.2%
Vitamin A, not less than ................ 15,000 I.U./lb.
Vitamin D₃, not less than ................ 3,000 I.U./lb.

INGREDIENTS
Dried Skimmed Milk, Dried Whey, Dried Whey Product, Dried Milk Albumin, Vegetable and Animal Fat (Preserved with BHA), Starch, Lecithin, Dextrose, Polyoxy-ethylene Glycol (400) Mono and Dioleates, DL-Methionine, L-Lysine, Vitamin A Supplement, Vitamin D₃ Supplement, Vitamin E Supplement, Vitamin K Supplement, Vitamin B₁₂ Supplement, Folic Acid, Choline Chloride, Riboflavin, Niacin, Calcium Pantothenate, Thiamine Mononitrate, Ascorbic Acid (Vitamin C), Calcium Carbonate, Copper Sulfate, Cobalt Sulfate, Zinc Sulfate, Ethylenediamine Dihydriodide, Sodium Selenite, Sodium Silico Aluminate, Manganese Sulfate, Magnesium Oxide, Artificial Flavor

Manufactured by
MILK SPECIALTIES CO.
DUNDEE, ILLINOIS 60118
50007

Figure 9. The composition of the basal diet fed to veal calves.
mineral supplementation. The second group (N = 15) also continued to receive the milk replacer diet, but with mineral supplementation of phosphorus fed as monosodium phosphate (Stauffer Chemical Co., Westport, CT). Calves were fed 9-12 g twice daily (18-24 g/day) and a total of 100 mg of vitamin E as dl-alpha tocopherol acetate coated vitamin E dry powder (Professional Veal Formulars; Milk Specialties Co., Dundee, Ill.) per calf per day.

Two days before Kosher slaughtering of the calves, blood constituents were re-analyzed as previously described. The pH of the abomasum fluid was measured on the kill floor and the semimembranosus muscle pH was determined at 15, 45, 90 minutes, 24 hours and 48 hours postmortem. The semimembranosus muscle (400 g approximately) samples were taken in the slaughter house, and transported over ice to The Ohio State University Meat Laboratory in a plastic package. Sensory color evaluation was determined at 2.2°C, 48 hours postmortem by six trained sensory panelists and an objective color value was determined by reflectance spectrophotometry at the same time. All muscle samples were trimmed of any visible subcutaneous fat and connective tissues, double wrapped in a freezer paper and stored at -25°C for further chemical analyses.

**Total Blood Hemoglobin and Packed Cell Volume**

Portion of the whole blood collected from the jugular vein of the calves after overnight fasting was used for the colorimetric determination of total blood hemoglobin and pack cell volume using the Sigma #525 procedure. (See Appendix A.1 for details.)
Serum Minerals

Essentially, the blood minerals were determined according to the procedure of Wise, et al. (1947), except for the inorganic phosphorus which was determined by the Sigma #670 procedure. Descriptions of the procedures used for the inorganic phosphorus determination are in Appendix A-11.

Serum Total Cholesterol

A portion of the serum was used for the total serum cholesterol colorimetrically according to Sigma #351 procedure, with some modification. The details are outlined in Appendix A. III.

Muscle Cholesterol

A modification of the gas chromatography procedure of Kunsman et al. (1981), for muscle tissue was used in this experiment for cholesterol analyses. A mass spectrophotometer was later used to confirm the separation of cholesterol. The Chromatogram and mass spectrum of the cholesterol from the veal muscle are shown in Figures 12a and 12b, respectively. The procedures are outlined in Appendix A. IV.

Muscle Pigments Determination

Myoglobin, hemoglobin and total muscle pigments of the veal muscle samples were determined using the carbon monoxide procedure of Fleeming et al. (1960). (See Appendix A. V for details.)

Muscle Minerals

The Atomic Absorption Spectrophotometry procedures (Anonymous, 1984) was used to analyze the soft tissue for iron, calcium, phosphorus,
sodium, potassium, copper, manganese, magnesium and zinc. (See Appendix A. VI for details.)

**Muscle pH Measurement**

About 10 g of the semimembranosus muscle was used for the surface pH determination at 15, 45, 90 minutes, 24 hours and 48 hours post-mortem using a contact probe electrode as described in Appendix A. VII.

**Abomasum Fluid pH Measurement**

The abomasum was pierced and contents collected in a 50 ml polyethylene bottle. pH of the fluid was determined using Ockerman (1980) procedure for slurry as outlined in Appendix A. VIII.

**Muscle Color Evaluation**

Reflectance procedure of Ockerman (1985) was used for objective evaluation while sensory color was accomplished as outlined in Appendix A. IX.

**Proximate Analyses**

Proximate analyses were determined as outlined by Ockerman (1981). (See Appendix A. X for details.)

**Statistical Analysis**

The data were analyzed using the General Linear Model, Stepwise and T-test pairwise comparison of SAS (1987). The mathematical model for the one-way classification with a regression used was as follows:

\[ y_{ij} = \mu + a_i + b(X_{ij} - \bar{X}) + e_{ij} \]

\[ i = 1, 2, \ldots, p \]

\[ j = 1, 2, \ldots, n_i \]
where $y_{ij} = \text{muscle color}$

$\mu = \text{overall mean}$

$a_i = \text{the treatment effect}$

$b = \text{partial regression coefficient of the dependent variable (yi) on the continuous independent variable (Xi)}$

$X_{ij} = \text{the fixed continuous independent variate for the corresponding yij observation}$

$\bar{X} = \text{the arithmetic mean of the Xij}$

$e_{ij} = \text{the random errors assumed to be normally distributed}$

**Variables**

- **ID** = Calf Identification number
- **TRT** = Treatment
- **PCVB** = Pack Cell Volume before treatment
- **PCVA** = Pack Cell Volume after treatment
- **HBB** = Hemoglobin before treatment
- **HBA** = Hemoglobin after treatment
- **THBB** = Total hemoglobin before treatment
- **THBA** = Total hemoglobin after treatment
- **MYO** = Myoglobin
- **HBM** = Muscle hemoglobin
- **TPG** = Muscle total pigment
- **REF** = Reflectance
- **PANCS** = Panel color score
- **ABOPH** = Abomasum pH
- **pH** = pH
- **VITE** = Vitamin E
BLDK = Blood potassium
BLDNA = Blood sodium
BLDCA = Blood calcium
BLDMG = Blood magnesium
BLDP = Blood phosphorus
BLDCHOLB = Blood cholesterol before treatment
BLDCHOLA = Blood cholesterol after treatment
MUSP = Muscle phosphorus
MUSZN = Muscle zinc
MUSCU = Muscle copper
MUSFE = Muscle iron
MUSCA = Muscle calcium
MUSK = Muscle potassium
MUSNA = Muscle sodium
MUSMG = Muscle magnesium
MUSMN = Muscle manganese
MOIST = Percent muscle moisture
FAT = Percent muscle fat
PROT = Percent muscle protein
ASH = Percent muscle ash
CALFAGO = Calf age on test
CALFAGS = Calf age at slaughter
CHAPTER IV
RESULTS AND DISCUSSION

This study was designed to determine the effects of feeding high level of phosphorus and vitamin E to calves on the quantity of blood pigments, blood composition, muscle composition and muscle color. As described in Figure 8, this research consisted of three major portions as follows:

I Blood analyses for percent hematocrit, total blood hemoglobin, blood minerals, serum cholesterol and serum vitamin E.

II Determination of pH of abomasum content, measurement of muscle minerals, muscle cholesterol, pH, myoglobin, total pigments and proximate analysis.

III Color evaluation of the muscle by sensory and objective methods.

Hematocrit, Serum Cholesterol and Vitamin E

Means and the standard errors for percent hematocrit, the hemoglobin values, serum cholesterol and serum vitamin E are shown in Table 1. Dietary supplementation of monosodium phosphate and alpha tocopherol did not result in any significant difference (P>0.05) in the blood hemoglobin values. The values of 9.62 vs. 9.64 g/100 ml whole blood
TABLE 1. MEANS (X) AND STANDARD ERRORS (S.E.) FOR SELECTED BLOOD CONSTITUENTS AS AFFECTED BY HIGH MONOSODIUM PHOSPHATE AND VITAMIN E SUPPLEMENTATION.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treated (N=15)</th>
<th>Control (N=15)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>28.86</td>
<td>28.93</td>
<td>.44</td>
</tr>
<tr>
<td>Hemoglobin g/100 ml</td>
<td>9.62</td>
<td>9.64</td>
<td>.16</td>
</tr>
<tr>
<td>Vitamin E ug/ml serum</td>
<td>7.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.28</td>
</tr>
<tr>
<td>Cholesterol mg/100 ml</td>
<td>142.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>152.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.54</td>
</tr>
</tbody>
</table>

<sup>c,d</sup>Means on the same line with superscript c are significantly higher (P<0.05) than those with superscript d.
(S.E. .16) reported in the treated and control groups, respectively, is in agreement with the report by Greatorex (1954), and Wrenn et al. (1980). In the Greatorex (1954) study, the majority of hemoglobin values reported from 233 calves were between 9.0 and 14.5 g/100 ml whole blood. There was no sign of anemia observed in the two groups studied in the present report. Holman (1956) reported three types of anemia based on the level of hemoglobin: pathological anemia occurs when calves hemoglobin values fall below 8 g/100 ml, mild anemia 7 g/100 ml, moderate anemia 5-6 g/100 ml and severe anemia 4 g/100 ml and less. In the present study, the nonsignificant (P>0.05) difference between the phosphorus supplemented and the control calves for the hemoglobin and percent hematocrit values is probably due to the ability of the growing calves to protect their hemoglobin synthesis. In separate studies (St. Laurent and Brisson, 1968, and MacDougall et al., 1973), increase in dietary level of iron from 10 to 50 ug Fe/g of milk powder almost eliminated anemia in veal calves, but resulted in no increase in the muscle pigment concentration, suggesting that hemoglobin formation takes precedence over myoglobin synthesis. Bhargava and Gabbe (1984) reported that rats were able to compensate blood loses of up to 5% of the total blood volume per day at the expense of histochemically graded iron stores without developing anemia when the animals are fed an iron-replete diet.

Means and standard errors for serum cholesterol and vitamin E is also depicted in Table 1. Level of cholesterol was significantly lowered (P<0.05) and vitamin E significantly higher (P<0.05) in the serum of the supplemented group than in the control after the
experimental treatments. The higher level of vitamin E in the serum of
the supplemented group agreed with work of Grifo et al. (1959), who re-
ported a higher (P<0.05) level of alpha tocopherol in the blood, liver,
kidney and heart of calves receiving dietary vitamin E supplementation.
Many controllable exogenous factors such as diet, exercise and uncon-
trollable factors such as genetic traits can affect cholesterol synthe-
sis and its subsequent level in the blood (Brown et al., 1981). It is
possible that some biochemical action of vitamin E might cause the
lowering of serum cholesterol in this study. Arneson (1984), reported
that vitamin E has been shown to reduce serum cholesterol in man. It
is also possible that inhibition of the major enzyme in cholesterol
synthesis (Hydroxymethyl glutaryl Coa reductase) in the liver by a
kinase enzyme utilizing phosphorus (Gibbons et al., 1982), coupled with
the action of vitamin E in the liver, might be the reason for the
lowering of the cholesterol observed in the treated group. Vitamin E
being a fat soluble vitamin might also have interfered with the lipid
metabolism of the calves, resulting in an increase in the production of
the bile salts which can lead to the lowering of plasma cholesterol.
Another possibility is that the calves might be reacting to high phos-
phorus and vitamin E intake by their detoxification mechanism resulting
in a reduction of cholesterol synthesis. Jackson et al. (1959) attrib-
uted the changes in the blood cholesterol level in cattle grazed on
alfalfa forage to the reaction of the animals to high intake of a toxic
constituent in the alfalfa. Also, since liver is the major site of
cholesterol synthesis and degradation (Brown et al., 1981), and since
most dietary vitamin E is accumulated in the liver (Grifo et al.,
1959), it is conceivable to think that the synergistic effect of vitamin E and phosphorus given to the calves in this study might be responsible for lowering of the blood cholesterol measured. It is also possible that the difference in cholesterol level between the two groups reported here is simply due to a type two errors. The role of vitamin E and phosphorus on cholesterol synthesis warrants further investigation.

**Blood Minerals**

The results of the analyses of the mineral constituents from the serum of some randomly selected veal calves treated and not treated with monosodium phosphate and alpha tocopherol are presented in Table 2. Inorganic phosphorus was significantly higher (P<0.05) in the serum of calves receiving monosodium phosphate and tocopherol supplementation. The increase in the level of serum inorganic phosphorus reported in Table 2 for the phosphorus supplemented group is a reflection of the dietary intake of this nutrient, and is consistent with previous studies Becker and Smith (1950), Miller et al. (1964a) and Reinhardt and Conrad 1980. There was no difference (P>0.05) in the level of blood potassium and magnesium measured in the two groups and this is probably because these elements are found mainly in the cellular components of the whole blood (Wise et al., 1947 and McCay, 1931) not in serum. In this study, one of our main objectives was to study the dietary effect of phosphate supplementation on the level of the inorganic phosphorus which is found mainly in the serum. This alone explains our choice of serum for the analyses.
TABLE 2. MEANS (\(\bar{X}\)) AND STANDARD ERRORS (S.E.) FOR BLOOD MINERALS AND VITAMIN E AS AFFECTED BY HIGH MONOSODIUM PHOSPHATE AND VITAMIN E SUPPLEMENTATION.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treated (N=10)</th>
<th>Control (N=10)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>18.4</td>
<td>19.9</td>
<td>.51</td>
</tr>
<tr>
<td>Na</td>
<td>251d</td>
<td>294c</td>
<td>4.65**</td>
</tr>
<tr>
<td>Ca</td>
<td>10.6c</td>
<td>9.76d</td>
<td>.05*</td>
</tr>
<tr>
<td>Mg</td>
<td>2.32</td>
<td>2.29</td>
<td>.02</td>
</tr>
<tr>
<td>P</td>
<td>9.01c</td>
<td>8.13d</td>
<td>.11*</td>
</tr>
</tbody>
</table>

1All variables are given in ug/ml serum.

c,dMeans on the same row with superscript c are significantly higher than those with superscript d.

**Significant (P<.01)

*Significant (P<.05)
There was a highly significant (P<0.01) reduction in level of sodium in the blood of treated calves. This amounts to approximately 15% reduction. It is possible that sodium, being an element found mainly in the extracellular fluid, is being lost via urination as a result of the animals reacting to the effect of high phosphorus intake. The significant (P<0.05) increase in level of calcium observed in the treated group might be due to sodium-calcium pump effect, since calcium might be replacing the sodium being lost from the systems. Forest et al. (1975) reported the possibility of a monovalent ion such as sodium being replaced by a divalent ion such as calcium during aging of meat. Roy et al. (1964) reported that high absorption of calcium could be partially accounted for by the increased digestibility of fat, and a concomitant decrease in a fecal excretion of calcium as calcium soap of fatty acids. In a different report, Church et al. (1972), reported that the presence of undigested fat in calves reduced digestion and absorption of minerals, particularly calcium and magnesium. Another possibility is the systematic effect of iron in increasing calcium retention as a result of an increase in oxygen supply to the tissues to compensate for the reduction in the muscle iron (Table 3) in the phosphate treated group. High level of blood calcium may be beneficial in reducing the incident of coronary heart disease in man. Lloyd et al. (1978) reported that incidences of coronary heart disease was higher in a soft-water community (rich in Na) than in a hard-water community (rich in Ca).
Muscle Minerals

Means and standard errors for the semimembranosus tissue minerals are presented in Table 3. There was no difference (P>0.05) in the level of phosphorus, zinc, manganese and magnesium measured in the muscle of the two groups. Thus, the only increase in inorganic phosphorus due to treatment occurred in the blood (Table 2). The level of muscle iron was significantly lowered (P<0.05) in the calves receiving monosodium phosphate and tocopherol supplementation. The lower level of iron might be due to the inhibitory action of high phosphorus intake for iron absorption. It is recognized that iron and phosphorus are interrelated nutritionally (Standish et al., 1971) and a decrease in iron absorption due to increase in dietary intake of phosphorus in lamb has been reported (Lassister, 1976). This alone might account for the reduction in muscle iron observed in this study. There is also the possibility that monosodium phosphate supplementation resulted in the formation of a non-absorbable iron phosphate complex. Formon (1974) reported that high intake of phosphorus interfered with absorption of iron probably by competing for metal-binding sites in the intestinal mucosa. In humans, desferrioxamine has been used successfully as a chelating agent to inhibit iron absorption by increasing its urinary excretion (Moeschlin and Schnider, 1962). It is also possible that the abomasum pH of (2.96 vs. 3.0 S.E. .05) reported in Table 5 is a factor that could favor the absorption of the iron complex formed in the gut. Conrad (1984), in his extensive review on iron metabolism in the ruminant, reported that the conditions for existence of iron
TABLE 3. MEANS ($\bar{x}$) AND STANDARD ERRORS (S.E.) FOR SEMIMEMBRANOSUS MUSCLE MINERALS AS AFFECTED BY HIGH MONOSODIUM PHOSPHATE AND ALPHA TOCOPHEROL SUPPLEMENTATION.

<table>
<thead>
<tr>
<th>Variables$^1$</th>
<th>Treated$^2$ (N=13)</th>
<th>Control$^2$ (N=13)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>1312.7</td>
<td>1310.2</td>
<td>9.81</td>
</tr>
<tr>
<td>Mg</td>
<td>171.2</td>
<td>170.2</td>
<td>1.41</td>
</tr>
<tr>
<td>Ca</td>
<td>30.3c</td>
<td>27.4d</td>
<td>1.09</td>
</tr>
<tr>
<td>Mn</td>
<td>0.08</td>
<td>0.08</td>
<td>.01</td>
</tr>
<tr>
<td>Fe</td>
<td>7.95d</td>
<td>9.14c</td>
<td>.32</td>
</tr>
<tr>
<td>Zn</td>
<td>15.7</td>
<td>15.9</td>
<td>1.09</td>
</tr>
<tr>
<td>Na</td>
<td>339.6</td>
<td>348.8</td>
<td>4.86</td>
</tr>
<tr>
<td>K</td>
<td>2254.0c</td>
<td>2213.0d</td>
<td>18.7</td>
</tr>
<tr>
<td>Cu</td>
<td>0.24d</td>
<td>0.30c</td>
<td>.01</td>
</tr>
</tbody>
</table>

$^1$All in ug/g of tissue.

$^2$Two samples/group were lost at the slaughterhouse.

$c,d$Means on the same row with superscript c are significantly higher ($P<0.05$) than those with superscript d.
utilization and absorption in the gut is pH dependent. Both ferrous and ferric iron are absorbed equally at the immediate pyloric area of the duodenum as long as iron remains in the ionized form at pH of about 2. However, pH of above 3 causes the formation of sparingly soluble ferric hydroxide preventing the absorption of this element (Forth and Rumel, 1974).

Causes of some changes observed in some of the minerals in this study cannot be easily explained, but might simply indicate some shifting of elements between tissues. The significant (P<0.05) increase in the level of calcium and potassium might be due to the same reasons given for these elements in the blood. The reasons for the lower level (P<0.05) of muscle copper in the treated group cannot be easily explained. Le Chatleir's (1978) stated principle that "if a constraint or stress is imposed on a system in equilibrium, the equilibrium will adjust itself to remove the effect of the imposed constraint" might be an appropriate physiological principle to explain some of the changes noted in the blood and muscle minerals (Tables 2 and 3). Eighteen grams of phosphorus fed per calf per day to the growing calves in this study is high and could be stressful. The NRC (1971) recommended daily requirement of phosphorus for 132-170 kg growing bull calf is 2.5 g/day. In this study, calculation by weight indicated that 18 g of NaH₂PO₄ will be supplying approximately 5.0 g of P per calf per day. Barber et al. (1955) reported that high dietary copper supplementation of diets for growing finishing swine produced no deleterious effects to the swine provided it is fed for short periods of time. This supports the
reason for feeding these calves the high phosphorus and vitamin E for only two weeks prior to slaughter. Thus, it is possible to think that some of the calves in this study tend to adjust their physiological systems to remove the probable "stress" that high phosphorus intake might cause. This is shown by high loss of the element sodium found mainly in the extracellular fluid, lower absorption of iron and compensatory effect of increased high calcium absorption.

Muscle Pigments, Sensory Color Score and Reflectance

The results of treatment effect on muscle myoglobin, hemoglobin, muscle total pigment, sensory color score, percent muscle reflectance and muscle cholesterol are presented in Table 4. All the muscle pigments reported were significantly (P<0.05) lowered in the phosphorus and tocopherol treated group than in the muscle of the control calves. The lower level of the major muscle pigment (myoglobin) might be attributed to the lower muscle iron reported in Table 3. Certain levels of dietary iron fed to veal calves resulted in higher myoglobin concentration and dark colored veal (Bray et al., 1959, Niedermier et al., 1955 and 1959). As hypothesized in this study, and as discussed in the literature review, phosphate could inhibit absorption and cause excretion of tissue iron based on the mechanism previously described. The significantly (P<0.05) lower level of copper in the treated group might have some synergistic effect with iron content in decreasing the myoglobin concentration in the tissue. Bray et al. (1959) reported a significantly higher myoglobin content in the longissimus dorsi muscle.
TABLE 4. MEANS (X) AND STANDARD ERROR (S.E.) FOR MUSCLE PIGMENTS, MUSCLE CHOLESTEROL, REFLECTANCE AND SENSORY COLOR SCORE FOR VEAL CALVES TREATED WITH OR WITHOUT MONOSODIUM PHOSPHATE AND TOCOPHEROL SUPPLEMENTATION.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treated (N=13)</th>
<th>Control (N=13)</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin mg/g muscle</td>
<td>1.53\textsuperscript{d}</td>
<td>1.89\textsuperscript{c}</td>
<td>.06</td>
</tr>
<tr>
<td>Hemoglobin mg/g muscle</td>
<td>1.17\textsuperscript{d}</td>
<td>1.43\textsuperscript{c}</td>
<td>.05</td>
</tr>
<tr>
<td>Total Pigment mg/g muscle</td>
<td>2.71\textsuperscript{d}</td>
<td>3.30\textsuperscript{c}</td>
<td>.11</td>
</tr>
<tr>
<td>Panel Score (PS)</td>
<td>1.86\textsuperscript{d}</td>
<td>2.33\textsuperscript{c}</td>
<td>.07</td>
</tr>
<tr>
<td>Reflectance (RF:685nm)%</td>
<td>49.8\textsuperscript{c}</td>
<td>46.6\textsuperscript{d}</td>
<td>.38</td>
</tr>
<tr>
<td>Cholesterol mg/100 g</td>
<td>39.19\textsuperscript{d}</td>
<td>43.13\textsuperscript{c}</td>
<td>1.63</td>
</tr>
</tbody>
</table>

\textsuperscript{c,d}Means on the same line with superscript c are significantly higher (P<0.05) than those with superscript d.

PS = Sensory panel score on scale of 1-4; 1=lightest and 4=darkest color (Figure 10).

RF = Higher reflectance indicates lighter muscle.
of veal calves on dietary iron and copper supplementation. The lowered levels of the muscle pigments resulted in a significant (P<0.05) increase in percent muscle reflectance (Table 4) in the phosphate treated group when compared with the control. The higher reflectance means lighter muscle color (Ockerman, 1980) and this result agrees with the report of Henry and Bratzler (1960), MacDougall (1973) and Claus (1984). The same trend was observed for sensory panel color scores (Table 4). The values for myoglobin (1.53 vs. 1.89, SE .06) reported in this study are higher than the 0.29 vs. 0.54 mg/g of myoglobin reported for veal (Bray et al., 1959). In that study, calves were slaughtered at 6 weeks of age as compared to 16 weeks in the present report, suggesting that other factors, such as age of the animal, might be an important factor affecting myoglobin concentration and subsequent color of meat. Lawrie (1950), Henry and Bratzler (1960), Forest et al. (1975) and Field et al. (1980), reported that myoglobin concentration increased with age and maturity of the animal. The significantly (P<0.05) higher level of the hemoglobin in the muscle of the control group compared with the treated group is very difficult to explain. Since all the calves were slaughtered by the same Kosher method, one would expect nearly the same level of the residual blood in the carcasses of the calves involved. However, factors such as variation in the animal's ability to withstand stress and differences in blood vascularity, might cause some of the animals to retain their blood more than the others. It must be mentioned that hemoglobin content of the tissue does contribute to muscle color (St. Laurent and Brisson, 1967; Warris and Rhodes, 1977). Warris
(1977), reported that the contribution of hemoglobin to the total heme pigment concentration is comparatively large in meat with a low concentration of myoglobin, such as pork or veal. Additional studies must be done to determine whether the differences in veal muscle myoglobin concentration reported in the literature were more influenced by age of the animals than by experimental treatments.

Muscle and Abomasum pH

The pH of the semimembranosus muscle of the veal calves treated with or without dietary phosphorus and vitamin E is presented in Table 5. There was no significant (P>0.05) difference between the groups in the pH measured at 15 minutes postmortem, but there was a significantly (P<0.05) lower pH value in the muscle of the phosphate group as the muscle aged to 48 hours postmortem. The nonsignificant difference (P>0.05) in initial pH determined might mean that calves underwent similar rate of postmortem glycolysis within the first 15 minutes after exsanguination. However, as the muscle ages, it is possible that the phosphate group metabolized their reserve glycogen more rapidly resulting in lower muscle pH in this group than the control group. The values of the ultimate muscle pH reported in Table 5 are in agreement with MadDougall et al. (1973) who reported the ultimate pH values of the veal muscle in the range of 5.3 to 5.6. The higher the muscle pH, the darker the muscle color (Lawrie, 1950, Ockerman, 1980 and Siederman, 1984). The mechanism by which the pH of the muscle could affect the subsequent muscle color was reported by Walters (1975). The low
### TABLE 5. MEANS (\( \bar{X} \)) AND STANDARD ERRORS (S.E.) FOR SEMIMEMBRANOSUS AND ABOMASUM pH OF VEAL CALVES TREATED OR NOT TREATED WITH MONOSODIUM PHOSPHATE AND ALPHS TOCOPHEROL

<table>
<thead>
<tr>
<th></th>
<th>Treated (( N=13 ))</th>
<th>Control (( N=13 ))</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min. postmortem</td>
<td>7.04</td>
<td>7.06</td>
<td>.02</td>
</tr>
<tr>
<td>90 min.</td>
<td>6.64(^d)</td>
<td>6.72(^c)</td>
<td>.01</td>
</tr>
<tr>
<td>24 hr.</td>
<td>5.57(^d)</td>
<td>5.65(^c)</td>
<td>.01</td>
</tr>
<tr>
<td>48 hr.</td>
<td>5.50(^d)</td>
<td>5.59(^c)</td>
<td>.01</td>
</tr>
<tr>
<td><strong>Abomasum pH</strong></td>
<td>2.96</td>
<td>3.04</td>
<td>.05</td>
</tr>
</tbody>
</table>

\(^{c,d}\)Means on the same line with superscript \( c \) are significantly higher (\( P<.05 \)) than those with superscript \( d \).
ultimate pH could cause the muscle fibrils to be more "open," and scatter light to produce a paler color meat. This would suggest that pH of veal is an important factor that might contribute to veal muscle color, confirming the previous reports (Swatland, 1985).

The nonsignificant (P>0.05) difference in the abomasum pH in Table 5 is probably due to acidity of this fluid which causes it to buffer itself from any treatment effect. There was more variation in the abomasum pH within the group than between the groups. The lowest abomasum pH measured was 1.64 in the treated group and the highest was 4.06 in the control group.

Muscle Cholesterol

A chromatogram and spectrogram of veal muscle cholesterol from the veal calf are depicted in Figures 10 and 11. The cholesterol standard curve used for quantification of muscle cholesterol is shown in Figure 12.

The significantly (P<0.05) lower muscle cholesterol in the calves treated with monosodium phosphate and vitamin E might be a reflection of the lower content of this sterol (Table 1) in the circulatory blood of the treated group compared to the control. It is also possible that the high density lipoprotein (HDL) is removing more extrahepatic cholesterol more rapidly from the tocopherol and phosphate group, recirculating this in form of chylomicron remnants back to the liver where cholesterol is degraded and excreted (Brown et al, 1981). Bauchart et al (1985) reported the values of 41 to 66 mg/100 g fresh tissue of
Figure 10. A chromatogram of cholesterol from the muscle of veal calf.
Figure 11. A spectrogram of cholesterol from the muscle of veal calf.
Figure 12. A cholesterol standard curve.
growing calves fed dietary sorbitol which seems to be in the range of
the muscle cholesterol reported in Table 4. Kunsm an et al. (1981) had
earlier noted that the range of cholesterol available in meat is wide
and is usually affected by not only dietary factors, age, and sex, but
also by analytical method used.

Stepwise Regression for Sensory Color Score

Correlation coefficients of individual variables as they affect
color reflectance and sensory score, is shown in Tables 6 and 7, respec­tively, while stepwise regression analysis of some variables as they
affect the veal color and as evaluated by sensory panelists is shown in
Table 8. These results show that blood hemoglobin, muscle pH, blood
magnesium, copper, iron and manganese are the best variables that con­tribute significantly ($R^2 = 0.9922$) to the model used. The contribution
of the residual blood or hemoglobin to the meat color has been reported
(Warris, 1977) and the influence of pH on the subsequent color of veal
has been discussed (Table 5). The effect of magnesium on muscle color
is obscure and cannot be easily explained at this time. The presence
of iron and copper in the model as they affect muscle color is in agree­ment with Bray et al. (1959) who reported that dietary iron and copper
supplementation of calves diet resulted in high myoglobin concentration
and dark color veal. From Table 8, it can be said that hemoglobin and
iron with the smallest standard errors $0.0331$ ($P>0.0079$) and $0.0240$
($P>0.0003$), and high values of $r$ and $R^2$, respectively, have a greater
effect on veal muscle color than other variables, especially copper
TABLE 6. CORRELATION COEFFICIENTS SHOWING THE SIMPLE CORRELATION (r) AND R² OF AN INDIVIDUAL VARIABLE CONSTITUTING TO THE BEST FITTING MODEL FOR PREDICTING COLOR BY REFLECTANCE METHOD.

<table>
<thead>
<tr>
<th>Single Variables</th>
<th>r</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>-0.86</td>
<td>0.74</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>-0.86</td>
<td>0.74</td>
</tr>
<tr>
<td>Hemoglobin, muscle</td>
<td>-0.85</td>
<td>0.72</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.62</td>
<td>0.38</td>
</tr>
<tr>
<td>Abomasum pH</td>
<td>-0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Muscle pH</td>
<td>-0.70</td>
<td>0.49</td>
</tr>
<tr>
<td>Potassium, muscle</td>
<td>-0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>Magnesium, muscle</td>
<td>-0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.35</td>
<td>0.13</td>
</tr>
</tbody>
</table>
TABLE 7. CORRELATION COEFFICIENT SHOWING THE SIMPLE CORRELATION (r) AND R² OF AN INDIVIDUAL VARIABLE CONSTITUTING TO THE BEST FITTING MODEL FOR PREDICTING COLOR BY SENSORY COLOR METHOD.

<table>
<thead>
<tr>
<th>Single Variables</th>
<th>r</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>0.81</td>
<td>0.65</td>
</tr>
<tr>
<td>Muscle pH</td>
<td>0.63</td>
<td>0.39</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Copper</td>
<td>-0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Iron</td>
<td>0.81</td>
<td>0.66</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.37</td>
<td>0.14</td>
</tr>
</tbody>
</table>
## TABLE 8. REGRESSION ANALYSIS SHOWING THE BEST SIX VARIABLES FOUND TO AFFECT VEAL MUSCLE COLOR AS EVALUATED BY SENSORY PANEL

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>6</td>
<td>7.7574</td>
<td>1.2929</td>
<td>107.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>5</td>
<td>0.0604</td>
<td>0.0120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7.8179</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td>-17.7080</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>B Value</th>
<th>Type II SS</th>
<th>Std. Error</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>0.1420</td>
<td>0.2214</td>
<td>0.0331</td>
<td>18.32</td>
<td>0.0079</td>
</tr>
<tr>
<td>Muscle pH</td>
<td>3.6876</td>
<td>1.5804</td>
<td>0.3224</td>
<td>130.79</td>
<td>0.0001</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-2.9545</td>
<td>1.0210</td>
<td>0.3214</td>
<td>84.50</td>
<td>0.0003</td>
</tr>
<tr>
<td>Copper</td>
<td>-4.2645</td>
<td>0.2234</td>
<td>0.9917</td>
<td>18.49</td>
<td>0.0077</td>
</tr>
<tr>
<td>Iron</td>
<td>0.2148</td>
<td>0.9680</td>
<td>0.0240</td>
<td>80.11</td>
<td>0.0003</td>
</tr>
<tr>
<td>Manganese</td>
<td>1.6270</td>
<td>0.1248</td>
<td>0.5063</td>
<td>10.33</td>
<td>0.0236</td>
</tr>
</tbody>
</table>

\[ R^2 = 0.9922 \]
and manganese with high standard errors of 0.9917 (P>0.0077) and 0.5063 (P>0.0236), and lower \( r \) values, respectively.

**Maximum R square regression for sensory color score**

The stepwise regression analysis was followed by a more useful Maximum \( R^2 \) (MAXR) improvement technique (SAS, 1987) to "screen" and find from all the variables the best one variable model that affects veal muscle color. When this analysis was performed, Table 9, the best variable found to affect veal muscle color as evaluated by sensory method was iron \( (R^2 = 0.6622) \) standard error \( (0.0584) \). This will confirm our hypothesis that iron regulation should be the major concern in veal production if acceptable pale color veal is to be produced. As has been discussed in this study, high dietary phosphorus supplementation could have a valuable practical effect as it reduces muscle iron (Table 3) resulting in lighter veal (Table 4 and Figure 15). The \( R^2 \) value of 0.6622 (Table 7) suggests that 66.22% of the variation affecting veal color as evaluated by the sensory method was accounted for by iron alone. Other variables such as animal age, fat content, moisture, lighting conditions and variation among the individual judge's ability to perceive color differently, account for only 33.78% of the variation. In Table 10, it can be seen that the second variable found to have additional effect on veal muscle color by MAXR improvement method is muscle pH and this increases the value of \( R^2 \) to 0.8186 or 81.86%. This result validates Swatland's (1985) hypothesis that, when considering veal muscle color, the ultimate postmorten muscle pH effect is an important
TABLE 9. REGRESSION ANALYSIS SHOWING MAXIMUM R-SQUARE (MAXR) IMPROVEMENT FOR PICKING OUT THE BEST ONE VARIABLE FOUND TO AFFECT VEAL MUSCLE COLOR AS DETERMINED BY SENSORY PANELISTS

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>5.1771</td>
<td>5.1771</td>
<td>19.61</td>
<td>0.0013</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>2.6406</td>
<td>0.2640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7.8178</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td>-0.1402</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B VALUE** | **TYPE II SS** | **STD. ERROR** | **F** | **P>F**
-------------|----------------|----------------|-------|-------
Muscle Iron   | 0.2589         | 5.1771         | 0.0584| 19.61 | 0.0013 |

$R^2 = 0.6622$
### TABLE 10. REGRESSION ANALYSIS SHOWING MAXIMUM R-SQUARE (MAXR) IMPROVEMENT FOR PICKING OUT THE BEST TWO VARIABLES FOUND TO AFFECT VEAL MUSCLE COLOR AS DETERMINED BY SENSORY PANEL

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>6.4002</td>
<td>3.2001</td>
<td>20.32</td>
<td>0.0005</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>1.4175</td>
<td>0.1575</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7.8178</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-18.6709</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>B VALUE</th>
<th>TYPE II</th>
<th>STD. ERROR</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 90 (min)</td>
<td>2.8328</td>
<td>1.2230</td>
<td>1.0166</td>
<td>7.77</td>
<td>0.0212</td>
</tr>
<tr>
<td>Muscle Iron</td>
<td>0.2172</td>
<td>3.2816</td>
<td>0.0475</td>
<td>20.83</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

\[ R^2 = 0.8186 \]
factor. All the values reported as (B) in the tables indicated regression of each of the variables reported in the model.

**Stepwise and max R square regression for reflectance**

Results of a stepwise regression on the variables that contributed to veal muscle color as measured by the reflectance method is depicted in Table 11. In Table 11, it can be seen that the variables with the lowest standard errors make the most significant contribution to veal muscle color by reflectance methods. They include hematocrit value (SE = 0.0028), myoglobin (SE = 0.0071) vitamin E (SE = 0.0053), moisture (SE = 0.0059) and muscle potassium (SE = 0.0011). Upon running the MAXR improvement procedure to find the best one variable affecting the veal color by reflectance procedure, hematocrit value (Table 12) was the best variable found (R^2 = 0.7485 or 74.85%). This result suggests that hematocrit value alone can be used as an index of iron status in veal production. St. Laurent and Brisson (1967) reported that calves with maximum amounts of hemoglobin might have enough iron to protect their essential iron in myoglobin and would be expected to have darker meat. In the present study, it was observed that any calf with percent hematocrit value equal or greater than 33% is very likely to produce dark colored veal and it might be wise to eliminate such animal from the veal program. Bernier et al. (1984) observed that color was regarded as being undesirable for veal when blood hemoglobin was greater than 10 g/100 ml whole blood. Practically, such animals could be placed on a control diet as has been reported in this study.

The centrifuge method used in this experiment for hematocrit analysis
### TABLE 11. REGRESSION ANALYSIS FOR PICKING UP THE BEST VARIABLES AFFECTING VEAL MUSCLE COLOR AS AFFECTED BY TREATMENT AND AS EVALUATED BY REFLECTANCE PROCEDURE

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>9</td>
<td>254.9162</td>
<td>140156.09</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.0004</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>254.9166</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>B VALUE</th>
<th>SS</th>
<th>STD ERROR</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>-0.6591</td>
<td>11.0561</td>
<td>0.0028</td>
<td>54709.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hemoglobin, muscle</td>
<td>0.3203</td>
<td>0.4051</td>
<td>0.0071</td>
<td>2004.9</td>
<td>0.0005</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>-0.7746</td>
<td>0.1482</td>
<td>0.0286</td>
<td>733.4</td>
<td>0.0014</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.7647</td>
<td>4.1474</td>
<td>0.0053</td>
<td>20523.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>pH, Abomasum</td>
<td>2.4770</td>
<td>4.2852</td>
<td>0.0170</td>
<td>21204.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>pH, muscle</td>
<td>-13.9186</td>
<td>6.5204</td>
<td>0.0774</td>
<td>32265.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Potassium, muscle</td>
<td>0.1293</td>
<td>2.4310</td>
<td>0.0011</td>
<td>12029.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Magnesium, muscle</td>
<td>14.0423</td>
<td>5.1753</td>
<td>0.0877</td>
<td>25609.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.8084</td>
<td>3.6696</td>
<td>0.0059</td>
<td>18158.7</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\[ R^2 = 0.9999 \]
### Table 12. Regression Analysis Showing Maximum R-Square (MAXR) Improvement for Picking Out the Best One Variable Found to Affect Muscle Color as Determined by Reflectance

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>190.1269</td>
<td>190.1269</td>
<td>29.35</td>
<td>0.0003</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>64.7896</td>
<td>6.4789</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>254.9166</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intercept**

<table>
<thead>
<tr>
<th>B Value</th>
<th>SS</th>
<th>STD. ERROR</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.7744</td>
<td>190.1269</td>
<td>0.1429</td>
<td>29.35</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

\[ R^2 = 0.7458 \]
is simple, involves inexpensive equipment, is rapid and can be handled without any high technical training. Statistically, using the quick hematocrit method (Appendix A.1) to predict reflectance (a measure of veal muscle color) may be more practical to run ($R^2 = 0.7458$) than to run analysis for iron (Table 9) with a lesser predictability ($R^2 = 0.6622$) for sensory color score. In Table 13, the best two variables found by this reflectance procedure are hematocrit and vitamin E. The inclusion of vitamin E in the model increases the $R^2$ to 87.35%. This alone will confirm that vitamin E also has some effect on heme pigments synthesis (Nair et al., 1972). Vitamin E deficiency had been attributed to deposition of brown pigments in the lipid depots of the animal which can affect muscle color (Lehninger, 1975).

Linear regression on iron and muscle pigments

Simple linear regression analysis on iron retained ($X$) in the muscle and myoglobin content ($Y$) is shown in Figure 13. The regression equation of $Y = 0.128X + 0.573$ suggests that, for every unit increase in iron content, myoglobin content goes up by 0.128 mg/g tissue. This relationship is linear over the range of iron content as shown by high direct correlation between the two variables ($r = 0.62; P<.01$). The same interpretation is true for the linear regression equation for iron content ($X$) and muscle total pigment ($Y$) shown in Figure 14. In this case, $Y = 0.252X + 0.827$ ($r = 0.72; P<.01$). The value of $r$ increases when total pigment (myoglobin + hemoglobin) was considered suggesting that residual blood does contribute to muscle color to some extent. In view of previous reports (Bray et al., 1959, Niedermier et
### TABLE 13. REGRESSION ANALYSIS SHOWING MAXIMUM R-SQUARE (MAXR) IMPROVEMENT FOR PICKING OUT THE BEST TWO VARIABLES FOUND TO AFFECT MUSCLE COLOR AS DETERMINED BY REFLECTANCE

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>222.6758</td>
<td>111.3379</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>32.2408</td>
<td>3.5823</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>254.9166</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B VALUE</th>
<th>TYPE II SS</th>
<th>STD. ERROR</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.6636</td>
<td>124.7270</td>
<td>0.1124</td>
<td>34.82</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.7098</td>
<td>32.5488</td>
<td>0.2354</td>
<td>9.09</td>
</tr>
</tbody>
</table>

$R^2 = 0.8735$
Figure 13. Simple linear regression analysis of myoglobin content (Y) on iron retained (X).

\[ Y = 0.128X + 0.573 \]
\[ r = +0.62 \]
\[ n = 26 \]
Figure 14. Simple linear regression analysis of total pigment (Y) on iron retained (X).

\[ Y = 0.252X + 0.827 \]
\[ r = +0.70 \]
\[ n = 26 \]
al, 1959, and Niedermier et al, 1959, and MacDougall et al, 1973), and the high direct significant (P<.01) correlation between iron and myoglobin content (r = 0.62) and iron and total pigment (r = 0.70), it is conceivable to conclude that low iron content (Table 3) was a substantial factor that influenced myoglobin concentration (Table 4) and subsequently caused lighter muscle color (Figure 15) in phosphorus and vitamin E treated group.

Considering the two methods used for veal color evaluation (Table 4) it can be seen that percent reflectance was significantly (P<.01) but inversely correlated with myoglobin content (r = -0.68) in Table 14. This will further suggest that the darker the muscle, the lower the amount of light reflected. Henry and Bratzler (1960) reported that incidence of fading decreases in pork tissues as myoglobin concentration increases when measured by optic spectrophotometer. A very strong and significant (P<.01) direct relationship occurred between myoglobin content and sensory panel scores (Table 14) for color (r = 0.92). This suggests the opposite interpretation of myoglobin vs. percent reflectance but with the same muscle color result. That is, as the myoglobin concentration increases, sensory color scores on the scale of 1-4 (Table 4) for 1 being the lightest and 4 being the darkest muscle also increases. The ability of the experienced sensory panelists used in this study alone, coupled with the use of the color standard (Figure 16) as a guide, might have led to the very high value of r (r = 0.92). The scale of 1-4 used for veal color score is an acceptable standard in the commercial plant.
Figure 15. A color picture of semimembranosus muscle samples from the veal calves treated or not treated with monosodium phosphate and alpha tocopherol supplementation.
### Table 14. Some Correlation Coefficient Between Some Variables Measured in the Veal Semimembranosus Muscle as Affected by High Phosphorus and Alpha Tocopherol Supplementation

<table>
<thead>
<tr>
<th>Variables</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Reflectance vs. Myoglobin</td>
<td>-0.68**</td>
</tr>
<tr>
<td>Panel Sensory Score vs. Myoglobin</td>
<td>+0.92**</td>
</tr>
<tr>
<td>Myoglobin vs. Copper</td>
<td>+0.33*</td>
</tr>
<tr>
<td>Copper vs. Iron</td>
<td>+0.48*</td>
</tr>
<tr>
<td>Calcium vs. Iron</td>
<td>-0.24</td>
</tr>
<tr>
<td>Calcium vs. Copper</td>
<td>-0.21</td>
</tr>
<tr>
<td>Manganese vs. Iron</td>
<td>0.24</td>
</tr>
<tr>
<td>Ash vs. Fat</td>
<td>-0.30*</td>
</tr>
<tr>
<td>Abomasum pH vs. Iron</td>
<td>-0.07</td>
</tr>
<tr>
<td>Iron vs. Potassium</td>
<td>-0.02</td>
</tr>
<tr>
<td>Fat vs. Potassium</td>
<td>-0.37*</td>
</tr>
<tr>
<td>Fat vs. Calcium</td>
<td>+0.46*</td>
</tr>
<tr>
<td>Cholesterol vs. Fat</td>
<td>+0.17</td>
</tr>
<tr>
<td>Serum cholesterol vs. Inorganic phosphorus</td>
<td>-0.30*</td>
</tr>
</tbody>
</table>

*Significant (P<.05).

**Significant (P<.01).
Figure 16. Muscle color standard used by the sensory panelists as a guide for scoring veal color.
Results of correlation coefficients

Simple correlation coefficients for some veal attributes measured with serum vitamin E are presented in Table 15. Myoglobin content was significantly (P<.01) but inversely correlated with vitamin E (r = -0.54) and this alone might be due to the direct effect of alpha tocopherol interfering in heme pigment synthesis. Alpha tocopherol has been shown to play some role in the biosynthesis of porphyrins and heme compounds (Nair et al., 1972). Also, since the terminal step in the pathway of heme synthesis (Figure 2) is completed intramitochondrially by the introduction of iron (Fe2+) into protoporphyrin IX by the enzyme ferrochelatase, then perhaps the lack of availability of tissue iron in the phosphate and tocopherol treated group (Table 3) might have caused some interruption in aerobic enzymatic activity necessary for heme synthesis, resulting in an inverse relationship between these two variables, and, hence, lower muscle pigments in the calves receiving high phosphate and tocopherol supplemented diet (Table 4). This explanation of potential interaction between vitamin E and myoglobin and the negative correlation reported is difficult to explain in any other way. This is particularly true since vitamin E and the major enzymes involved in heme synthesis: alanine synthase and alanine dehydratase were not assayed from the liver of the calves used in the present study. Iron content was negatively correlated with vitamin E (Table 15), but this relationship was not significant (P>.05; r = -0.18). Melhorn and Gross (1971a) reported the possible interfering of vitamin E and iron with each other during their metabolism in the intestine. A similar inverse relationship
TABLE 15. THE SIMPLE CORRELATIONS BETWEEN VITAMIN E AND SOME VEAL ATTRIBUTES MEASURED IN VEAL CALVES TREATED WITH OR WITHOUT PHOSPHORUS AND TOCOPHEROL SUPPLEMENTED DIET

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Correlation with Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin Content</td>
<td>-0.54**</td>
</tr>
<tr>
<td>Iron Content</td>
<td>-0.175</td>
</tr>
<tr>
<td>Copper, muscle</td>
<td>-0.21</td>
</tr>
<tr>
<td>Muscle Calcium</td>
<td>+0.22</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td>+0.55**</td>
</tr>
<tr>
<td>Manganese, muscle</td>
<td>+0.055</td>
</tr>
<tr>
<td>Serum Cholesterol</td>
<td>-0.14</td>
</tr>
<tr>
<td>Serum Phosphorus</td>
<td>+0.26</td>
</tr>
</tbody>
</table>

**Significant (P<.01).
was observed between vitamin E and muscle copper content (Table 14). Copper and iron are related functionally as indicated by the same inverse relationship they have with vitamin E (Table 14). In Table 14, it can be seen that there was a significant (P<.01) direct relationship between muscle copper and muscle iron content (r = 0.48). Both copper and iron are necessary for hemoglobin formation even though, unlike iron, copper is not part of hemoglobin molecule.

The negative but non-significant (P>.05) correlation between serum vitamin E and serum cholesterol (Table 15) would suggest that vitamin E intake might be responsible for the reduction in serum cholesterol reported in Table 1. Chupukcharven et al. (1985) reported an accumulation of cholesterol in muscle as well as elevation of plasma cholesterol in vitamin E deficient rabbits. The accumulation of both plasma and muscle cholesterol in rabbits was attributed to a decrease in cholesterol 7-alpha-dehydroxylase activity, a rate limiting enzyme in the degradation of cholesterol to bile acid in the liver. Also deficiency of vitamin E might have caused a reduction in the level of cytochrome-P-450 which might, in turn, lead to reduction in the activity of cholesterol 7-alpha-hydroxylase. Luzio (1972) reported a reduction in plasma lipids which contained conjugated dienes, the composition of which include phospholipids, triglycerides and cholesterol in human patients receiving 1.5 g of mixed tocopherol per day for a period of 7 days, while maintaining normal diet. In a separate study, Deuel et al. (1955) reported that deficiency of vitamin E caused an elevation of plasma and muscle cholesterol in rats and guinea pigs. The significant
direct relationship between vitamin E and serum calcium content (Table 15) \((r = 0.55; P<.01)\) probably reflects the improvement of calves nutrition by dietary vitamin E and phosphorus intake. It can be seen that vitamin E (Table 1) and calcium (Table 2) were significantly higher \((P<.05;\) in the serum of the calves receiving the phosphorus and tocopherol supplementation. More studies involving the measuring of vitamin E content in the liver, cholesterol content in the liver, assays of plasma lipoprotein, especially the high density lipoprotein (HDL) in the pre-ruminant, ruminants and different species of animals treated with alpha tocopherol and phosphorus, will be beneficial.

The inverse relationship between iron retained in the muscle and the abomasum pH of the pre-ruminant animals used in this study is depicted in Table 14. This will suggest that the solubility of iron either in a ferrous or ferric form is pH dependent. As the pH of the abomasum increases, the solubility and availability of iron decreases because a sparingly insoluble ferrous hydroxide and ferric hydroxide are formed. Literally, this interpretation means that acid conditions favor iron absorption.

**Analysis of variance on selected muscle components**

Analysis of variance on myoglobin, as influenced by treatment and muscle mineral compositions, is depicted in Table 16. Treatment by phosphorus and alpha tocopherol has a significant effect \((P<.05)\) on myoglobin concentration. The same is true for muscle phosphorus and muscle iron. The relationship between iron retained and myoglobin concentration (Tables 3 and 4), myoglobin and vitamin E (Table 15) have
TABLE 16. ANALYSIS OF VARIANCE ON MYOGLOBIN AS AFFECTED BY SOME MINERAL VARIABLES MEASURED IN VEAL

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>2.01049</td>
<td>2.01049</td>
<td>4.14*</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>2.08503</td>
<td>2.08503</td>
<td>4.30*</td>
</tr>
<tr>
<td>Zn</td>
<td>1</td>
<td>0.27516</td>
<td>0.27516</td>
<td>0.57</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>0.00014</td>
<td>0.00014</td>
<td>0.00</td>
</tr>
<tr>
<td>Fe</td>
<td>1</td>
<td>1.97178</td>
<td>1.97178</td>
<td>4.06*</td>
</tr>
<tr>
<td>Ca</td>
<td>1</td>
<td>0.00790</td>
<td>0.00790</td>
<td>0.02</td>
</tr>
<tr>
<td>K</td>
<td>1</td>
<td>0.09976</td>
<td>0.09976</td>
<td>0.21</td>
</tr>
<tr>
<td>Na</td>
<td>1</td>
<td>0.00579</td>
<td>0.00579</td>
<td>0.01</td>
</tr>
<tr>
<td>Mg</td>
<td>1</td>
<td>0.12602</td>
<td>0.12602</td>
<td>0.26</td>
</tr>
<tr>
<td>Mn</td>
<td>1</td>
<td>0.00281</td>
<td>0.00281</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>1</td>
<td>0.06215</td>
<td>0.06215</td>
<td>0.13</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>6.79273</td>
<td>0.48519</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Corrected total = 25 because 2 samples/groups were lost during processing.

*Significant (P<.05).
been discussed in this report. The significant effects of these variables noticed in Table 14 is a further confirmation that iron control is important if light veal is to be produced. Analysis of variance on reflectance (Table 17) showed an additional interesting result. Treatment has a high significant effect ($P<.01$) on the amount of light reflected by the veal muscle in this study. Myoglobin concentration also affected reflectance ($P<.05$). The amount of hemoglobin and meat moisture would be expected to affect amount of light reflected from the muscle. This was shown to be true to some extent in the analysis Table 17).

When panel scores were considered, analysis of variance (Table 18) also showed significant effects treatment ($P<.10$) and myoglobin ($P<.25$).

**Proximate Analyses**

Proximate analysis of the veal semimembranosus muscle is shown in Table 19. There was a significantly higher percent moisture ($P<.05$) in the treated group than control. This was accompanied by lower percent fat ($P<.05$) in the treated group than the control. The higher moisture content in the treated group might be one of the factors contributing to higher percent reflectance and subsequent lighter color perception of the treated group (Table 4) than the control. Craig et al. (1959) reported that high moisture content in meat contributed to its pale appearance unlike the dry surface which might be due to surface migration of the pigment, resulting in dark appearance. The level of protein was not different ($P>.05$) in the two groups. The higher ash content in the treated group ($P<.05$) than the control might be a
### TABLE 17. ANALYSIS OF VARIANCE ON MUSCLE COLOR REFLECTANCE AS AFFECTED BY SOME VEAL VARIABLES.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>64.65384</td>
<td>65.65384</td>
<td>5.74**</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1</td>
<td>39.39247</td>
<td>39.39247</td>
<td>3.50*</td>
</tr>
<tr>
<td>Hemoglobin, muscle</td>
<td>1</td>
<td>17.11588</td>
<td>17.11588</td>
<td>1.52</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>2.72475</td>
<td>2.72475</td>
<td>0.24</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>0.39952</td>
<td>0.39952</td>
<td>0.04</td>
</tr>
<tr>
<td>Fe</td>
<td>1</td>
<td>6.09613</td>
<td>6.09613</td>
<td>0.54</td>
</tr>
<tr>
<td>Moisture</td>
<td>1</td>
<td>23.16917</td>
<td>23.16917</td>
<td>2.06$</td>
</tr>
<tr>
<td>Fat</td>
<td>1</td>
<td>0.06374</td>
<td>0.06374</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>1</td>
<td>2.25828</td>
<td>2.25828</td>
<td>0.20</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>180.16462</td>
<td>11.26028</td>
<td></td>
</tr>
</tbody>
</table>

Corrected Total = 25

Corrected total = 25 because 2 samples/groups were lost during processing.

**Significant (P<.01).

* Significant (P<.05)
TABLE 18. ANALYSIS OF VARIANCE ON PANEL COLOR SCORE AS AFFECTED BY SOME VARIABLES MEASURED IN VEAL

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>1.46895</td>
<td>1.46895</td>
<td>3.01*</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1</td>
<td>1.12753</td>
<td>1.12753</td>
<td>2.31</td>
</tr>
<tr>
<td>Hemoglobin, muscle</td>
<td>1</td>
<td>0.37456</td>
<td>0.37456</td>
<td>0.77</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>0.19328</td>
<td>0.19328</td>
<td>0.40</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>0.02739</td>
<td>0.02739</td>
<td>0.06</td>
</tr>
<tr>
<td>Fe</td>
<td>1</td>
<td>0.27639</td>
<td>0.27639</td>
<td>0.57</td>
</tr>
<tr>
<td>Moisture</td>
<td>1</td>
<td>0.19080</td>
<td>0.19080</td>
<td>0.39</td>
</tr>
<tr>
<td>Fat</td>
<td>1</td>
<td>0.00654</td>
<td>0.00654</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>1</td>
<td>0.03564</td>
<td>0.03564</td>
<td>0.07</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>7.80870</td>
<td>0.48804</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant (P<.10).

1Corrected total = 25 because 2 samples/group were lost during processing.
TABLE 19. MEANS (X) AND SE OF THE PROXIMATE ANALYSIS OF VEAL SEMIMEMBRANOSUS MUSCLE AS AFFECTED BY PHOSPHORUS AND TOCOPHEROL SUPPLEMENTATION.

<table>
<thead>
<tr>
<th>% Variables</th>
<th>Treated (N=13)</th>
<th>Control (N=13)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>75.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>Fat</td>
<td>1.82&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein</td>
<td>21.84</td>
<td>21.26</td>
<td>0.57</td>
</tr>
<tr>
<td>Ash</td>
<td>0.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.69&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Means on the same line with superscript c are significantly (P<.05) higher than those with superscript d.*
reflection of total muscle minerals in the phosphate group (4131.77 ug/g tissue) than the control (4095.02 ug/g tissue) in Table 3.
CHAPTER V
SUMMARY AND CONCLUSIONS

The main objective of this study was to explore the possibility of improving veal muscle color by using a suitable iron chelator such as monosodium phosphate and an antioxidant such as alpha tocopherol in order to reduce iron and myoglobin concentration in the muscle. The possibility of vitamin E interfering with heme synthesis and cholesterol metabolism in the calves treated with monosodium phosphate were also studied. The experiment involved using thirty Holstein bull calves which were all placed on a milk replacer diet. The treated calves received also the phosphate and tocopherol supplement for two weeks prior to slaughter. All calves were on feed for a total of 16 weeks and were slaughtered at the average weight of 170 kg.

The veal muscle color as affected by monosodium phosphate and tocopherol supplementation were evaluated by both the sensory and reflectance methods. Muscle pigments were determined by using the carbon monoxide colorimetric method. The hematocrit and hemoglobin were determined using both a centrifuge and colorimetric methods. All the blood and muscle mineral analyses were determined using the atomic absorption spectrophotometry procedure, except the serum inorganic phosphorus which was determined by Fiske Subbarow colorimetric procedure. Total serum cholesterol was determined colorimetrically, while
muscle cholesterol was quantified by both the gas chromatography and mass spectrophotometer.

The significant differences of all the variables studied between the two groups were determined by t-test paired comparison, and analysis of variance procedures. A stepwise regression analysis was run for each variable and combined variables to determine the variables best useful in predicting veal muscle color.

The t-test analysis indicated that there was no treatment effect (P >.05) on the level of hematocrit and hemoglobin in the blood of calves treated with monosodium phosphate and alpha tocopherol when compared with the control calves. However, an opposite result was observed in the muscle where iron content and all pigments measured were significantly (P <.05) lower in the treated group when compared with the control. This means that it could be possible to regulate the amount of iron retained in the tissue to minimize the myoglobin concentration without making the calves anemic. Both the sensory and reflectance methods for color evaluation indicated that muscle samples from the treated group were significantly (P <.05) lighter than the control group.

The stepwise regression analysis indicated that variables such as hematocrit quantity, hemoglobin, muscle iron, muscle copper, amount of moisture in the tissue, and muscle pH affect ultimate veal muscle color. Veal muscle color could be predicted most accurately by using the level of the hematocrit content in the veal program. Result in this study indicated that any calf with hematocrit higher than 33% is very likely to produce dark meat. Such animals should be placed on a dietary controlled program or could be eliminated from the veal program.
Also, simple linear regression analysis gave the equation of \( Y = 0.128X + 0.573 \), meaning that, as the level of iron retained increases by one unit, the myoglobin concentration also increases by 0.128 mg/g tissue.

In this experiment, routine monitoring of the blood composition resulted in many interesting results, such as:

1. A significant (P<.05) reduction in both serum and muscle cholesterol in favor of the calves treated with monosodium phosphate and alpha tocopherol. These results were explained by the possibility of the kinase enzyme using the phosphorus to inactivate 3-hydroxy-3 methylglutaryl (HMA)-CoA reductase, the major enzyme involved in cholesterol synthesis in the liver. The correlation coefficient of inorganic phosphorus vs. serum cholesterol showed an inverse relationship (\( r = -0.30; P<.05 \)). Another possible reason for the reduction in the serum cholesterol might be due to vitamin E intake. It is possible that tocopherol activity might cause a promotion of the action of cholesterol 7-alpha dehydroxylase, the major enzyme involved in cholesterol degradation into bile acids also in the liver. The correlation coefficient of serum cholesterol and vitamin E also showed an inverse relationship (\( r = -0.15; P>.05 \)).
2. A highly significant (P<.01) reduction in the level of blood sodium in the calves receiving monosodium phosphate and alpha tocopherol resulted when compared with the control group. This was probably due to the chelating activity of phosphate causing the treated calves to urinate more, resulting in more rapid loss of the extracellular element (sodium).

3. The levels of calcium were significantly (P<.05) higher both in the blood and muscle of the treated group than the control.

In conclusion, it was demonstrated in this study that high phosphorus fed as monosodium phosphate and alpha tocopherol could interfere with iron metabolism in veal production. If fed late in the feeding program, such a treatment could cause a depletion in amount of iron retained, and amount of myoglobin concentration in the muscle without any adverse effect on blood hemoglobin.

Also, it was demonstrated that both high vitamin E and monosodium phosphate intake could affect cholesterol and mineral balance in favor of the calves treated with the two nutrients. It could be possible that the combination of reduction in serum cholesterol and sodium, and an increase in blood calcium, might have a beneficial medicinal implication in regulating the incidence of arteriosclerosis and high blood pressure in man. In addition, it is interesting to notice that when a certain mineral is altered in the animal system, mineral shifting does occur
in the animal body to balance the effect of the alteration. In this study, high intake of phosphorus led to loss of sodium and an improvement in calcium absorption. Therefore, it might be nutritionally valuable to undertake more study on the effects of various levels of monosodium phosphate and tocopherol supplementation on cholesterol and mineral compositions on animals of various species and varying maturity.
APPENDIX A

ANALYTICAL PROCEDURES

A.I. Total blood hemoglobin (Adapted procedure of Sigma 525 (1984))

Equipment:
Bausch and Lomb Spectronic 20.
Eppendorf pipette 20 ul, 10 ml serological pipet.
Volumetric flasks (1000 ml).
Amber bottle.
Cuvets.
Blood collecting tubes.

Reagents: As supplied in the kit (Sigma # 525).
Hemoglobin Standard: Contains methemoglobin (Human) 18 g hemoglobin/100 ml blood. This reagent is reconstituted with 50.0 ml Drabkin’s solution.
Drabkin’s Reagent: 1.25 g/vial. Containing 1.0 part of sodium bicarbonate, 20 parts potassium ferricyanide and 5 parts of potassium cyanide,
Brij 35 Solution: Containing polyoxyethlene 23 laury ether (30% w/v).

Procedure:
1. Add about 0.9 g of EDTA to all the blood collecting tubes.
2. Obtain blood from right jugular vein after overnight fasting of the calves.


5. Read values with the aid of lens on the instrument and express values as percentage of whole blood (Pack Cell Volume).

6. PCV/3 = Hemoglobin value.

7. For colorimetric determination, allow blood to thaw at room temperature if frozen. Vortex to mix. For the analyses, set the cuvets as follows:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td>Add 5.0 ml Drabkin's reagent. Also 5.0ml of Drabkin's.</td>
</tr>
<tr>
<td>b) None</td>
<td>Add 40 ul of whole blood to test tubes using Eppendorf pipette. Vortex gently.</td>
</tr>
<tr>
<td>c)</td>
<td>Allow the tubes to stand for 20 minutes and with the aid of parafilm to cover the tubes, invert gently to mix immediately before reading the absorbance.</td>
</tr>
<tr>
<td>d)</td>
<td>Read absorbance A at 540 nm using blank to calibrate spectrophotometer and then read A of the samples.</td>
</tr>
</tbody>
</table>
Preparation of the standard curve:

For the hemoglobin standard curve, set the test tubes up as follows:

<table>
<thead>
<tr>
<th>Tube Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Solutions (ml)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

e) Mix contents gently by inversion using parafilm to cover the tubes.

f) Read absorbance, at 540 nm, of tubes 2-4 using tube 1 as a reference.

g) Plot a calibration curve of standard absorbance values vs. blood hemoglobin (g/100 ml whole blood) in column 4. Curve is linear passing through origin.

h) Determine total hemoglobin concentration (g/100 ml whole blood) of the samples from the calibration curve.

A.II. Serum Phosphorus Measurement (Adapted from Sigma 670 Procedure).

Equipment and Materials:


Pyrex centrifuge 40 ml tubes.

Eppendorf pipette (20-1000 ul).
Cuvets.
Ashless filter paper (Whatman # 42).
Volumetric flasks (1000 ml).
Demineralized water.
Centrifuge.

Reagents: Trichloroacetic acid prepared fresh by dissolving 200 g in 700 ml demineralized water and made to volume in a 1000 ml volumetric flask. This gives 20% w/v. Mix contents thoroughly (corrosive, do not pipette by mouth).
Fiske and SubbaRow Reducer (Sigma # 661-8): Containing 1-Amino-2-naphthol-4-sulfonic acid, sodium sulfite and sodium bisulfite.
Fiske and SubbaRow Solution: This reagent is prepared by dissolving 5 g of Fiske and SubbaRow Reducer in 31.5 ml deionized water and filter into an amber bottle and store at room temperature (18-26°C).
Phosphorus Standard Solution (Sigma # 661-9): Containing 20 μg inorganic P/ml as potassium phosphate monobasic dissolved in 0.05 N HCl. Store tightly capped in refrigerator (2-6°C).
Acid Molybdate Solution (Sigma # 661-11): Prepared by dissolving 1.25 g of hydrated ammonium molybdate in a 100 ml 2.5 N sulfuric acid. This reagent is stored at room temperature.
Procedure:

1. Pipette accurately 0.5 ml (500 ul) of serum into 40 ml centrifuge tubes.

2. Add 2.5 ml demineralized water.

3. Add 2.0 ml trichloroacetic acid (20% w/v).

4. Vortex vigorously to mix and allow to stand for 10 minutes.

5. Centrifuge at 10,000 rpm for 5 minutes.

6. Label test tubes BLANK, Test 1, 2, -------10 and set tubes up as follows:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Tests</th>
</tr>
</thead>
</table>
   a) Add 2.0 ml trichloroacetic acid | None                       |
   b) None                    | Pipette 2.0 ml upper layer from centrifuge tube from step 5 into the tubes |
   c) Add 3.0 ml demineralized water | Add 3.0 ml water           |
   d) Add 1.0 ml acid molybdate solution | Also add 1.0 ml of molybdate solution |
   e) Vortex all tubes to mix contents very gently.

7. Add 0.25 ml Fiske and SubbaRow solution to each tube, including the blank.

8. Mix tubes by inversion using parafilm to cover tubes. Allow to stand for 10 minutes for color development.

9. Using blank as a reference to calibrate spectrophotometer, read absorbance of tests at 660 nm. Must complete readings within 10 minutes after color development.
10. Determine the inorganic phosphorus from the calibration curve.

Preparation of the calibration curve:

11. Use 2 tubes for duplication as in samples. Label tubes S1----S6 and pipette into each tube the reagents indicated below in columns 2 and 3.

<table>
<thead>
<tr>
<th>Tube Numbers</th>
<th>Phosphorus Standard (ml)</th>
<th>Water (ml)</th>
<th>Serum Inorganic Phosphorus mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>5.00</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>4.75</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>4.50</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>4.25</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>4.00</td>
<td>10.0</td>
</tr>
<tr>
<td>6</td>
<td>1.25</td>
<td>3.75</td>
<td>12.5</td>
</tr>
</tbody>
</table>

12. Add 1.0 ml of acid molybdate solution to the tubes and mix by swirling.

13. Add 0.25 ml of Fiske and SubbaRow solution and mix by inversion. Allow to stand for 10 minutes for color development.

14. Read absorbance at 660 nm using tube 1 as a reference. Prepare a calibration curve by plotting the absorbance values vs. the corresponding inorganic phosphorus
concentration in mg/100 ml provided in column 4. Determine the samples' phosphorus concentration from the curve.

A.III. Serum Cholesterol Measurement (Adapted Procedure of Sigma # 351).

**Equipment and Materials:**

- Bausch and Lomb Spectronic 20.
- Cuvets.
- Parafilm.
- Eppendorf pipette (20-1000 ul and 5 ml sizes with tips).
- Dispensette bottle.
- Water bath.
- Magnetic stirrer and 100 ml beakers.

**Reagents:**

- Sodium chloride crystals.
- Demineralized water.

**Cholesterol Aqueous Standard (Sigma # 350-200):** Containing 200 mg of Cholesterol/100 ml water with 0.1% sodium azide added as preservative. This reagent is stored at room temperature.

**Cholesterol Reagent Solution (Catalog # 351-20):** The reagent contains 0.1 ml/liter of phosphate buffer, 0.8 mmol/liter of 4-aminoantipyrine, 20 mmol/litre of p-hydroxybenzene-sulfonate, cholesterol oxidase (microbial) > 200 ul, esterase (microbial) and peroxidase (horseradish) > 150 ul.
Analysis:
1. Label cuvets BLANK, STANDARD, TEST_a, TEST_b, etc.
2. Turn spectrophotometer on at least 30 minutes before readings and set wavelength at 500 nm.
3. Turn water bath on and set temperature at 37°C.
4. Reconstitute Cholesterol reagent bottle with 15.0 ml demineralized water using dispensette bottle. Inverse bottle to dissolve. DO NOT SHAKE.
5. Prepare saline solution by dissolving 0.9 g sodium chloride crystal into 100 ml demineralized water to give 0.9% (w/v) solution. Magnetically stir to dissolve.
6. If serum is frozen, thaw at room temperature and vortex to homogenize. Set cuvets up as follows:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>b) To each cuvet, add 2.2 ml reconstituted enzymatic reagent (including blank). Vortex very gentle. Start timing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Put cuvets in the 37°C water bath to incubate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d) At 20 minutes, add 1.4 ml saline solution (to remove any available plasma) to cuvets, vortex gently.</td>
<td></td>
<td></td>
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<tr>
<td>e) Read absorbance A of the samples against the blank as a reference.</td>
<td></td>
<td></td>
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</tbody>
</table>
f) Add 2 drops of cholesterol decolorizing agent to each tube and vortex gently. Read A again.

6. Calculation:

\[
\text{mg Total Cholesterol/100 ml Sera} = \frac{(A \text{ Sample} - A \text{ Cleared Sample})}{(A \text{ Standard} - A \text{ Cleared Standard})}
\]

7. Then multiply the result by concentration of the standard given on the bottle.

8. Example:

Concentration of standard = 200 mg/100 ml sera.

Sample = 0.17
Cleared Sample = 0.04
Standard = 0.26
Cleared Standard = 0.04

Then:

\[
\text{mg Total Cholesterol/100 ml sera} = \frac{(0.17 - 0.04) \times 200}{(0.26 - 0.04)}
\]

= 118 mg Total Cholesterol/100 ml sera.

A.IV. Muscle Cholesterol Analysis (Adapted Procedure of Kunsman et al. (1981)).

Equipment:

A Gas Chromatograph Model (Varian 2100) with Integrator (#3390A, Hewlett-Packard 5880A, Avondale, PA).

Nitrogen supply source.

Glass vials (1.5 ml) with teflon caps.
A 10 - 50 ul syringe.
Pasteur Pipettes.
Heating blocks that can accommodate vials.
Freeze dry equipment.
Rotoevaporator.
Glass jars (3 oz.) or 100 ml beakers.
Scalpel.
Volumetric flasks (10 or 25 ml sizes).
Membrane filters.
Gas chromatography column for cholesterol (0. V. 17).
Round bottom flasks.
Vacuum evaporator.
Reagents: All reagents must be analytical standard.
Chloroform.
Hexanes.
Potassium hydroxide.
Cholesterol standard.
5-alpha-cholestane as Internal Standard.
bis (Trimethylsilyl) Trifluoroacetane (BSTFA).
Dimethylformamide (DMF)
Procedure:
a) Lipid Extraction (Modified).
   1. Tare the 100 ml beakers or 3 oz. glass jars on the balance.
   2. With scalpel, shave frozen veal samples to obtain approximately 10 g.
3. Place sample in freeze dry flasks and put in freezer for about 4 hours, then freeze dry.

4. Re-weigh flasks using original tare. Can calculate % moisture.

\[
\text{% moisture} = \frac{\text{wt in grams after freeze dry}}{\text{original sample wt.}} \times 100
\]

5. Mince samples into pieces, add 50 ml chloroform and cap.

6. Magnetically stir for 90 minutes at room temperature.

7. Filter through pre-washed filter paper into 200 ml round bottom flasks.

8. Add about 40 ml extracting solvent to jar or beaker, shake and pour through filter paper.

9. Rotoevaporate, and re-extract lipid with hexane.

10. Purify lipid by passing through 1 micron solvent inert membrane filter (Miller et al., 1979) into a 10 or 25 ml volumetric flasks.

11. Make content to volume with hexane.

12. Aliquots can be filtered through .5 micron filter if desired.

\[
\text{% Crude Lipid} = \frac{\text{wt. of lipid (10 or 25 ml/aliquot taken)}}{\text{wt. of veal extracted}} \times 100
\]

13. From the above information, volume of the aliquot needed to obtain about 40 mg of lipid for cholesterol analysis can be calculated.
14. **Conditions of gas chromatography for the analysis:**

a) Glass column 2-3% OV 17; 1/8" O.D.

b) Injector port = 300°C.

c) Column temperature was between 250-300°C.

d) Column loaded with a 100-200 mesh.

15. Place about 40 mg of lipid in each vial using different pipette to dispense volume.

16. Add an appropriate amount of internal standard (5-alpha-cholestane) to each vial. e.g., 0.8 ul.

17. Add about 0.5-1.0 ml 50% KOH.

18. Add about 2 ml 95% ethanol.

19. Heat the tightly capped vial at 100°C for 1 hr.

20. Let cool to room temperature.

21. Add about 2 ml distilled water.

22. Add about 2 ml hexanes, cap, shake vigorously and let stand for few minutes.

23. Transfer most of the hexane layer (upper layer) into a clean vial with the aid of pasteur pipette.

24. Re-extract with 2 ml hexane two more times and transfer the hexanes layer as in 23.

25. Dry by warming (60-80°C) the vials under hood with a steady flow of nitrogen gas. Let the vials cool to room temperature.

26. Add about 30-60 ul of dry DMF to each vial.
27. Cap the vials and warm at 80°C for 10 min. or until residue dissolves. Allow to cool to room temperature.

28. Add 30-60 ul bis-(Trimethylsilyl)-Trifluoroacetane (BSTFA) and cap immediately.

29. Heat at 80°C for 30 min. Let it cool to room temperature.

30. Inject 1 ul of the standards first, then inject the same quantity of the samples into the gas chromatograph.

31. Preparation of the cholesterol standard for the gas Chromatograph:

a) Dissolve 15.0 mg of 5-alpha-cholestane in 3.0 ml chloroform. Shake to dissolve. Thus the stock solution contains 5 mg/ml.

b) Dissolve 30.0 mg of cholesterol in 3.0 ml chloroform. Stock solution contains 10 mg/ml.

c) Need 5 vials (1.5 ml sizes).

d) Add 0.8 ul of cholestane to each vial very carefully with a syringe.

e) Add (appropriate) 2, 4, 8, 16 and 32 ul of cholesterol standard prepared into vials 1 to 5, respectively.

f) Evaporate chloroform very carefully under nitrogen gas.
g) Add 60 ul BSTFA and immediately cap vial. Heat at 80°C for 30 minutes and let it cool to room temperature.

h) Inject 1 ul each standard into gas chromatograph with proper rinsing of syringe with hexane before injecting subsequent standards.

i) Prepare the standard curve by plotting Peak Area Ratio given by the Integrator (Model 3390A) vs. Concentration of Standard ug/ul and determine the sample cholesterol quantity from the curve.

j) Peak Area Ratio (PAR) =

\[
\frac{\text{Cholesterol area from chromatogram}}{\text{Area of internal standard used}}
\]

k) Quantification:

\[\text{mg Cholesterol/100 g sample} = \frac{\text{from standard curve}}{\text{g/ml sample used for derivation}}\]

l) Convert the value obtained in k to percent by multiplying by 100.

A.V. Muscle Pigment Measurements (Adapted from Procedure of Fleeming, et al. (1960).

Reagents:

1. A stock solution of acetate buffer prepared by adding 11.45 ml acetic acid to water and dilute to 2 liters. This gives 0.1N of the solution.
2. Sodium acetate solution prepared by dissolving 16.408 g of sodium acetate in water and dilute to 2 liters (0.1N).

3. Prepare 0.01N buffer solution as follows:
   
   180 ml water
   10 ml 0.1N acetic acid
   10 ml 0.1N sodium acetate.

4. Disodium phosphate crystals (Na₂HPO₄·7H₂O).

5. Sodium hydrosulfite crystals (Na₂S₂O₄).

6. Carbon monoxide compressed gas.

Equipment:

7. Waring blender.

8. Centrifuge and centrifuge tubes.


11. Glass manifold tube for attachment of a rubber tube used for bubbling carbon monoxide through sample.

Procedure:

12. Cut the veal samples into pieces (1.90 cm) and blend for 30 seconds. Re-blend for another 30 seconds or until sample is homogenized.

13. Weigh approximately 25 g of the homogenized meat in a tared 150 ml beaker.

14. Blend the sample with 60 ml of cold 0.01N acetate buffer solution for 5 minutes.
15. Pour slurry into a 250 ml polyethylene bottle. Rinse blender with buffer and pour into bottle. Centrifuge at 3000 rpm at 4°C for 15 minutes.

16. Filter the supernatant into a 250 ml Erlenmeyer flask through a funnel containing a wad of cotton.

17. Re-extract the sample residue with 40 ml of the buffer solution by blending for 3 minutes. Pour slurry into same bottle and centrifuge slurry for 15 minutes. Filter supernatant into the previous filtrate.

18. Filter the extract through Whatman Qualitative No. 3 filter paper into a 250 ml volumetric flask and make content to volume with 0.01N acetate buffer.

19. Pippette 25 ml of the extract into each of two Erlenmeyer flasks containing 1.01 g of Na₂HPO₄·7H₂O. This makes M/15 solution which offsets the acidifying effect of sodium hydrosulfite.

20. Magnetically stir the solution very gently until all the phosphate has dissolved.

21. Fill a 40 ml centrifuge tube with the pigment solution and centrifuge for 15 minutes at 10,000 rpm in Serval centrifuge.

22. Filter through Whatman No. 3 filter paper.

23. Rinse the Dickens Simer reaction tube with the sample solution and pour approximately 15 ml into the tube. With the aid of spatula, place a small (pinch) amount
of sodium hydrosulfite in the side arm of the tube. Avoid getting the sodium hydrosulfite in the pigment solution.

24. Bubble carbon monoxide through the solution for 5 minutes. Then invert the tube to wash the sodium hydrosulfite into the tube. Bubble carbon monoxide for another 5 minutes.

25. Rinse a cuvette with a portion of the solution and quickly fill the cuvette to the top and cover with a parafilm.

26. Read absorbance of the solution at 538 and 568 nm against a blank of M/15 disodium phosphate solution prepared.

27. Calculations:

Myoglobin (Mb)  
\[ D = \text{Optical density} \]
\[ E = \text{Extinction coefficient} \]
\[ [\text{MbCo}] = \frac{[D568 \times E_{Hb538}] - [D538 \times E_{Hb568}]}{E_{Mb568} \times E_{Hb538}} - \frac{[D538 \times E_{Hb568}]}{E_{Mb568}} \]
\[ [\text{MbCo}] = \frac{[D568 \times 14.8]}{17 \times [250 \text{ ml volume}]} - \frac{[D538 \times 14.5]}{39.96} \]
\[ \text{Mg Mb/g Veal sample} = \frac{[\text{MbCo}] \times 17 \times [250 \text{ ml volume}]}{\text{Wt. of veal sample used}} \]

Hemoglobin (Hb)  
\[ [\text{HbCo}] = \frac{[D568 \times E_{Mb538}] - [D538 \times E_{Mb568}]}{E_{Hb568} \times [14.8]} - \frac{[E_{Hb568} \times E_{Mb568}]}{E_{Mb568}} \]
\[ [\text{HbCo}] = \frac{[D568 \times 14.8]}{39.96} - \frac{[D538 \times 11.8]}{39.96} \]
\[ \text{Mg Hb/g Veal sample} = \frac{[\text{HbCo}] \times 4250}{\text{Wt. of sample}} \]
Total Pigment = mg Mb/g + mg Hb/g

\[
\% \text{ Myoglobin} = \frac{mg \text{ Mb/g}}{\text{Total pigment}} \times 100
\]

\[
\% \text{ Hemoglobin} = \frac{mg \text{ Hb/g}}{\text{Total pigment}} \times 100
\]


**Reagents:**

1. Deionized or triple distilled water.
2. Concentrated HCl 6N.
3. Five percent Lanthinum Oxide (La$_2$O$_3$) prepared by dissolving 58.65 g of Lanthinum Oxide in 250 ml concentrated 6 N HCl and make to 1000 ml with triple distilled water.

**Analyses:**

4. Approximately 10 g of ground veal is weighed in a tared clean crucible in duplicate.
5. Dry ashing of the samples was done by placing the crucibles into furnace at 200-300°C for 2 hours and increase temperature to 550°C until samples are carbon free.
6. Cover the crucibles with lids and allow to cool for at least 2 hours.
7. Wet the ash with 6 N HCl and allow to stand for about 1 hour.
8. Quantitatively transfer the crucible content to a 25 ml volumetric flask and make content to volume with 6 N HCl.
9. For individual mineral analyses dilution for each cation is done as follows:
Iron
Read Fe in P-E Atomic Absorption Spectrophotometer directly from original sample.

Calcium
Place 5 ml of sample in duplicate into 10 ml volumetric flask, add 2 ml of 5% La2O3 and make to volume with triple distilled water.

Magnesium
Place 1 ml of sample into 100 ml volumetric flask, add 2 ml 5% lantinum oxide and make the content to volume with triple distilled water. Read Mg on spectrophotometer.

Zinc
Pipette 2 ml of the sample into 10 ml volumetric flask and make content to volume with triple distilled water. Read absorbance.

Calculations
a) Convert percent absorption to absorbance using conversion tables.
b) Calculate a "b" value using absorbance of standard curve using the following formula:

\[ b = \frac{\sum XY - \frac{\sum X \cdot \sum Y}{N}}{\sum X^2 - \left(\frac{\sum X}{N}\right)^2} \]
\[ a = Y - bX \]
\[ b = \text{slope of standard curve} \]
\[ X = \text{absorbance of particular concentration} \]
\[ Y = \text{concentration of cation in ppm} \]
\[ a = \text{Y intercept of standard curve} . \]

Cation in ppm = Absorbance \( \times b + a \)

Cation in mg/100 g veal sample =
- Calcium = ppm \( \times 5 \times 1/\text{wt} \).
- Zn = 12.5 \( \times \) ppm \( \times 1/\text{wt} \).
- Mg = 250 \( \times \) ppm \( \times 1/\text{wt} \).
- Fe = 2.5 \( \times \) ppm \( \times 1/\text{wt} \).

A.VII. Muscle pH Measurement (Adapted Procedure by Ockerman, 1980)

**Equipment:**
1. A Flat-Surface Contact Probe pH electrode (Fisher Scientific Co., Pittsburgh, PA Cat. # 13-639-83).
2. Corning pH meter Model 7 (Corning Medical and Sci., Medfield, MA).
3. Weighing papers.

**Reagents:**
4. pH buffer of 5 (OSU reagent laboratory).
5. Demineralized water.
6. Acetone solution for rinsing of the probe to prevent fat accumulation.
7. Connect probe to Corning pH meter and calibrate at pH 5. Allow meter to warm for about 30 minutes and recalibrate.
8. Place about 10 g of the muscle sample on weighing paper moistened lightly with demineralized water and allow contact gently but firmly with tip of the probe and read pH of the sample in triplicate.
9. Read subsequent muscle samples after rinsing the probe with water and acetone solutions.

A.VIII. Abomasum Fluid pH Measurement

**Equipment:**
1. Any laboratory pH meter is suitable.
2. Polyethylene bottles with lids.

**Reagents:**
3. Two buffer standards of pH 2.0 and 5.0 (OSU reagent laboratory).
4. Demineralized water.
5. The veal abomasum tissue was pierced with a boning knife and content collected in the polyethylene bottles.
6. Read pH of the fluid after calibration of the meter with the two buffer standards. Rinse electrode thoroughly with demineralized water after each reading.

A.IX. Muscle Color Evaluation

Basically three separate procedures were used:
Sensory Method:

1. In the first method, about 40 g of veal muscle samples were cut 30 minutes prior to color evaluation by six panelists.
2. Samples were displayed at 2.2°C and color scored using the same grading system used in the commercial plants with a color standard as a guide. The color standard used is shown in Figure 10.
3. The scoring system used was on the scale of 1-4 for:
   1 = white
   2 = light red
   3 = red
   4 = red-red
4. The second procedure is called "Rating Method":
   a. An untrained person who is exclusively outside the meat area arranged the same samples scored by the trained sensory panelists from the lightest samples to the darkest samples.
   b. A trained panelist grouped the samples on the same scale of 1-4 as described and the percent in each color group based on total samples was calculated.
   c. Muscle Color Measurement by Reflectance (Adapted Procedure by Ockerman, 1985).

Equipment and Procedure:

1. Spectronic - 20. (Bausch and Lomb) with power supply unit and sample holder.
2. Set wave length to 685 nm.
3. Allow the equipment to warm for at least 30 minutes.
4. Immediately after sensory color scoring, wrap the veal samples in reflectance film (Cellophane film type).
5. Set needle at 0% on top scale with knob C.
6. With the opening covered by flap, slowly depress switch and set proper percent with knob A:

<table>
<thead>
<tr>
<th>Wavelength Used</th>
<th>Reflectance Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>570</td>
<td>82.6</td>
</tr>
<tr>
<td>650</td>
<td>81.0</td>
</tr>
<tr>
<td>685</td>
<td>80.0</td>
</tr>
</tbody>
</table>

7. Release switch H.
8. Open flap E (never open with switch depressed).
9. Place veal sample in the meat holder and place sample container over opening E.
10. Depress switch H and read % reflectance of the sample.
11. Note that the higher the reflectance, the lighter the samples.

A.X. Proximate Analyses (Adapted Procedure of Ockerman, 1981)
Percent total moisture and lipid were determined as outlined under the muscle cholesterol analyses (Appendix A.IV). For the protein analyses, the procedure used was as follows:
1. A piece of ashless # 42 Whatman filter paper was tared and then approximately 1.5 g of veal sample was weighed.
2. Sample and the paper were then placed in a clean 800 ml Kjeldahl flask.
3. Add 1 g of the catalyst mixture, 3 glass beads and 25 ml of concentrated H₂SO₄.
4. Start fan and light burners on digestion rack (low flame).
5. Digest the content until solution becomes blue-green and digest for additional 30 minutes. COOL FLASK!!.
6. To cold Kjeldahl flask, add 400 ml of distilled water while the flask is held in an inclined position.
7. Add 50 ml of 4% Boric Acid containing indicator to a 300 ml Erlenmeyer flask. Place this flask under the condenser with the glass tube immersed in the liquid.
8. Add two pieces of mossy zinc to Kjeldahl to prevent bumping.
9. Carefully layer 75 ml NaOH (19.6 N app.) in the Kjeldahl flask. Do not allow to mix. Connect the flask to the distillation apparatus, mix and distill over 200 ml into Erlenmeyer flask.
10. Remove Erlenmeyer flask and replace with 400 ml of distilled water. Turn the heat off.
11. Titrate the boric acid mixture with an HCl solution to a steel gray or nearly colorless endpoint. Pink color is too far.
12. Determine a blank which will contain everything but the sample. Blank will eliminate any N that might be present in the chemicals and water.
13. **Calculation:**

\[
\text{% Protein} = \frac{(\text{ml HCl sample} - \text{ml for blank}) \times 1.4 \times 6.25 \text{ N of HCl}}{\text{Weight of veal digested}}
\]

**Ash content determination (Adapted Procedure of Ockerman, 1981)**

1. Dry a porcelain evaporating dish overnight at 525°C in an ash oven.
2. Cool in desicator and weigh.
3. Add approximately 10 g of ground veal to the dish and re-weigh.
4. Dry overnight in drying oven at 100°C.
5. Heat on a hot plate in a hood until completely charred and smoking stops.
6. Place in ash furnace for 18 hours at 525°C.
7. Cool in desicator and weigh. Be careful not to blow the ash away when the desicator is opened.
8. Percent ash = \(\frac{\text{weight of residue}}{\text{Sample Weight}} \times 100\)


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