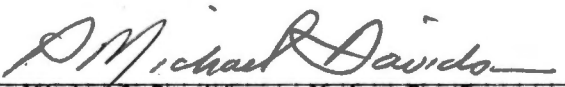
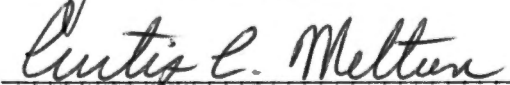


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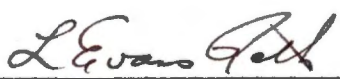
I am submitting herewith a thesis written by Melinda Elena Chen entitled "Sensory and Microbiological Characteristics of Grass-Fed Beef Formulated with Beef Fat and/or Pork Trim." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.


P. Michael Davidson, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

SENSORY AND MICROBIOLOGICAL CHARACTERISTICS
OF GRASS-FED BEEF FORMULATED WITH
FAT BEEF OR PORK TRIM

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Melinda Elena Chen

December 1982

3065144

DEDICATION

I would like to dedicate this thesis to my parents, Mr. and Mrs. Homer M. Chen, to whom I wish to express my utmost appreciation for their encouragement, continual support, immeasurable patience and understanding throughout the various challenging endeavors I have undertaken.

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VENI, VIDI, VICI.

ABSTRACT

Microbiological and sensory quality of grass-fed ground beef formulated with fat beef or pork trim was assessed. Samples were packaged in retail over-wrap, freezer wrap and a vacuum type film and stored under different conditions. Nine tests were conducted to detect potential spoilage and pathogenic microorganisms in the raw meat. Similar initial and final microbial plate counts were found for retail over-wrap samples analyzed during storage (4°C) on days 0, 2, 4, 6 and 8, and freezer wrap samples evaluated after 4 weeks in the frozen state (-29°C) and thawed (4°C) for 0, 1, 3, 5 and 7 days. However, the all grass-fed beef formulation in the retail over-wrap packaging had reduced psychrotrophic growth compared to the meat formulations containing grass-fed beef and fat grain-fed beef trim, grass-fed beef and fat pork trim, and all grain-fed beef. This did not occur with other packaging methods. Lipid deterioration was measured by the 2-thiobarbituric acid test (TBA). Difference in degree of oxidation of the various meat types during storage could be attributed to variation in the fatty acid composition from different muscle and fat sources. TBA values for the beef-pork mixes were significantly higher than the 3 other meat types, but were comparable or lower than the figures reported by previous investigators. Sensory evaluation of the meat patties indicated average scores in the acceptable range with beef-pork mixes possessing the lowest sensory attributes. Results from the present study show that the addition of fat beef to grass-fed beef

potentially improves its palatability. Microbiological data suggest that grass-fed beef mixes may be used safely as ground meat products.

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CHAPTER I

INTRODUCTION

World grain shortages during the past few years have increased foreign demand and resulted in higher prices for grain. Ground beef consumption in the United States has increased in recent years (USDA, 1979). Eighty-eight percent of consumers serve this ground product two or more times per week (Law et al., 1965). As a result, interest has developed in marketing the less expensive forage-fed steer as an alternative to all grain-fed beef products. However, grass-fed beef has been found to have a less desirable flavor than grain-fed beef and thus, has limited acceptability at the wholesale and retail levels.

A possible approach to improving the flavor and acceptability of grass-fed beef is to mix the edible tissue (fat and muscle) from the fat, thinly muscled cuts (flank, plate and brisket) of overly-finished beef carcasses with the edible tissue from grass-fed beef carcasses. Another alternative might be the addition of fat pork trim (50% lean--50% fat) to the grass-fed beef.

Information on the microbiological effects of mixing muscle and fat from different animals or different feeding regimens to produce ground beef is virtually unknown. Microbiological examination of this mixed raw meat product is necessary to provide data concerning its initial quality and subsequent shelf life.

Oxidative rancidity is a major cause of deterioration in the

quality of meat. Undesirable changes in flavor, color and nutritive value may occur as meat lipids oxidize. Frozen raw meat is generally resistant to oxidation, but adverse changes in flavor and color may result during freezing and thawing. The development of rancidity may be accelerated by the wide fluctuations in temperature and insufficient protection against oxygen.

The purpose of this investigation was to determine: (1) the microbial quality of ground beef formulated with grass-fed beef, grain-fed beef and/or pork, stored under various environmental conditions; (2) the susceptibility of this product to oxidative rancidity with different storage times and packaging methods; and (3) the consumer acceptability of grass-fed beef formulated with fat beef or pork trim.

CHAPTER II

REVIEW OF LITERATURE

A. Grass-Fed Beef Versus Grain-Fed Beef

Interest in grass-fed beef by livestock producers tends to occur during periods of feed grain shortage. However, Bowling et al. (1977) reported that forage-finished beef does not compare favorably at the retail level with grain-finished beef. Characteristics which limit acceptability at the retail and wholesale level are the color differences (muscle and/or fat) and poor palatability (flavor and/or tenderness). Brown et al. (1979) reported that meat packers discriminate against grass-fed beef because of lower dressing percentages, higher cooler shrinkage and lower quality grades.

The color of the fat of grass-fed beef tends to be more yellow than grain-fed beef (Hunt et al., 1953; Kropf et al., 1975; Sathirakul, 1976; Brown et al., 1979). Cabell et al. (1943) attributed this yellow color to the higher concentration of carotene in the feed of forage-finished beef. They also suggested that forage-fed beef may have a higher rate of carotene absorption than grain-fed beef.

In a study on muscle color, Kropf et al. (1975) found the longissimus muscle of grass-fed beef to be darker than grain-fed beef. It has been suggested by Schupp et al. (1976) that this color may be due to the higher susceptibility of forage-finished cattle to pre-slaughter stress and to the dark cutting beef syndrome.

Research has shown that grass-fed beef was tougher and had

higher Warner-Bratzler shear values than grain-fed beef (Meyer et al., 1960; Bowling et al., 1977; Dyer, 1980). These higher shear forces may result from the extensive cold shortening of the sarcomeres in grass-fed beef muscle due to an extremely thin fat cover on the carcass (Schroeder et al., 1980).

Low flavor scores for grass-fed beef have been related to one or more of the following: (1) fatty acid composition (Amiri, 1980; Black, 1981; Melton et al., 1982); (2) low fat content (Oltjen et al., 1971); (3) weight and grade differences of grass-fed beef versus grain-fed beef (Bidner, 1975); (4) lack of a typical beef fat flavor (Amiri, 1980; Melton et al., 1982); (5) off-flavors (dairy, milky, grassy, metallic, fishy) (Schroeder et al., 1980; Black, 1981; Melton et al., 1982).

Data reported by Waldman et al. (1968) and Dryden and Marchello (1970) indicated a relationship of fatty acid composition to meat palatability. Brown et al. (1979) found that grass-fed beef contained higher levels of stearic and linolenic acids in the polar lipids and lower amounts of palmitic acid in the neutral and polar lipids than grain-fed beef. These researchers postulated that linolenic acid (an unsaturated fatty acid highly susceptible to oxidation) contributed to low flavor scores of grass-fed beef. High positive correlation coefficients between flavor score and either intramuscular oleic acid or intramuscular total unsaturated fatty acids were reported (Westerling and Hedrick, 1979). High negative correlations with flavor scores were found with other intramuscular fatty acids (palmitic, stearic, linoleic) and total saturated fatty acids.

Additional research is needed to determine the economic feasibility of producing and marketing forage-finished beef as an alternative to increased grain costs (Hodgson, 1971). Medical controversies have also arisen surrounding the consumption of beef with high amounts of fat in relation to heart disease (Clark et al., 1980). Studies should be conducted in the areas of flavor improvement and microbiological quality of grass-fed beef as they relate to acceptability and good shelf life for marketing purposes.

B. Spoilage Problems of Meat

Microbial Spoilage of Fresh Meat

Little research is available concerning the microbiological analysis of grass-fed beef. Studies which have been conducted contain contradicting data concerning both the types and numbers of microorganisms found in this type of beef. For example, Amiri (1980) found higher numbers of psychrophilic and lipolytic microorganisms on grass-fed beef than steers finished on grain. In contrast, Reagan et al. (1977) showed that feeding regimens did not significantly affect microbial numbers on subcutaneous fat and lean tissue. Because the data on the microbial quality of forage-fed beef is limited, a review of the spoilage of grain-fed beef will be presented for background information.

Comminuted fresh meat such as ground beef invariably has higher numbers of microorganisms than noncomminuted meat such as steaks. A variety of reasons have been reported for the higher bacterial populations in ground meat: (1) the process of manufacturing results in

trimmings from various cuts which represent multiple handling of the product (Emswiler et al., 1976); (2) greater surface area usually results in an increased exposure of meat surfaces stimulating the growth of aerobic spoilage microorganisms; (3) inadequate sanitation promotes build-up of microbial numbers on the processing equipment (Berry and Chen, 1976); and (4) formulation of meat using fat with contaminated lymph nodes causes increased microbial numbers after the meat is passed through the grinder (Lepovetsky et al., 1953).

Spoilage of refrigerated fresh meat is primarily the result of the growth and metabolism of the following genera: Pseudomonas, Alcaligenes, Acinetobacter, Moraxella and Aeromonas (Jay, 1978). Research by Stringer et al. (1969) demonstrated that Pseudomonas fragi was one of the predominant species involved in the spoilage of aerobically stored ground beef. These psychrotrophic microorganisms compete well for oxygen and the result of their growth is often slime formation, and rancid off-flavors and odors (Kirsch et al., 1952). Therefore, while these microorganisms are generally non-pathogenic to man, they are an important problem in the meat industry because they decrease the shelf life of fresh meats and cause economic losses.

"Aromabakterien" was the first term used to describe the rancid, sweetly aromatic esterlike odor of spoiled meat (Glage, 1901). This aroma is indicative of incipient spoilage as it occurs a few hours before large numbers of minute, translucent, moist colonies appear. According to Ayres (1960), a microbial load exceeding $3 \text{ to } 6 \times 10^7/\text{cm}^2$ results in a uniform sticky, or slimy layer on the meat. After the bacteria

utilize the carbohydrates present in the meat, ammonia-like odors appear as amino acid degradation occurs (Jay, 1972; Gill and Newton, 1978).

Smith and Alford (1968) also reported that Pseudomonas species produced large amounts of alkanals, 2-alkenals and 2-alkenes which contributed to off-flavors in meat products.

The shelf life of ground meat is related to its initial contamination level. Ayres (1960) found that meat stored at 5°C exhibited sliminess within 6 days when the initial bacterial load exceeded $10^5/\text{cm}^2$. When the initial load was less than $100/\text{cm}^2$, sliminess was delayed until the 12th day.

Ayres (1951) also investigated the changes in microbial flora during the storage of fresh meat at 0, 5, and 10°C. Enterobacter, Flavobacterium, chromogenic bacteria, spore-forming organisms, molds and yeasts together comprised 20 to 50% of the total population of the meat at the beginning of the low temperature storage period. As the storage period lengthened, however, the microbial flora shifted to where Pseudomonas, Microbacterium and Micrococcus were the predominant genera. Because of their ability to grow at cold temperatures and compete well for oxygen, the pseudomonads have a generation time 30% shorter than other species on meat surfaces at temperatures between 2 and 15°C (Ayres, 1960).

Many surveys have been conducted to determine the microbial quality of commercial ground beef. The meat industry and regulatory agencies have proposed the development of quality assurance programs to provide the consumer with meat and meat products of the highest quality (Surkiewicz et al., 1975). Studies such as these present background

information for establishing quality standards for ground beef.

Duitschaever et al. (1973) conducted a study on 213 ground beef samples purchased directly from display cases at 2°C. Mesophilic and psychrotrophic counts on 64% of the samples were in excess of 10^7 /g. The average range for the coliforms was 1.4×10^3 /g to 1.9×10^4 /g and enterococci counts ranged from 10 to 10^4 /g. In 98% of the samples, staphylococci were present at 1×10^3 /g. Salmonella was not detected in any sample which may be attributed to the relatively low pH (5.6-5.8) of the meat. In an early study, Weinzirl and Newton (1914) suggested a total plate count of 10^6 /g as an upper limit standard for raw hamburger meat. If this figure were used as the standard, 64% of the samples in the Duitschaever study would be unacceptable.

In 1975, Al-Delaimy and Stiles surveyed the microbial quality of raw ground beef from retail counters in Edmonton, Alberta. They reported that the average total plate count and psychrotrophic counts were approximately equivalent (3.8×10^6 /g after 4 days at 5°C) suggesting that the psychrotrophs were the principal organisms on the meat. Staphylococcal counts on 58 samples of ground beef ranged from 4.3×10^2 /g to 1.1×10^5 /g. These counts indicated the absence of a food poisoning hazard. Ninety-eight percent of the samples tested contained an excess of 100 coliforms/g. Yeast and mold counts had 67 to 4.1×10^3 /g as the average population. These numbers were insignificant relative to the total microbial count.

In a study to determine the importance of yeasts to the spoilage of fresh ground beef, Jay and Margitic (1981) analyzed 111 samples for total, gram-negative and yeast counts. They found 9.5×10^6 /g for the

mean aerobic plate count, 3.5×10^6 /g for the mean gram-negative count, and 8.7×10^3 /g for the mean yeast count. Therefore, the yeasts comprised only a small percentage of the total population. They concluded that yeast counts were generally low in fresh ground beef because they did not compete well with the bacterial population.

One hundred fifty-five samples of raw ground beef obtained from markets throughout the United States were analyzed for aerobic mesophiles and coliforms (Goepfert, 1976). Samples stored at 5°C gave an average aerobic plate count (APC) in the range of 1×10^5 /g to 5×10^7 /g, and an average coliform count of 1×10^3 /g. Another bacteriological analysis of ground beef in Maryland was conducted to provide information concerning bacterial standards on fresh meats. Westhoff and Feldstein (1976) reported a total plate count of 7.9×10^6 /g (28°C) and 2.0×10^6 /g (35°C). Samples were stored at -15.5°C and analyzed on days 0, 1, 2, and 6. The average coliform, fecal coliform and Escherichia coli count per gram was 10^2 , 10, and 5, respectively. In another recent study, Emswiler et al. (1976) evaluated storage time at $-1.7 \pm 0.6^\circ\text{C}$ on the microbial flora of ground beef. The initial microbial load for total aerobic and psychrotrophic bacteria was 10^6 or less/g. Nearly all of the samples contained 100 or fewer coliforms, E. coli, coagulase-positive Staphylococcus aureus and Clostridium perfringens per gram. After 18 days of storage, the total aerobic and psychrotrophic bacterial counts had increased by 1 log, the coliforms and E. coli had decreased, and the S. aureus and C. perfringens had remained constant. Rogers and McClesky (1957) noted an average total plate count of 3.28×10^7 /g for 96 ground beef samples

from retail stores in Baton Rouge, Louisiana. Micrococcus, Bacillus and yeasts were prevalent at the initial storage period. However, after 14 days storage at 7°C, Pseudomonas became the predominant microorganism. Research by Law et al. (1971) calculated a mean range of 1.9×10^6 to 4.6×10^8 /g as the microbial level for acceptance.

Bacterial numbers reported by the previous investigations have provided data to set maximum microbial standards for ground beef based on the appearance of off-odors and slime formation on the meat surface (Kotula, 1970). Some states have already established standards. A legal maxima of 5×10^6 /g and 50/g for the aerobic plate count (APC) and E. coli assay, respectively, has been followed by Oregon (Goepfert, 1976). Exceeding these limits in 3 successive samples makes the supermarket manager liable to criminal prosecution. The state of New York has stringent limits of 1×10^6 /g (APC) and 10 E. coli/g, and Maryland has proposed standards of 1×10^7 /g (APC) and 50 fecal coliforms/g (Westhoff and Feldstein, 1976).

While some samples in the previous studies exceeded standards for microbial numbers, the counts used in the standards do not necessarily reflect hazard levels. According to Jay (1978), coliform counts in excess of 1000/g indicate possible fecal contamination with a concurrent possibility of the presence of enteric pathogenic microorganisms. High numbers (10^3 /g) of S. aureus would also imply improper handling and poor sanitation practices. It is obvious that there are still many questions to be answered concerning microbial standards for fresh meat.

Microbial Spoilage of Vacuum Packaged Meat

An anaerobic environment such as that found with vacuum packaged meats creates an "ecosystem" which is selective with respect to the level and type of microbial flora (Pierson et al., 1970). Anaerobic conditions suppress the growth rate of the common meat spoilage bacteria such as Pseudomonas and Achromobacter species and allow the growth of facultative anaerobes such as lactobacilli and Leuconostoc species (Halleck et al., 1958; Ordal, 1962; Shaw and Nicol, 1969; Seideman et al., 1976). Past studies have found lactobacilli as the predominant microorganism on anaerobically packaged fresh meat (Jaye et al., 1962; Ingram, 1962; Sutherland et al., 1975; Patterson and Gibbs, 1977). However, recent studies have also shown that the organism Brochothrix thermosphactum occurs on this type of meat along with the homo- and heterofermentative lactobacilli (Grau, 1980). Initially, B. thermosphactum was found to dominate the microflora during storage at 0°C; however, lactic acid bacteria eventually grew to higher numbers than the Brochothrix (Gardner, 1966; Campbell et al., 1979).

In contrast to the studies above, Christopher et al. (1979) isolated species of oxidative type-pseudomonads, Acinetobacter and Moraxella during the first 4 weeks of storage of a vacuum packaged meat. By the 7th week, the fermentative Enterobacteriaceae dominated the microflora and by the end of storage at 17 weeks most of the group belonged to the Enterobacter genus. While the findings of Christopher et al. (1979) are possible, it is more likely they were an aberration since Lactobacillus spp. have been found to grow faster than both Enterobacter and B. thermosphactum spp. under anaerobic conditions (Roth and Clark, 1972).

These contradictory findings may possibly be due to differences in initial contamination, type of meat tested (for example, pork vs. beef) and competitive growth rate (Jay, 1978).

Research by Baltzer (1969) has shown that vacuum packaged meat has certain microbiological advantages over aerobically stored meat. Possible reasons for this are: (1) total bacterial counts increase more slowly; (2) putrefaction and slime formation are reduced; and (3) final bacterial counts after storage are usually lower than comparable samples packaged in oxygen-permeable films. As an example, in a study conducted by Roth and Clark (1972), vacuum packaging prevented microbial spoilage of fresh beef for 32 days, while the same product had a shelf life of only 3 days after exposure to oxygen.

While vacuum packaging extends the shelf life of fresh meat and minimizes shrink loss, discoloration and microbial growth (Baran et al., 1969; Roth and Clark, 1972), there are disadvantages. Some of these include a defect which can cause greening of the meat through the formation of sulphmyoglobin. Nicol et al. (1970) showed that organisms resembling Alteromonas putrefaciens produced this green defect at 10^6 /g. Another contaminant on vacuum packaged meat is the formation of brown-black spots on the fat surface (Grau, 1978). Saccharomycopsis lipolytica has been implicated as the source of these spots.

Baran et al. (1969) reported that consumer acceptance of the color of anaerobically stored meat may also be a potential problem. Lanier et al. (1978) found acceptability of vacuum packaged meat to be reduced by color changes--the red oxymyoglobin is converted to the brown

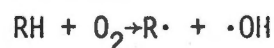
metmyoglobin by oxidation, and is not as esthetically acceptable to the consumer.

Lipid Deterioration in Meat

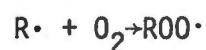
In addition to microbial spoilage, lipid oxidation is also a major cause of deterioration in the quality of fresh meat and meat products. Undesirable flavors and odors arise from the aldehydes, ketones and hydrocarbons produced from the peroxidation of unsaturated fatty acids in stored triglycerides or tissue phospholipids (Love and Pearson, 1971). This results in rancid flavors (or warmed-over flavor in cooked meats) in meat and meat products (Sato and Hegarty, 1971; Greene and Cumuze, 1981).

Oxidation of unsaturated fatty acids has been well documented (Labuza, 1971). The autoxidation reaction can be divided into three parts, initiation, propagation and termination:

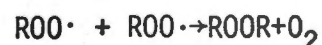
Initiation



Propagation



Termination



RH refers to any unsaturated fatty acid in which the removal of H takes place at the carbon atom adjacent to a double bond. Labuza (1971)

reported oleate, linoleate and linolenate as the principal unsaturated fatty acid moieties found in foods. A free radical, $R\cdot$, is formed by removal of a labile hydrogen.

The 2-thiobarbituric acid (TBA) procedure is the chemical test most often used for the assessment of oxidative rancidity in red meat, poultry and fish (Gray, 1978). Malonaldehyde, a 3-carbon compound and decomposition product of lipid oxidation, complexes with TBA to form a red pigment with a maximum light absorption at 530-538 nm (Sinnhuber and Yu, 1958). These researchers also proposed that this chromagen was formed through the condensation of 2 molecules of TBA with 1 molecule of malonaldehyde (Figure 1).

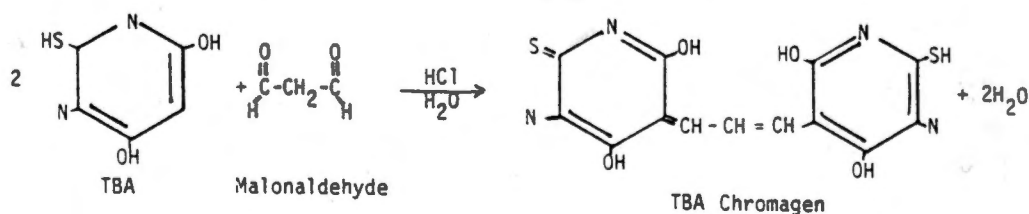


Figure 1. Condensation of 2 TBA molecules with 1 molecule of malonaldehyde.

According to Pearson (1968), the TBA procedure has two advantages over the other oxidation spoilage tests: (1) TBA measures deterioration in both extractable and non-extractable lipids; (2) the other chemical assessment tests measure the peroxide level which is only an indication of the intermediates in the oxidation decomposition of unsaturated fatty acids whereas the TBA test measures malonaldehyde which is an end product of the oxidation. Measurements of lipid

deterioration can be made over a period of time because the materials reacting with TBA accumulated without further decomposition.

Studies have shown this test to be useful in predicting the keeping quality (shelf life) of fatty foods, and correlating TBA numbers (mg TBA reacting substances/kg tissue) with trained sensory panelists' scores for rancid odor (Turner et al., 1954; Younathan and Watts, 1959; Tarladgis et al., 1960). Turner et al. (1954) were the first to apply the TBA method to test for rancidity in meat products. The sample was heated with an acid solution for 30-50 minutes and the pigment was extracted with a suitable solvent. A distillation procedure was developed by Sidwell et al. (1955) for determining oxidative changes in dairy products which eliminated the long heating periods and solvent extractions of the Turner method. Tarladgis et al. (1960) adapted the procedure of Sidwell et al. (1955) to meat products. Four years later, Tarladgis et al. (1964) reported that the acid-heat treatment was not necessary for the condensation of TBA with malonaldehyde nor for maximum color development. The compounds formed from such a treatment with TBA were found to exhibit the same wavelength absorbancy as the TBA-malonaldehyde colored complex. Another potential problem with the acid-heat distillation procedure is the ability of TBA to interact with various aldehydes at room temperature to form insoluble precipitates in the presence of acid (Plaisance, 1917). Vyncke (1975) compared the direct TBA method with the distillation method for the determination of rancidity.

The author concluded that the simpler and quicker method could be used as high correlations between the two procedures were observed.

Several studies have reported TBA values for fresh meat and meat during storage. Keskinel et al. (1964) reported an increase in TBA numbers of ground beef refrigerated at 5°C. TBA values were 0.52 at 1 day refrigeration and 7.21 after 10 days refrigeration. Pearson (1968) conducted a study on fresh meat and meat stored at 5°C for 20 days. Meat with 11.2% fat had TBA values that increased from 0.02 mg malonaldehyde/kg meat at day 0 to 2.05 at day 20. Fatty tissue meat with 70.2% fat had TBA values that increased from 0.38 to 0.72. High TBA values in lean beef were probably attributed to the high phospholipid content. A study on the chemical deterioration of frozen bovine (beef round) muscle at -4°C showed a progressive rise in TBA number during an 8 week storage period: 0.02 at day 0 to 0.27 at day 56 (Awad et al., 1968). Greene (1969) used the TBA test to demonstrate lipid oxidation in raw beef. Ground beef stored at 4°C for 2 to 8 days gave TBA numbers of 5.7 and 12.5, respectively. Keller and Kinsella (1973) determined TBA values for ground beef stored at -18°C for 16 weeks. A TBA value of 0.53 was obtained prior to freezing and a value of 1.46 was found by the 16th week. Jakobsson and Bengtsson (1973) also found a significant increase in TBA values for frozen ground beef (19.8 for 1 month and 27.6 for 9 months), and a decrease in sensory flavor scores. In a study on raw ground beef, Abo-gnah (1978) found TBA values and microbial count to increase during storage while taste panel scores decreased. TBA values of 1.2 to 3.8 were found acceptable to taste panelists from day 0 to day 6. However,

after 9 days of refrigerated storage, a taste panel score of 4.0 (maximum score = 10) and a TBA value of 4.2 indicated rejection of the meat on the basis of a putrid flavor. Lopez-Lorenzo et al. (1980) found that TBA numbers increased during a 20 day (1°C) storage of ground pork meat as follows: 1.0 at day 0, 8.8 at day 10 and 13.0 at day 20. Variability in TBA numbers as shown by these studies makes it difficult to suggest a critical TBA limit of acceptability. Factors such as appearance and odor of the meat must also be taken into consideration in the assessment of lipid deterioration.

An increase in TBA numbers during storage of ground meat may be attributed to an increase in surface area and resultant incorporation of oxygen into the meat during grinding (Keskinel et al., 1964). The rate of oxidation is dependent upon the meat type. Differences in TBA numbers between meat types may be attributed to two factors: (1) nature of the lipids and (2) heme catalysis. Pork has been reported to have a higher unsaturated fatty acid content than beef (Keskinel et al., 1964) and thus, should be more susceptible to autoxidation. Lipid oxidation in meat has been reported to be catalyzed by hematin compounds (Tappel, 1955). Heavily pigmented beef would be more susceptible to heme catalysis than pork or lamb.

Oxidation rate is also dependent upon storage temperature since frozen meat (14½ weeks at -18°C) was found to have lower TBA values than refrigerated meat (3 weeks at $5 \pm 1^{\circ}\text{C}$) (Keskinel et al., 1964). This suggested that unfrozen tissue fluids provide a better medium than the frozen flesh for the passage of dissolved oxygen.

CHAPTER III

MATERIALS AND METHODS

A. Meat Preparation

Meat Source

The meat used for this study was purchased from two commercial packing plants and boned at The University of Tennessee, Knoxville, Meat Laboratory. The grass-fed beef samples were taken from a U. S. Standard, Yield Grade 1 beef forequarter. U. S. Choice beef chucks were used for the grain-fed beef samples. Fat beef trim was composed of the briskets, flanks and plates of Yield Grade 4 and 5 grain-fed beef carcasses. Boneless fat pork trim was obtained from a commercial packing plant. Both fat trims were expected to contain approximately 50% lean and 50% fat.

Meat Formulation

Each of the boneless meat samples was ground separately through a $\frac{1}{4}$ inch (0.06 cm) plate, and mixed in a Leland mixer. The Modified Babcock procedure of Ockerman (1969) was used to approximate the fat content of the lean ground muscle and fat trims. Four meat mixes were then formulated: (1) beef-beef mix, prepared from grass-fed beef and fat grain-fed beef trim; (2) beef-pork mix, prepared from grass-fed beef and fat pork trim; (3) regular mix, prepared from grain-fed beef; (4) grass-fed beef mix, prepared from a forage-fed beef side with all external fat removed prior to boning. All meat formulations were

finally ground through a 1/8 inch plate to produce mixes that contained approximately 20, 25 or 30% fat. The appropriate ratios of fat to lean were determined by the Pearson Square Method (Terrel, 1971) and the final fat content analyzed by the crude fat (Soxhlet extraction) procedure (AOAC, 1975). Each mix was made into 4 ounce (113 g) patties.

Packaging

Three types of packaging were employed in this study: (1) a retail over-wrap using polyvinyl chloride 914 Reynolds film (Reynolds Food Service Packaging, Richmond, Virginia); (2) vacuum packaging employing a 2.0 mil thick flexible material (W. R. Grace Company, Cryovac Division, Duncan, South Carolina) with an oxygen permeability of 30 to 40 cc/m² in 24 hours at 1 ATM and 73°F; (3) a freezer wrap using lite laminated freezer food wrap (American Can Company, Greenwich, Connecticut).

B. Microbiological Analysis

A representative 25 g sample of meat was weighed into a stomacher bag (Dynatech Laboratory, Alexandria, Virginia). With the exception of the Salmonella analysis, all of the microbiological assays employed 225 ml sterile 0.1% peptone as the diluent. Selenite Cystine (SC) broth (225 ml) was added to meat samples when testing for the presence of Salmonella. The meat mixture was blended in the stomacher for 2 minutes. Appropriate serial dilutions of the homogenate were then used for microbiological assays.

Nine tests were conducted to detect potential spoilage and pathogenic microorganisms in the raw meat (Table 1). Methods of

Table 1. Tests Used to Evaluate the Microbiological Quality of Grass-Fed Ground Beef Formulations.

Microorganisms	Enumeration Technique	Medium	Incubation Conditions
Mesophilic Aerobes and Facultative Anaerobes	Pour-plate	Standard Methods	35°C, 48 hr
Gram-negative Psychrotrophic Aerobes	Pour-plate	Crystal Violet Tetrazolium agar (CVT)	30°C, 48 hr
Coliforms	Pour-plate	Violet Red Bile agar (VRB)	35°C, 24 hr
Yeasts and Molds	Pour-plate	Potato dextrose agar (PDA)	20-25°C, 5 days
Lactic Acid Bacteria	Pour-plate	All Purpose Tween agar (APT)	30°C, 48 hr
Fecal Streptococci	Pour-plate	KF Streptococcal agar (KF)	35°C, 24 hr
<u>Clostridium perfringens</u>	Pour-plate	Tryptose Sulfite Cycloserine-Egg Yolk Free agar (TSC-EY)	35°C, 24 hr (anaerobic) ^a
<u>Staphylococcus aureus</u>	MPN	Trypticase Soy Broth with NaCl (10%) (TSB-NaCl)	35°C, 48 hr
	Pour-plate	Baird-Parker agar	35°C, 48 hr
<u>Salmonella</u>	Pour-plate	Brilliant Green agar (BG)	35°C, 24 hr
	Slant	Triple Sugar Iron agar ^b (TSI)	35°C, 24 hr

a = Oxoid Anaerobic System (Oxoid U.S.A., Inc., Columbia, Maryland).

b = Serological flagellar (H) screening test conducted on presumptive positive TSI slants (Sperber and Deibel, 1969).

microbiological analysis used in the study conformed to those published by Compendium of Methods for the Microbiological Examination of Foods (Speck, 1976). Commercially prepared media used in this study were obtained from BBL, Division of Becton, Dickinson and Company, Cockeysville, Maryland.

Samples were stored for analysis under the following conditions: (1) retail over-wrap samples stored at 4°C for 0,2,4,6,8 days; (2) vacuum packaged samples stored at 4°C for 0,7,14,21,28 days; (3) freezer wrap samples stored at -29°C and 4°C for 0,1,3,5,7 days. Three zero times were carried out for the freezer wrap samples: (1) fresh meat prior to freezing; (2) meat frozen at -29°C for 4 weeks; (3) meat thawed at 4°C for 24 hours after 4 weeks. The microbiological assays were determined by packaging method and media type as shown in Table 2.

Salmonella testing involved incubation at 35°C for 24 hours in selective Selenite Cystine enrichment broth. Loopfuls of the broth were plated on Brilliant Green agar and incubated at 35°C for 24 hours. Suspect colonies were cultured onto Triple Sugar Iron agar (TSI) slants at 35°C for 24 hours. The serological flagellar (H) screening test was done on presumptive positive TSI agar cultures (Sperber and Deibel, 1969).

C. Chemical Analysis

Modified Babcock Test

This screening test measured the fat content of the meat sources. Each sample was treated with concentrated sulfuric acid to digest all non-adipose material. The sample was then centrifuged and the fat

Table 2. Microbiological Assay by Packaging Method and Media Type.

Packaging Method	Media Type
Retail Over-Wrap	SMA, CVT, VRB, PDA, TSB-NaCl, BG(TSI)
Vacuum	SMA, CVT, VRB, APT, TSC-EY, TSB-NaCl, BG(TSI)
Freezer Wrap	SMA, CVT, KF (Frozen samples) SMA, CVT, VRB, PDA, KF, TSB-NaCl, BG(TSI) (Thawed samples)

percentage read directly from the calibrated neck of a paley bottle.

Soxhlet Extraction Test

This analysis accurately measured percent fat (AOAC, 1975). Dried meat samples were heated in a Soxhlet apparatus for several hours as ethyl ether extracted the fat from the meat. The loss in weight of the extracted samples was calculated as percentage of fat.

Thiobarbituric Acid Test

The 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1964) was used to quantitatively assess the end products of lipid oxidation in the meat mixes. In this test, malonaldehyde, a 3-carbon compound produced during the oxidative breakdown of unsaturated fatty acids, reacts with TBA to give a measure of rancidity.

A 10 g sample was blended 2 minutes with 50 ml of distilled water in a Stomacher for 2 minutes. The slurry was quantitatively transferred to a beaker with an additional 50 ml of distilled water and filtered into a 100 ml volumetric flask. A portion of the clear filtrate (5 ml) was pipetted into a test tube and 5 ml of TBA reagent added. The tube was stoppered and set aside for 15 hours at room temperature. The optical density of the TBA-malonaldehyde samples was read at 538 nm on a Shimadzu Double-Beam Spectrophotometer UV-190 (Shimadzu Corporation, Kyoto, Japan).

A standard curve was prepared by determining the absorbance of solutions containing 1×10^{-8} to 8×10^{-8} moles of malonaldehyde per 5 ml after the formation of the TBA-malonaldehyde complex. Absorbance values for the TBA-malonaldehyde complex were converted to mg

malonaldehyde per 1000 g of meat and referred to as "TBA numbers."

Samples packed in retail over-wrap at 4°C were analyzed on 0,4 and 8 days. Vacuum packaged samples at 4°C were analyzed on 0,14 and 28 days. Samples frozen for 4 weeks at -29°C were thawed and analyzed after 1,3,5 and 7 days of storage at 4°C.

D. Sensory Evaluation

Sensory evaluation was conducted in the sensory evaluation laboratory of the Food Technology and Science Department. A difference test was done using a 10-member experienced taste panel to determine if there were differences between meat type and fat level of the 12 meat samples. The number of samples served for a subsequent consumer taste panel was dependent on the results of this test. Patties were evaluated for flavor, juiciness and tenderness on an 8-point scale where 8=extremely intense (beefy) and 1=extremely bland for flavor; 8=extremely juicy and 1=extremely dry for juiciness; and 8=extremely tender and 1=extremely tough for tenderness (Appendix A).

Statistical analysis indicated a difference ($P < 0.05$) between the meat types but not fat level ($P > 0.05$). Therefore, a fat level of 25% was selected for use with a consumer taste panel. In the consumer panel, a hedonic scale was used in an attempt to determine the overall acceptability of the 4 meat products. Scores ranged from 9=like extremely to 1=dislike extremely (Appendix B).

Meat patties for the sensory tests were thawed overnight in a 1°C cooler prior to cooking. Samples were then cooked in a pre-heated Presto Burger Hamburger Cooker for 3 minutes. Cooked patties were

divided into 6 portions and kept warm (approximately 54°C) on a heated serving tray. Each panelist was served 1/6 of the cooked sample on a coded white plate under red lights in individual booths.

Methods of sensory analysis used in the study conformed to those published in Methods for Sensory Evaluation of Food (Larmond, 1970).

E. Statistical and Computer Analysis

The entire experiment was replicated at least twice. Analysis of variance was used to statistically analyze the data (Snedecor and Cochran, 1971). Main effects (meat type and fat level) and interactions were analyzed according to the General Linear Model procedure (Barr et al., 1979). If significant differences existed between mean effects or interactions, Duncan's Mean Separation technique (Duncan, 1955) was used to determine differences among means. Significance was reported as $P < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

A. Microbiological Quality

In cases where there was no significant effect of meat type or fat level, only the log counts of the sample containing 25% fat were shown. The remaining data was compiled in Tables 10-12 (Appendix C).

Figure 2 depicts the log aerobic plate count of the retail over-wrap samples for the 8 day storage period at 4°C. Samples were analyzed on days 0,2,4,6 and 8. Meat type ($P>0.05$) and fat level ($P>0.05$) did not have a significant effect on the number of microorganisms found.

Results obtained from these samples were in agreement with previous investigations on the microbial quality of ground beef. For example, Ayres (1960) reported that a microbial load exceeding $3 - 6 \times 10^7/\text{cm}^2$ (log 7.48 - log 7.78) by day 6 led to an unacceptable ground meat product since slime appeared on the surface of the meat. In the present study, off odors and slime were also encountered at day 6 when the average log count for all samples was 7.50. Duitschaeffer et al. (1973) also found total plate counts of ground beef samples obtained at retail outlets to be in excess of $10^7/\text{g}$ (log 7.00). In research conducted by Al-Delaimy and Stiles (1975), the total plate count was $3.8 \times 10^6/\text{g}$ (log 6.58) for raw ground beef in display cabinets at 2°C.

In contrast to the samples plated on SMA, meat type ($P<0.05$) and fat level ($P<0.05$) did have a significant effect on the psychrotrophic bacterial growth for the samples packaged in retail over-wrap film

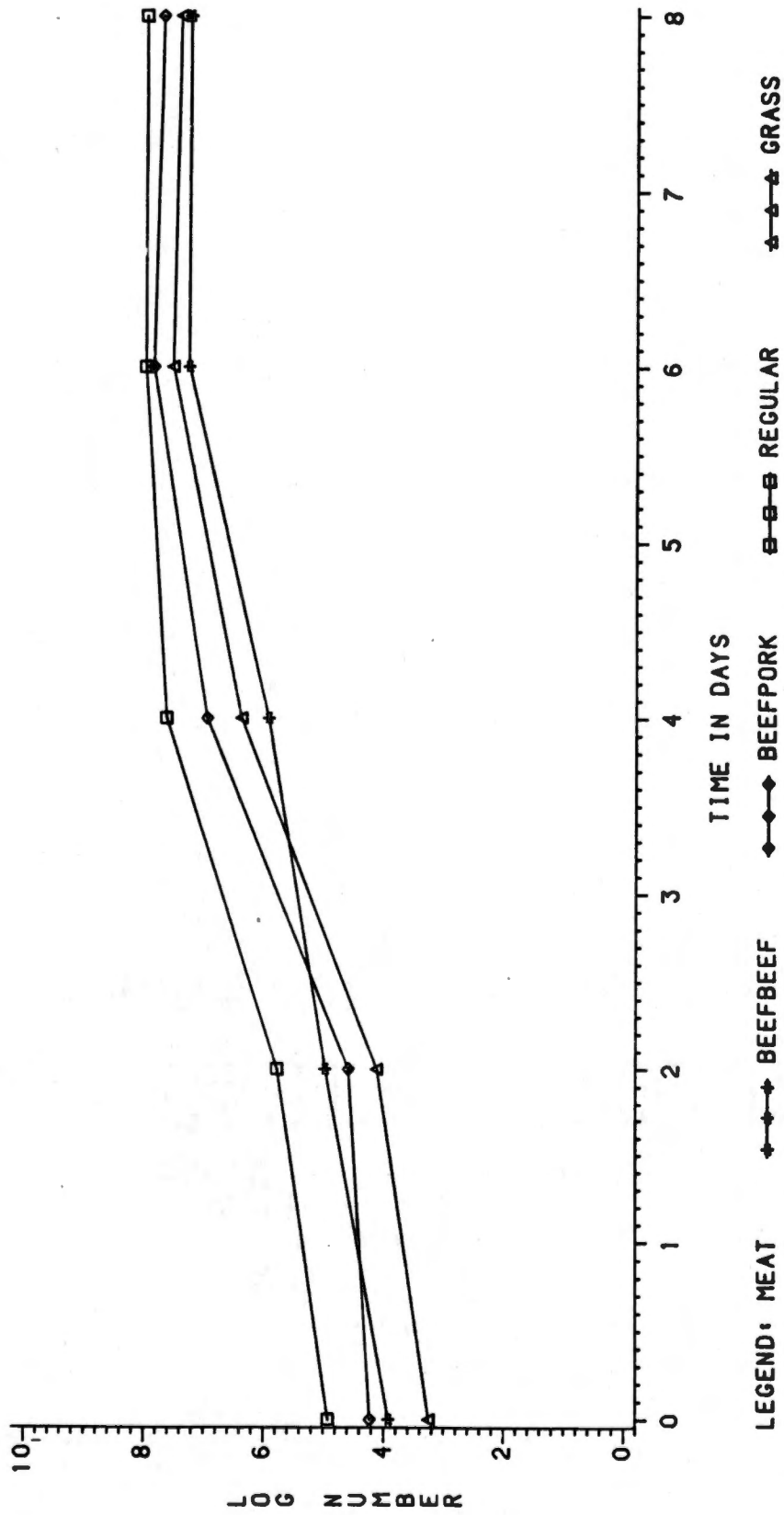


Figure 2. Log counts of retail over-wrap ground meat samples at 25°C and plated on SMA for 0, 2, 4, 6 and 8 days.

(Figures 3-5). The Duncan Mean Separation test showed that the 30% fat level (Figure 5) had significantly higher overall mean counts than the 25% and 20% fat levels. The mean log count of the grass-fed beef mix over time was found to be significantly lower (4.33) than the other three meat formulations which had a mean count of 6.16 (regular mix, $\bar{X}$ = 6.23; beef-pork, $\bar{X}$ = 6.16; beef-beef, $\bar{X}$ = 6.09). While the grass-fed beef had a lower microbial load than the grain-fed beef, it was interesting to note that the addition of the fat trim (pork and/or beef) to the grass-fed beef generally increased both the initial load and growth rate of the microorganisms present in the newly formed mixes. Addition of this extraneous fat also appeared to decrease the shelf life of the products by 2-4 days. For example, as shown in Figure 3, a log of 5.15 at day 8 for the forage beef mix was approximately equivalent to the microbial count from day 2-4 for the three other meat blends.

Part of the reason for the lower microbial counts for the grass-fed beef could be attributed to the texture differences between grain and grass-fed beef. According to previous studies, grass-fed beef is tougher and has higher Warner-Bratzler shear values (Meyer et al., 1960; Bowling et al., 1977; Dyer, 1980). This would suggest that psychrotrophic microorganisms could have a more difficult time penetrating the meat due to closer arrangement of the muscle fibers (Bowling et al., 1977). Ground meat with 30% fat led to higher microbial growth than the 20% and 25% meat patties as a result of more fat added in the meat formulations. It is suggested that the extraneous fat sources used lead to greater susceptibility to bacterial contamination. Previous studies have also reported lipolytic activity by the psychrotrophic

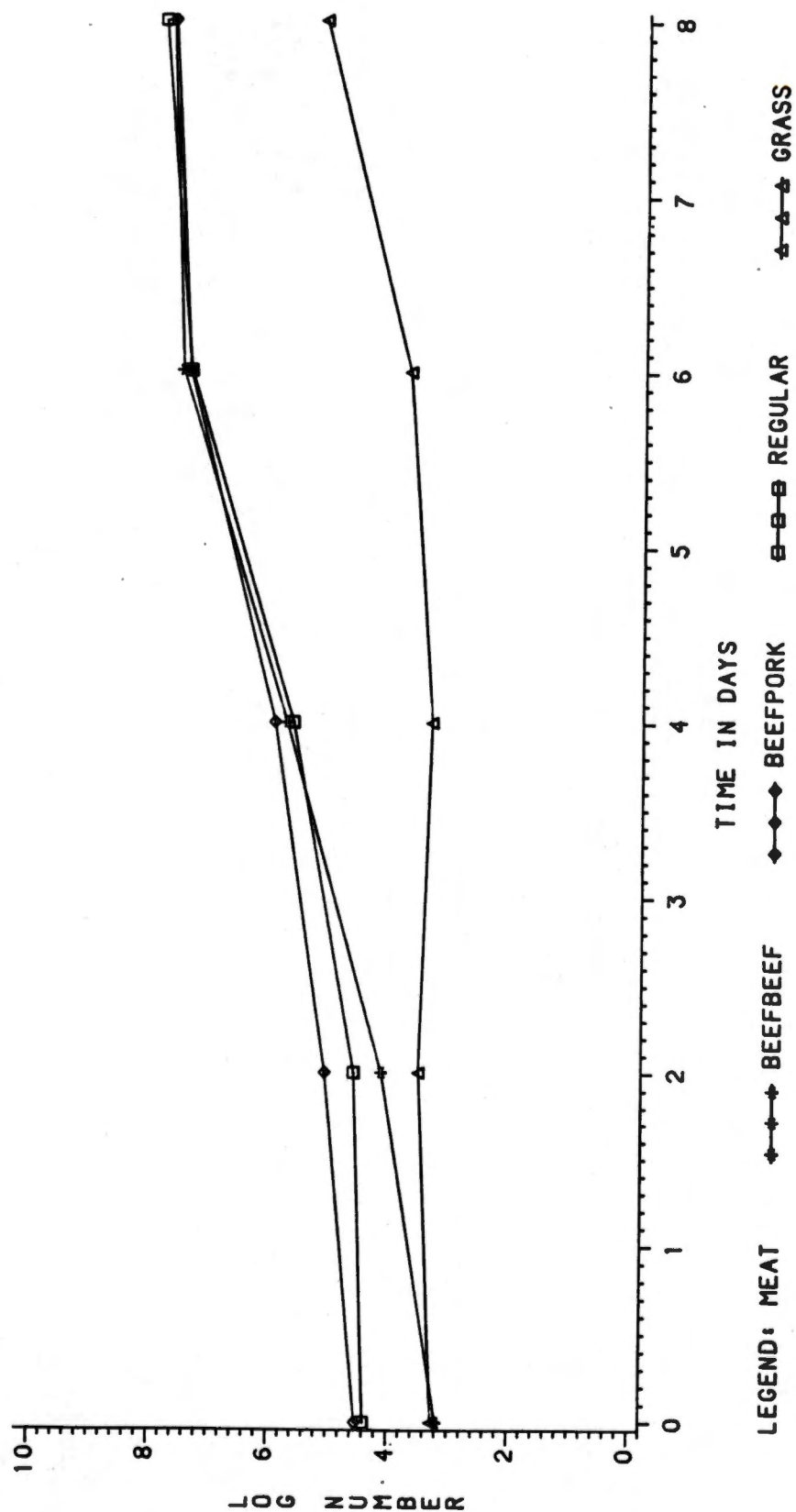


Figure 3. Log counts of retail over-wrap ground meat samples at 20% fat stored at 4°C and plated on CVT for 0,2,4,6 and 8 days.

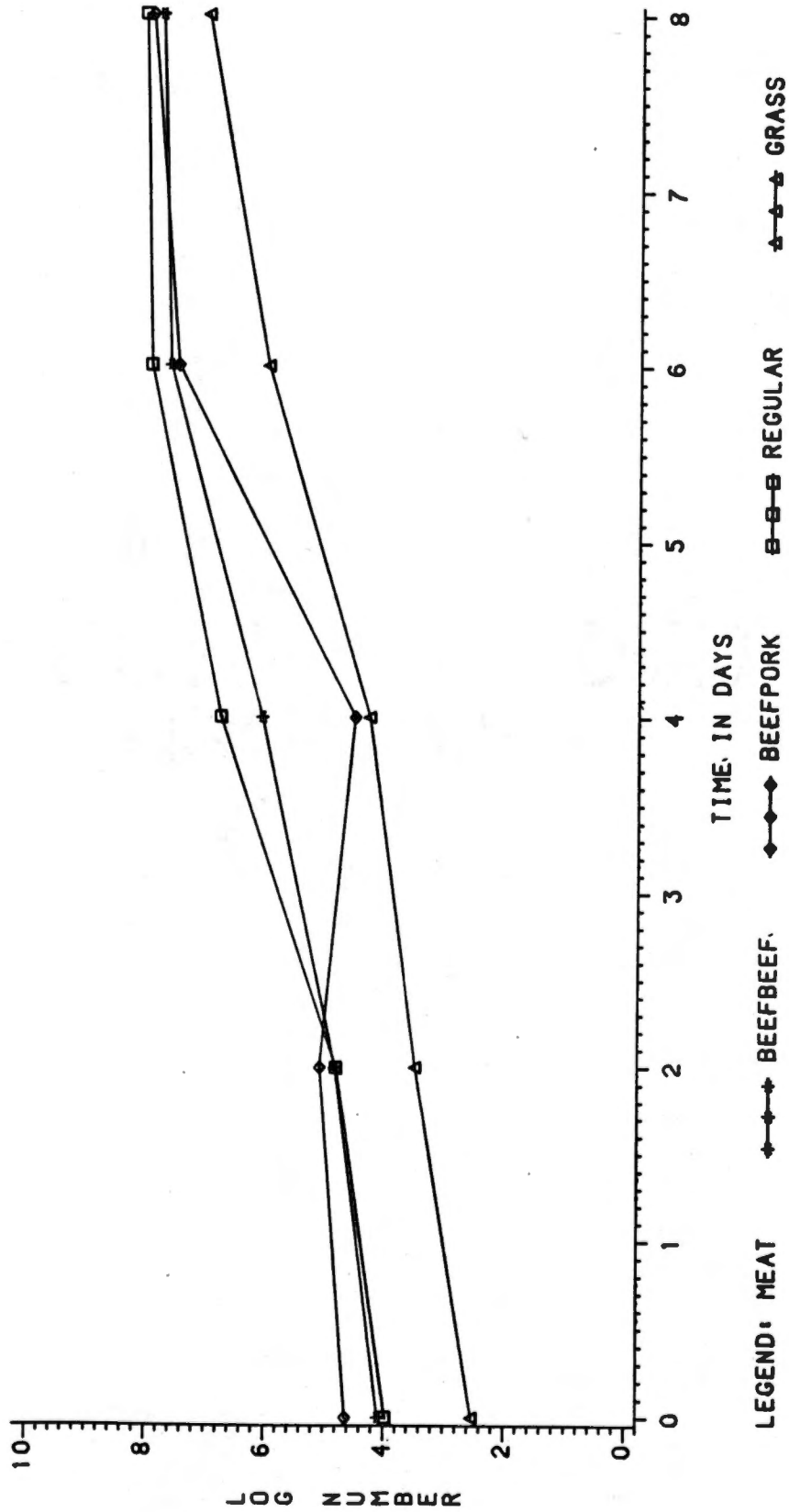


Figure 4. Log counts of retail over-wrap ground meat samples at 25% fat stored at 4°C and plated on CVT for 0, 2, 4, 6 and 8 days.

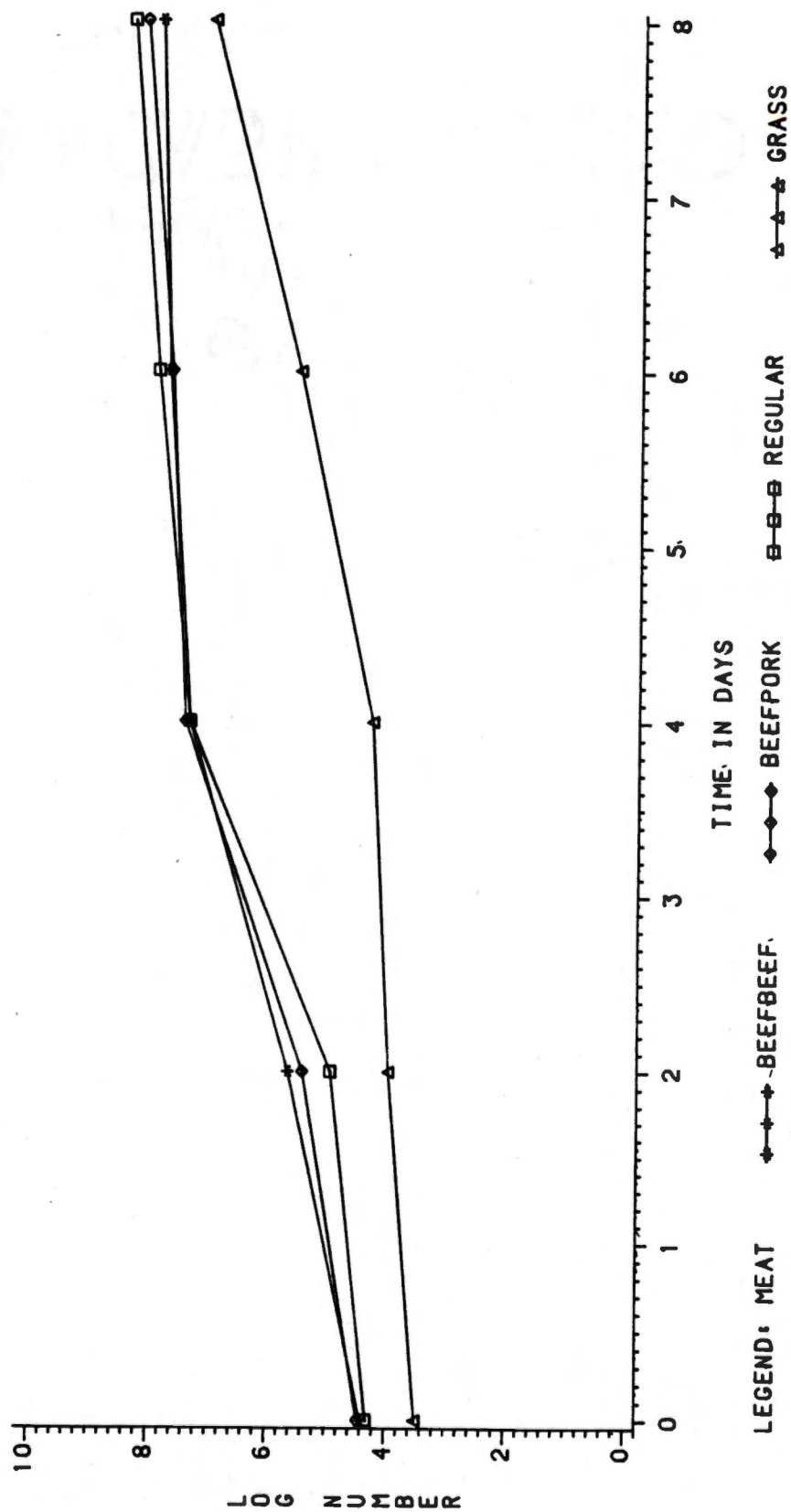


Figure 5. Log counts of retail over-wrap ground meat samples at 30% fat stored at 4°C and plated on CVT for 0, 2, 4, 6 and 8 days.

Pseudomonas (Forrest et al., 1975). Therefore, increased fat content results in a more accessible substrate for the microorganism to utilize.

No significant difference was found between the various meat types stored in retail over-wrap and plated on VRB. Jay (1978) reported that initial coliform counts in excess of 1000/g (log 3.00) indicated possible fecal contamination of raw meat with a concurrent possibility of the presence of enteric pathogenic microorganisms. In the present study, coliform counts attained a log of 3.00 only after incubation for 3 to 5 days (Figure 6).

In general, coliform counts on ground beef are usually quite low. Goepfert (1976) found an average initial coliform count of log 3.00 on 155 samples of raw ground beef. Westhoff and Geldstein (1976) conducted a bacteriological analysis of ground beef in Maryland where the average initial coliform count was 100/g. Emswiler et al. (1976) evaluated commercial ground beef samples and found the initial coliform count to be log 1.24/g.

Meat type and fat level also did not affect the yeast and mold counts for the retail samples plated on PDA (Figure 7). The average aerobic plate count was log 7.5 (Figure 2, page 27) while the mean count of all samples on PDA was approximately log 4.23 at day 8. This shows that the yeast and mold population was only a very small percentage of the total count.

The total aerobic plate count for the freezer wrap meat samples is shown in Figure 8. After statistical analysis, it was found that meat type and fat level again did not significantly affect the microbial

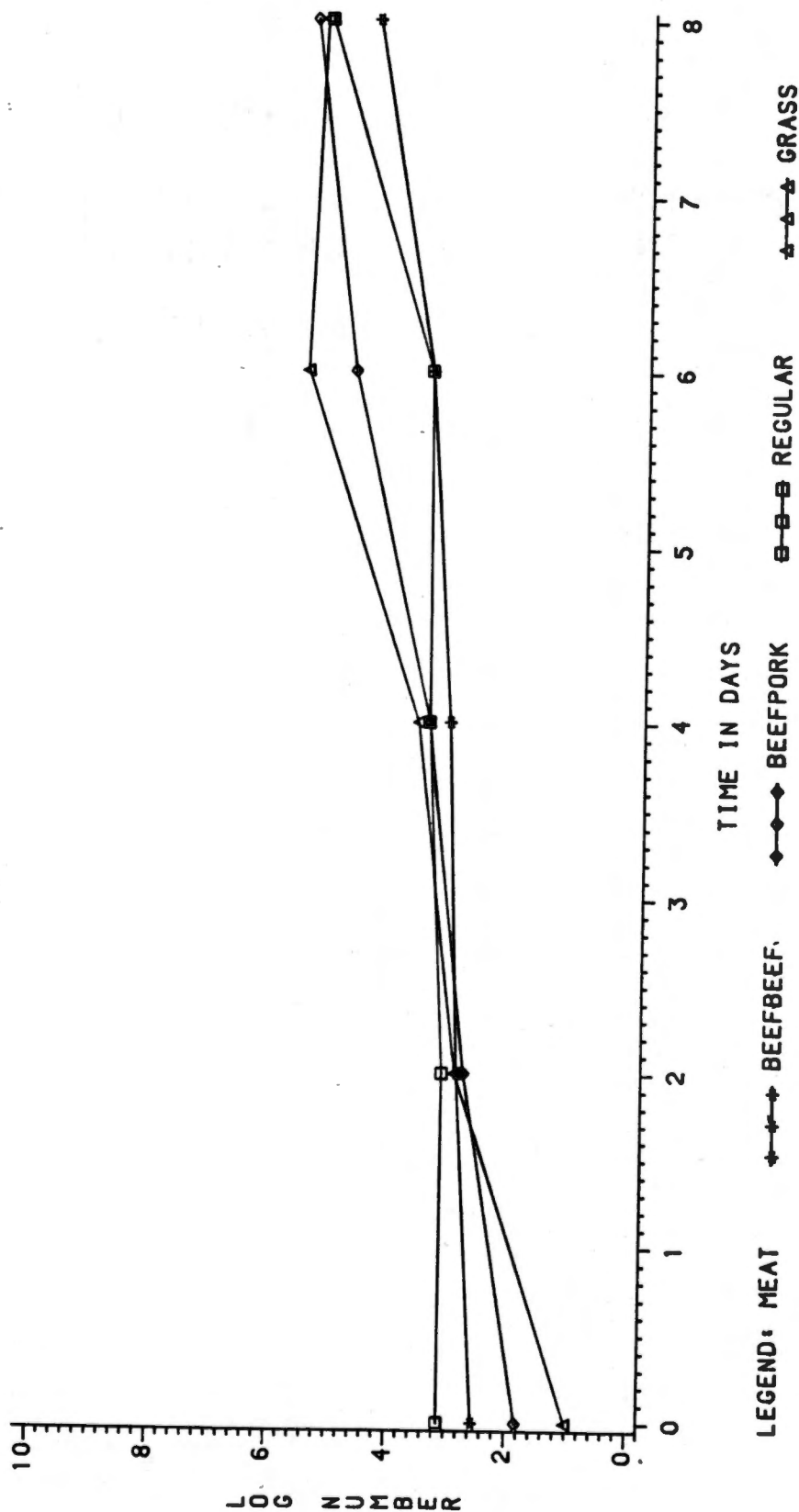


Figure 6. Log counts of retail over-wrap ground meat samples at 25% fat stored at 4°C and plated on VRB for 0,2,4,6 and 8 days.

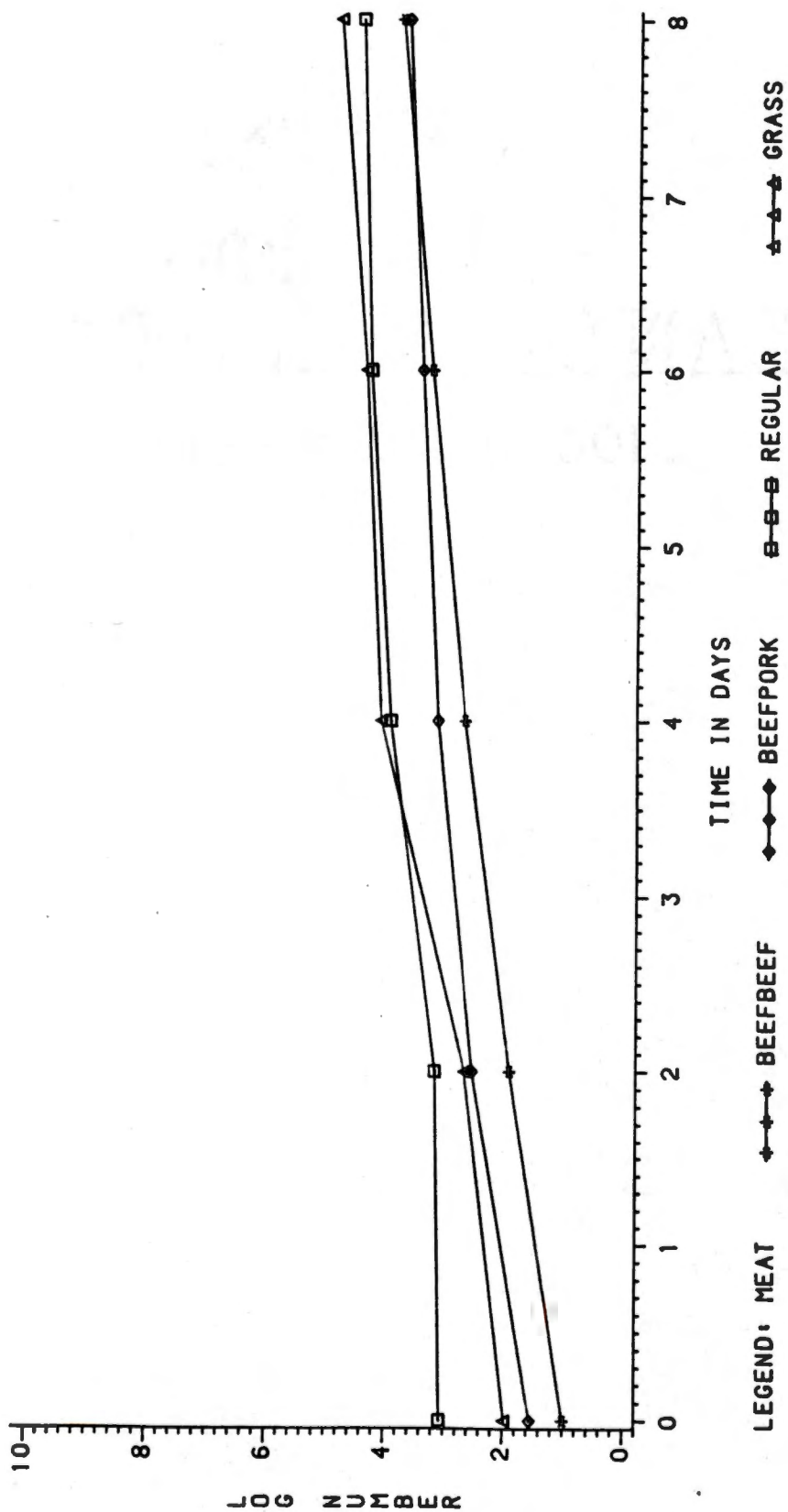


Figure 7. Log counts of retail over-wrap ground meat samples at 25% fat stored at 4°C and plated on PDA for 0,2,4,6 and 8 days.

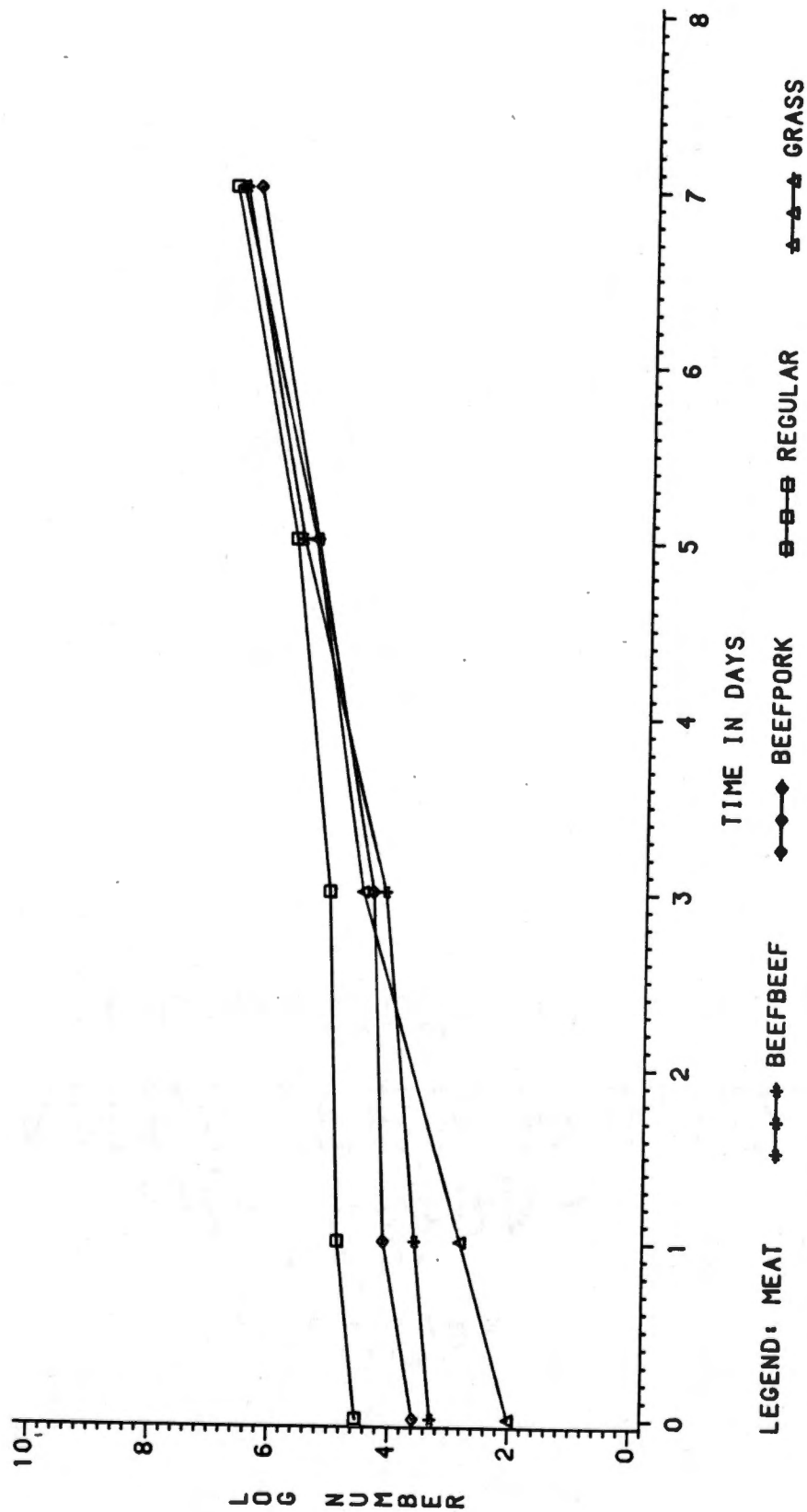


Figure 8. Log counts of freezer wrap ground meat samples at 25°C and plated on SMA for 0, 1, 3, 5 and 7 days.

count. Thawed freezer wrap samples on SMA had comparable growth rates to the retail over-wrap samples on SMA (Figure 2, p. 27).

While the retail grass-fed beef samples were significantly different from the other meat types, the microbial counts on the frozen-thawed mixes plated on CVT were not affected by meat type or fat level (Figure 9). Freezer wrap grass-fed beef samples fostered a faster growth rate than retail over-wrap meat. A possible explanation for this difference could be attributed to the disruption by ice crystal formation of the protein structure during the freezing process of grass-fed beef samples. Therefore, psychrotrophic microorganisms could have been allowed easier access to the amino acid sources provided by the meat proteins.

In the frozen-thawed samples, no significant difference was found in the number of coliforms or yeast and molds on the different types of meat (Figures 10 and 11).

Microbial levels for vacuum packaged samples plated on SMA ranged from 3.98-5.11 at day 0 to 6.65-6.84/g after 28 days at 4°C (Figure 12). No significant difference was found; however, between the number of microorganisms on the different types of meat. In a study by Reagan et al. (1977), the initial aerobic plate counts of vacuum packaged steaks ranged from 6.30 to 6.49/cm² which was much higher than the counts in the present study. After 28 days of storage at 1-3°C, the range was 7.06 to 7.55/cm² which was only slightly higher than the 6.65 to 6.84/g found for the ground meat samples. Since the initial number was lower on the ground meat, these results suggest

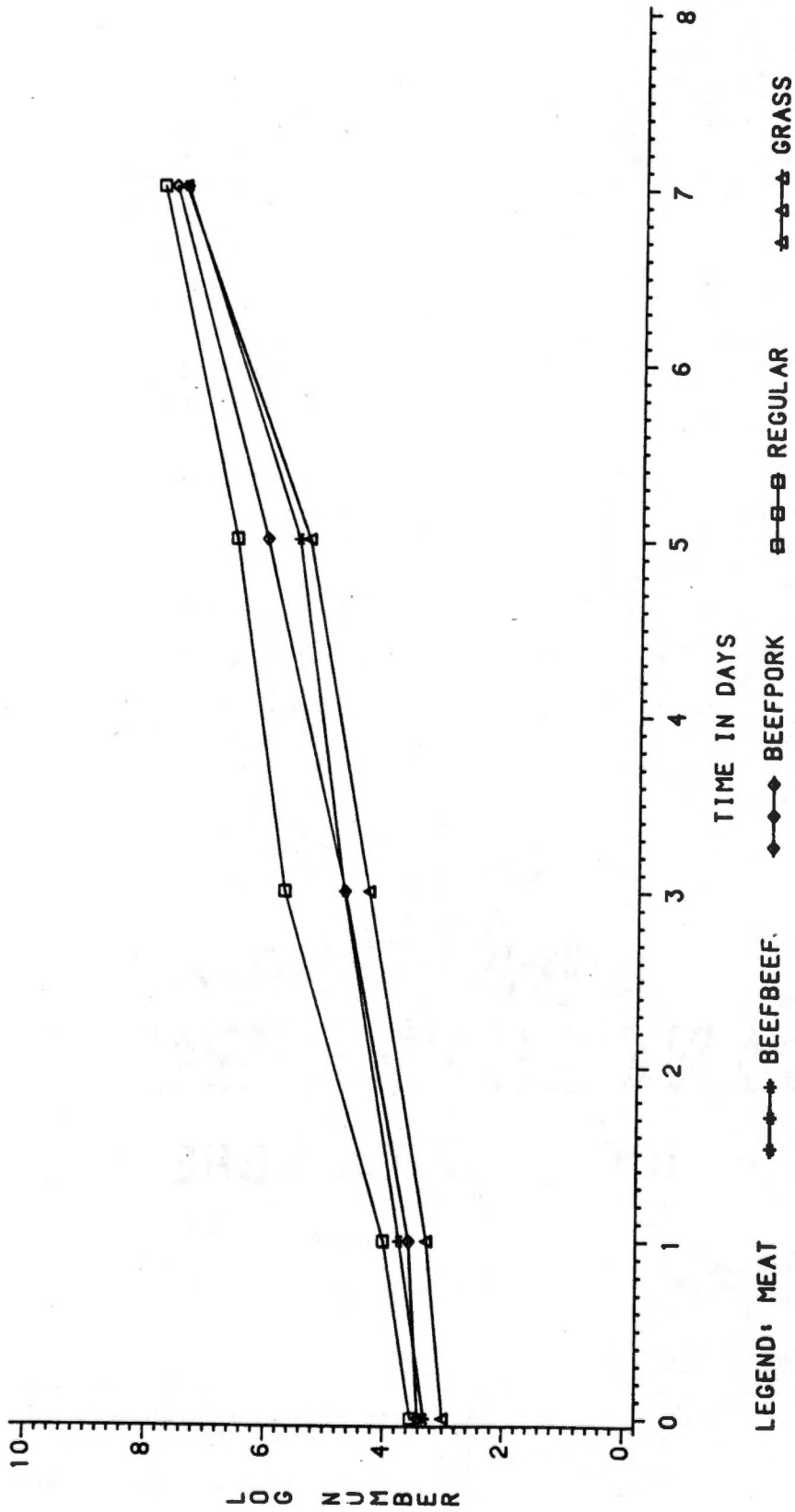


Figure 9. Log counts of freezer wrap ground meat samples at 25% fat stored at 4°C and plated on CVT for 0,1,3,5 and 7 days.

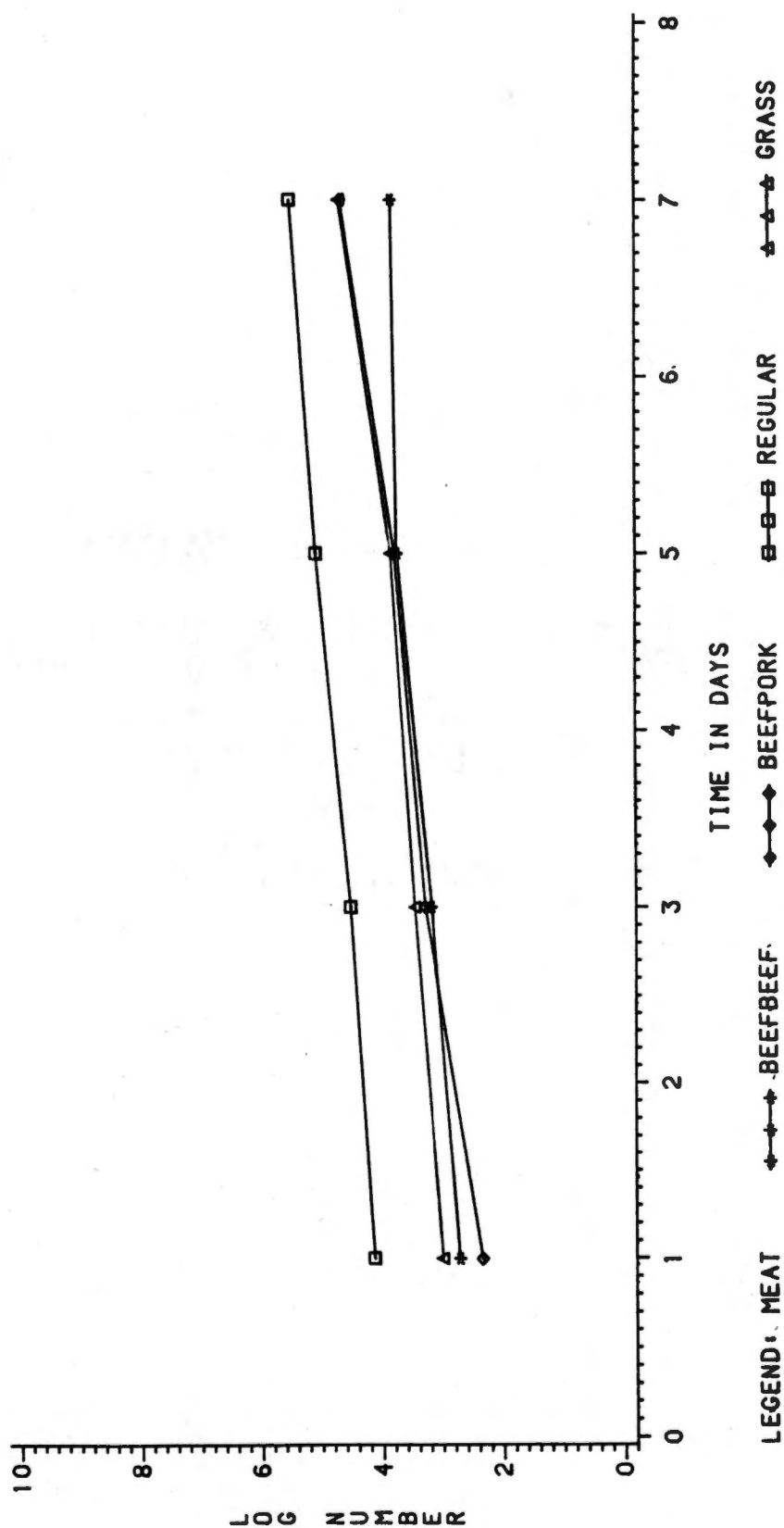


Figure 10. Log counts of freezer wrap ground meat samples at 4°C and plated on VRB for 0,1,3,5 and 7 days.

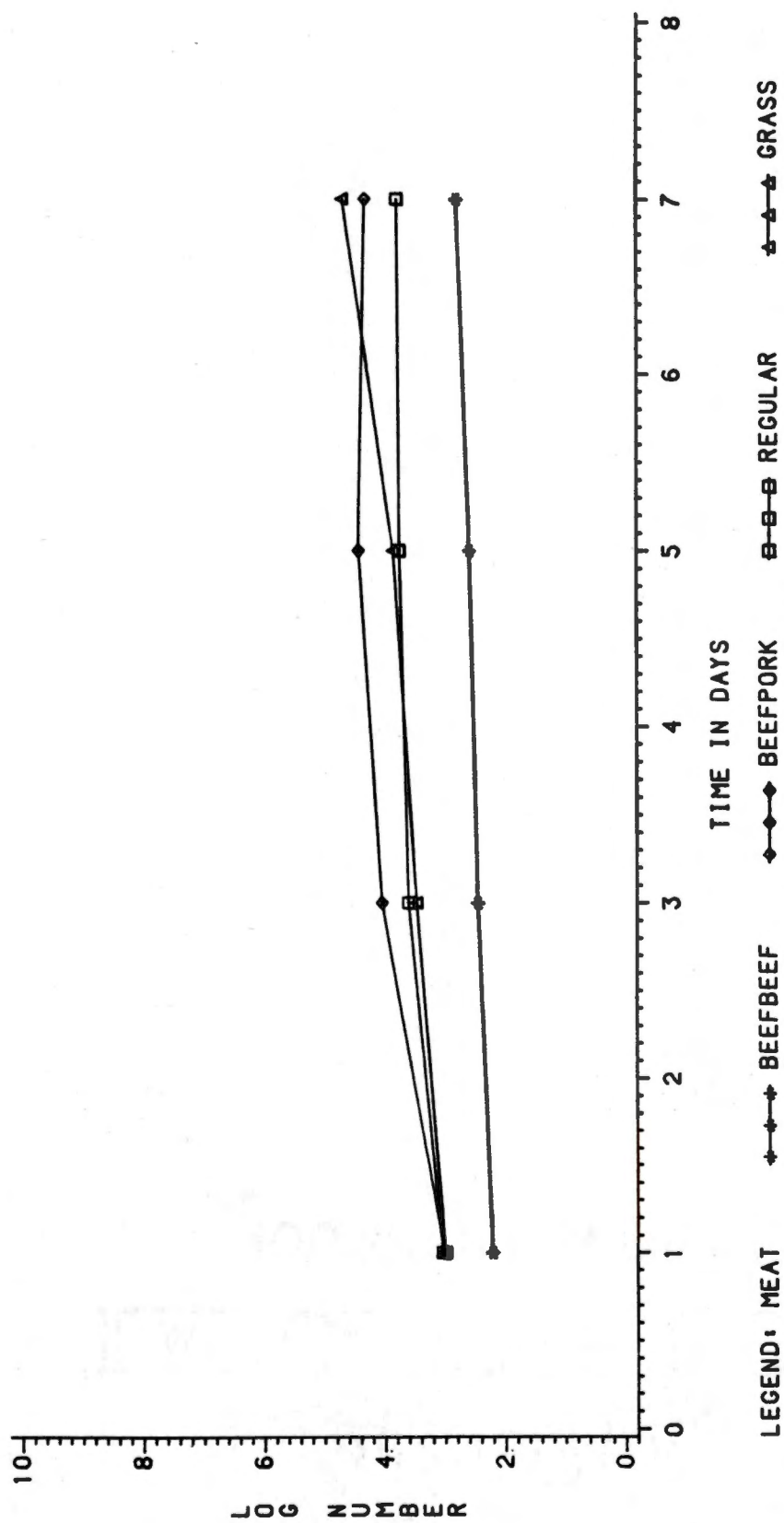


Figure 11. Log counts of freezer wrap ground meat samples at 25% fat stored at 4°C and plated on PDA for 0,1,3,5 and 7 days.

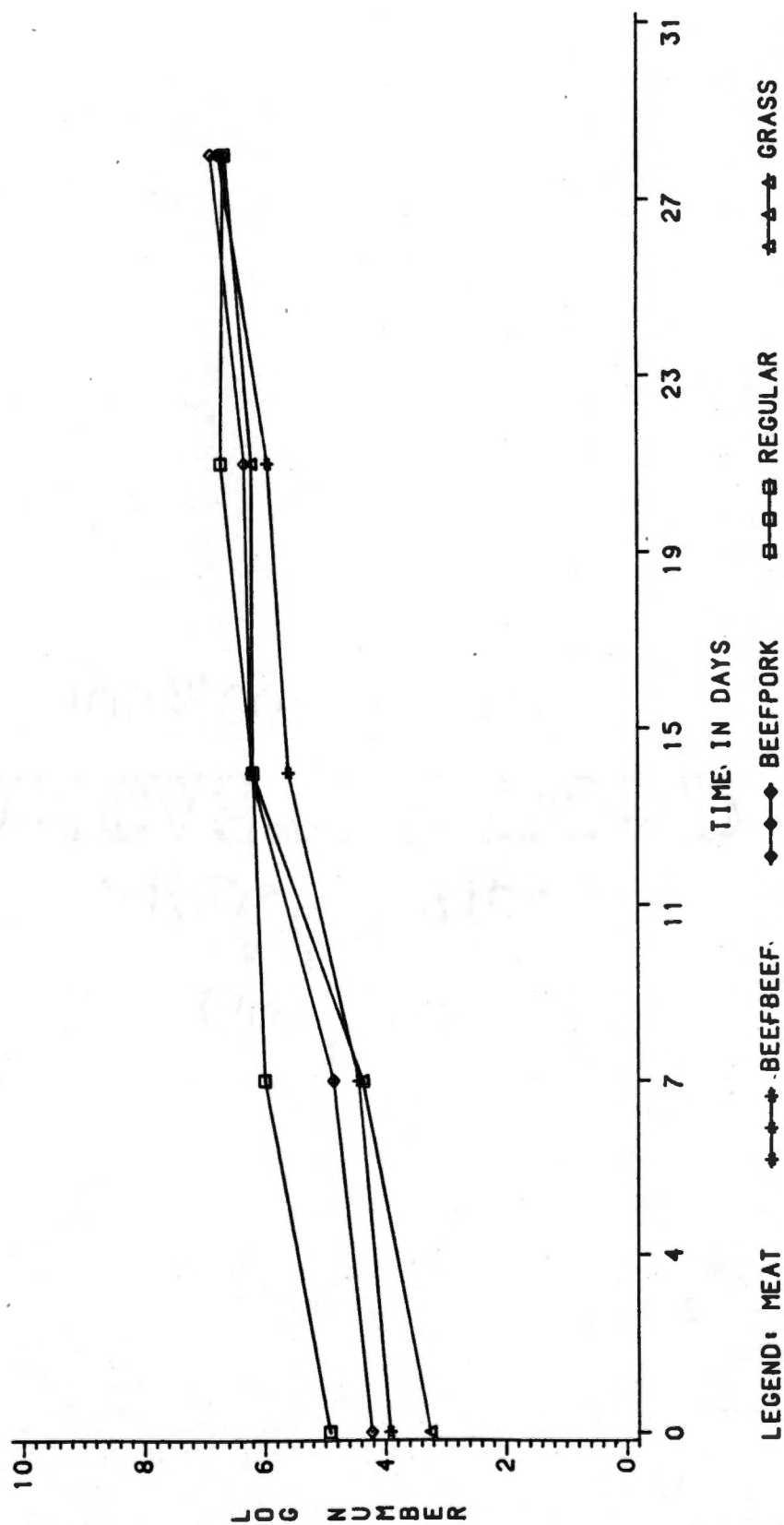


Figure 12. Log counts of vacuum packaged ground meat samples at 25% fat stored at 4°C and plated on SMA for 0, 7, 14, 21 and 28 days.

that the growth rate could have been more rapid on the comminuted product.

Psychrotrophic growth was not affected by meat type or fat level in samples under vacuum storage (Figure 13). The microbial growth rate was slower in comparison to the samples in retail over-wrap (Figures 3-5, pp. 29-31) and freezer wrap (Figure 9).

Vacuum packaged meat samples at 20, 25 and 30% fat were analyzed periodically for coliforms by plating on VRB (Figures 14-16). Both the type of meat mix and the level of fat significantly influenced the log counts. Samples containing 30% fat had overall mean counts which were significantly higher than samples with 20 or 25% fat. Even though there was significant difference across time, there was little difference in the final coliform counts.

Vacuum packaged meat is often spoiled by members of the genus Lactobacillus (Mol et al., 1971; Roth and Clark, 1972; Gill and Newton, 1978). Meat type and fat level did not have a significant effect on the samples plated on APT (Figure 17). Initially, the lactobacilli are probably a minor percentage of the microbial flora; however, they are able to predominate through competitive outgrowth.

The combination of restricted oxygen levels (<1%) and carbon dioxide concentration (about 20%) which develops in vacuum packs serves to inhibit growth of the gram-negative aerobes (Gill and Newton, 1978). Large populations of lactobacilli have also been reported to inhibit the growth of other species on meat through production of antimicrobial agents (Gill and Newton, 1978; Shelef, 1977). Smith et al. (1980) have demonstrated that Lactobacillus produces lactic acid, hydrogen peroxide

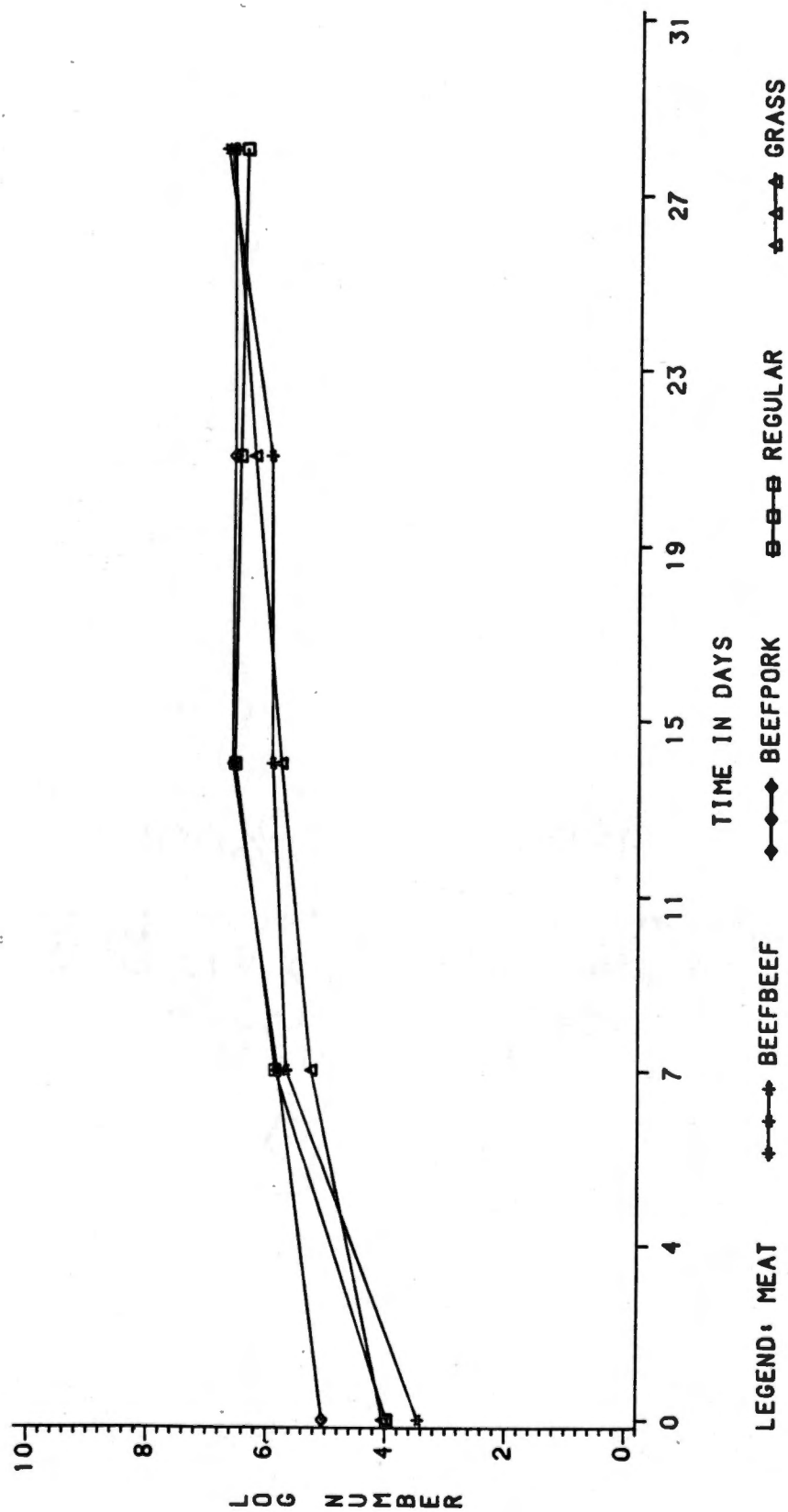


Figure 13. Log counts of vacuum packaged ground meat samples at 25°C and plated on CVT for 0,7,14,21 and 28 days.

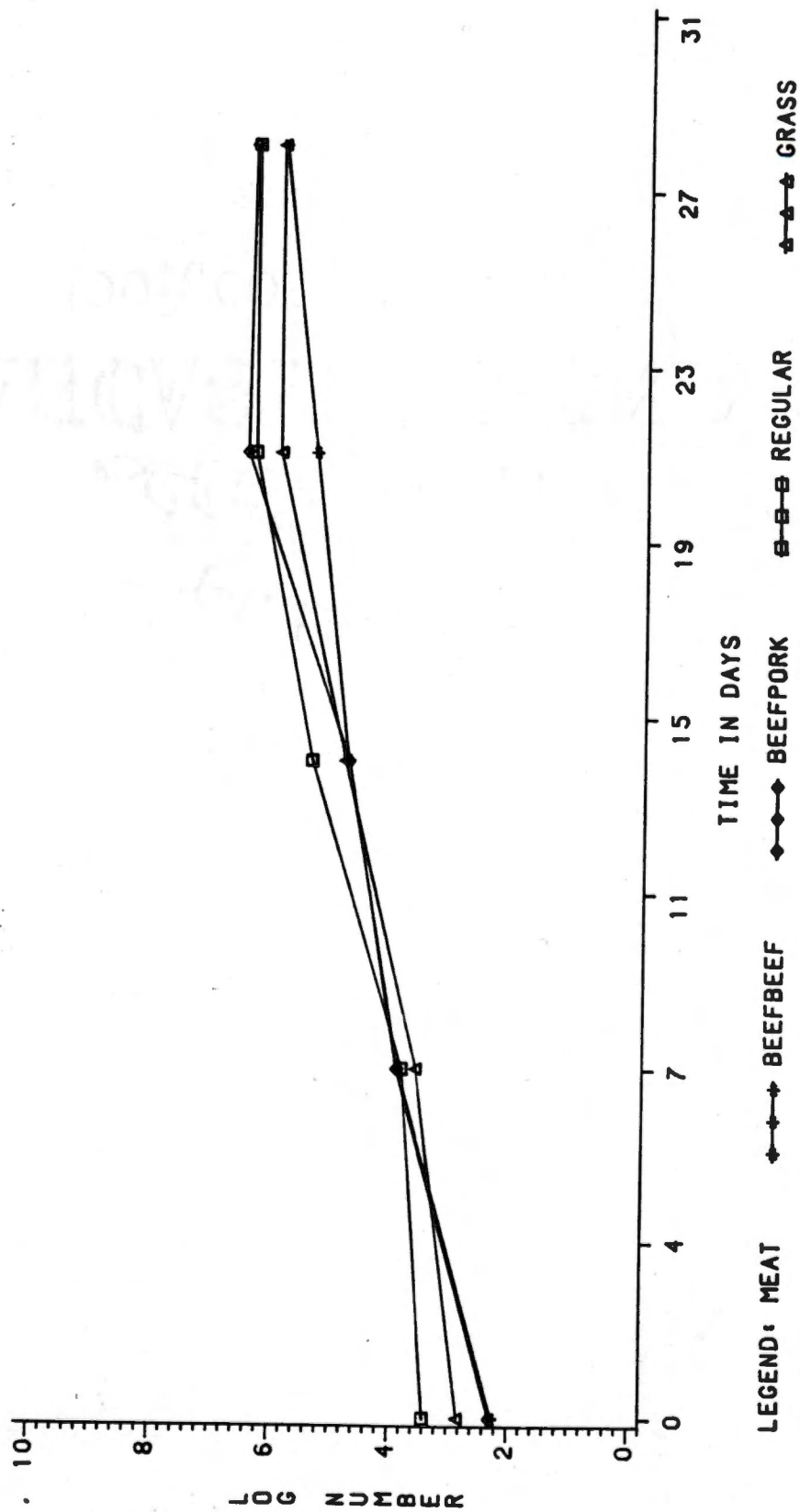


Figure 14. Log counts of vacuum packaged ground meat samples at 20% fat stored at 4°C and plated on VRB for 0, 7, 14, 21 and 28 days.

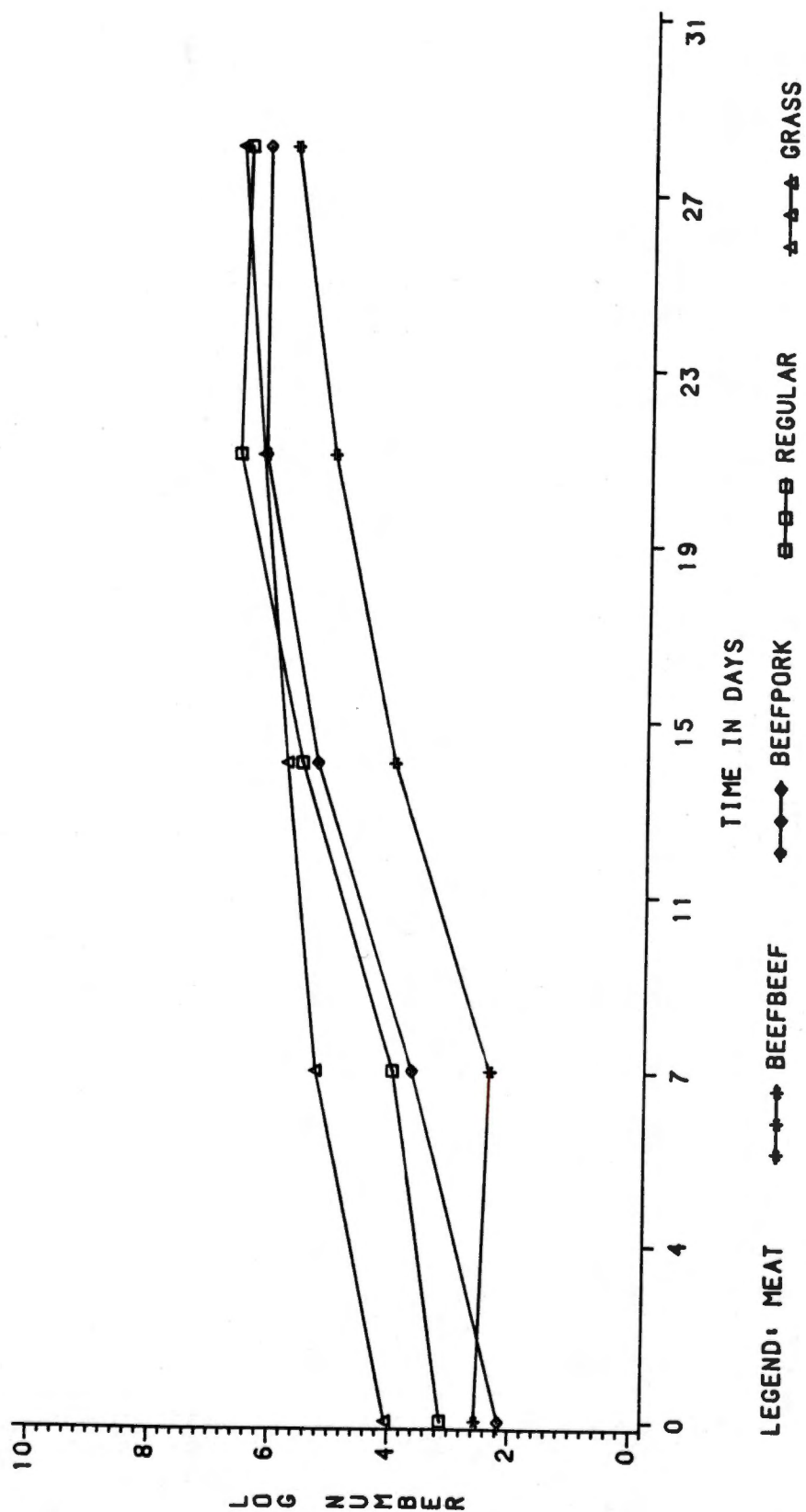


Figure 15. Log counts of vacuum packaged ground meat samples at 25% fat stored at 4°C and plated on VRB for 0, 7, 14, 21 and 28 days.

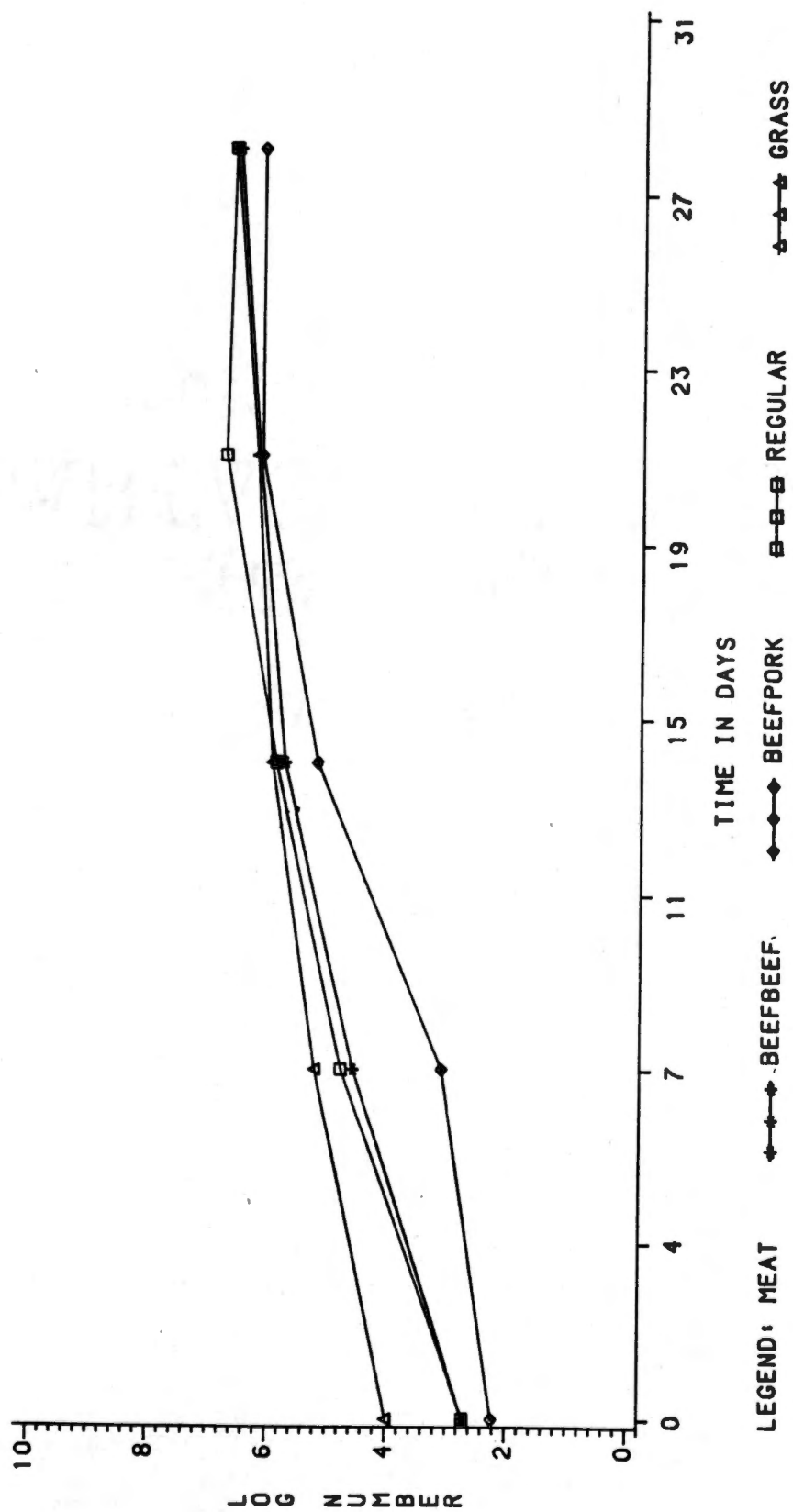


Figure 16. Log counts of vacuum packaged ground meat samples at 30°C and plated on VRB for 0,7,14,21 and 28 days.

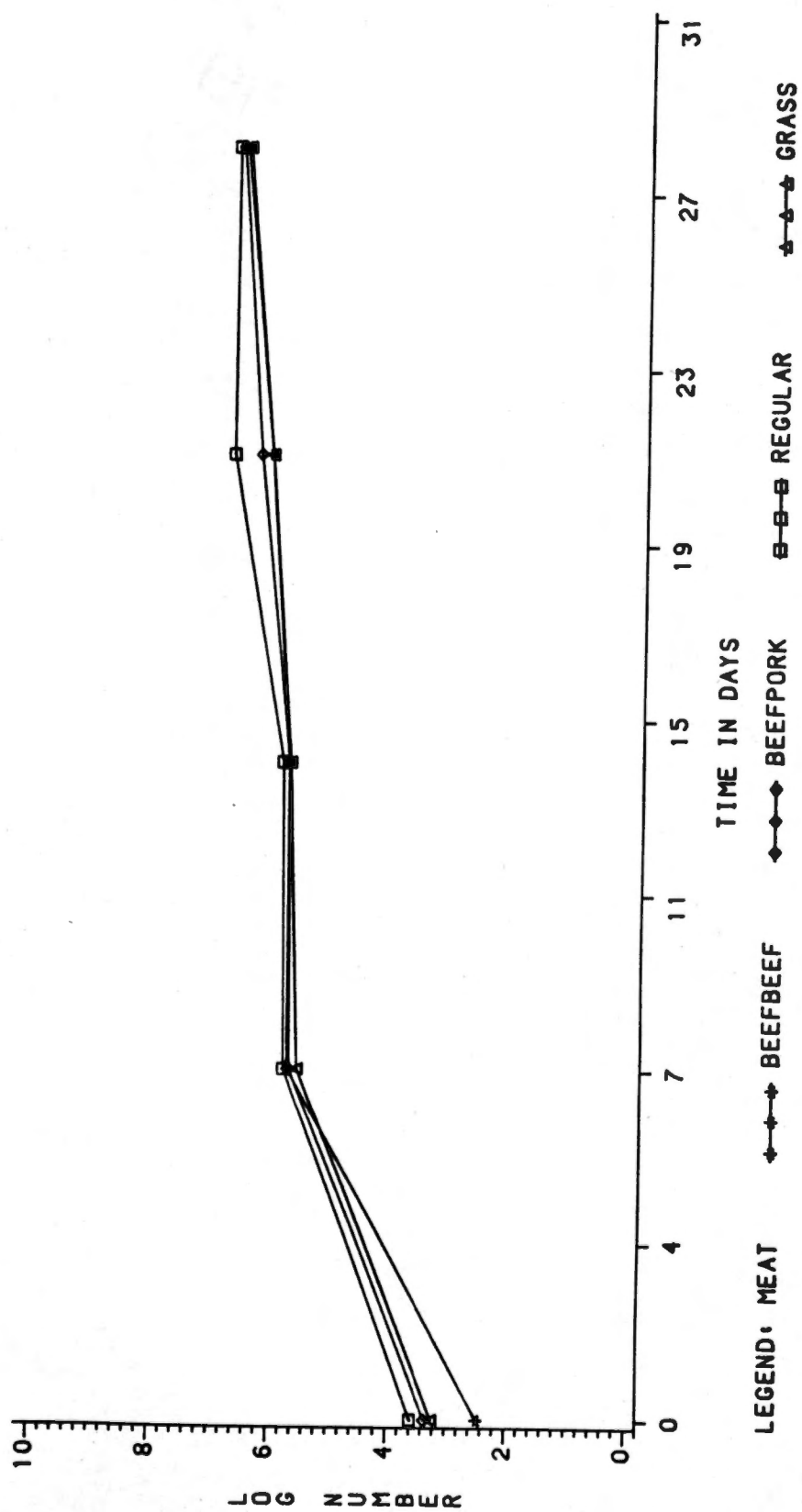


Figure 17. Log counts of vacuum packaged ground meat samples at 25°C and plated on APT for 0,7,14,21 and 28 days.

and an antibiotic which has been attributed to the nature of the antimicrobial effects of Lactobacillus.

Microbiological analysis showed that S. aureus counts remained very low (log 1.48-2.41) during the 8 day storage in retail over-wrap samples. Freezer wrap samples ranged up to log 2.35 at 7 days and vacuum packaged samples rose to log 2.17 after a 28 day storage period. For any packaging type, there was no significant difference found in the counts due to the meat type or level of fat (Table 3). These results compare well with previous studies which showed an average population count of less than 100 cells/gram of S. aureus on ground beef samples (Duitschaeffer et al., 1973; Westhoff and Feldstein, 1976). No evidence of Clostridium perfringens or Salmonella was found on any of the samples in the microbiological assays.

B. Lipid Deterioration Measured with the TBA Test

The effect of storage time on TBA values for each of the meat mixes at the 3 fat levels for the retail over-wrap samples is shown in Table 4. The TBA numbers were affected significantly by meat type ($P < 0.05$) and fat level ($P < 0.05$). An increase in the TBA values over the storage period was probably due to the incorporation of air into the meat samples through the retail over-wrap oxygen-permeable film (polyvinyl chloride).

Differences in degree of oxidation of the various meat types during storage could be attributed to: (1) nature of the lipids; and/or (2) heme catalysis (Keskinel et al., 1964). Pork has been reported to have a greater degree of unsaturated fatty acids than beef which may have accounted for its significantly higher TBA numbers (Hornstein et al., 1961). Labuza (1971) reported that 50-60% of the

Table 3. Log Counts of Ground Meat Samples with Fat Contents of 20, 25, and 30% in TSB NaCl.

		Log Number (colony forming units/g)											
		<u>Beef-Beef</u>			<u>Beef-Pork</u>			<u>Regular</u>			<u>Grass</u>		
		20	25	30	20	25	30	20	25	30	20	25	30
		----- fat % -----											
Retail													
Day	0	1.52(0.07) ^a	1.48(0.00)	1.48(0.00)	1.56(0.07)	1.52(0.07)	1.52(0.07)	1.52(0.07)	1.56(0.07)	1.56(0.07)	1.48(0.00)	1.48(0.00)	1.48(0.00)
	8	1.91(0.11)	1.95(0.10)	1.91(0.11)	2.35(0.02)	2.41(0.16)	2.40(0.16)	1.33(0.25)	1.33(0.25)	1.33(0.25)	1.33(0.25)	1.33(0.25)	1.33(0.25)
Freezer Wrap													
Day	1	1.04(0.00)	1.03(0.16)	1.07(0.22)	1.41(0.21)	1.27(0.08)	1.27(0.16)	1.45(0.15)	1.41(0.08)	1.32(0.14)	1.16(0.02)	1.06(0.18)	1.00(0.17)
	7	2.15(0.00)	2.12(0.03)	2.02(0.14)	2.35(0.09)	2.26(0.09)	2.22(0.09)	1.97(0.13)	2.02(0.14)	1.88(0.06)	2.21(0.79)	2.19(0.12)	2.25(0.09)
Vacuum													
Day	0	1.52(0.07)	1.48(0.00)	1.48(0.00)	1.56(0.07)	1.52(0.07)	1.52(0.07)	1.52(0.07)	1.56(0.07)	1.56(0.07)	1.48(0.00)	1.48(0.00)	1.48(0.00)
	28	1.85(0.00)	1.73(0.21)	1.52(0.07)	2.17(0.13)	2.15(0.05)	2.15(0.05)	2.17(0.02)	2.17(0.02)	2.12(0.07)	2.05(0.17)	1.88(0.31)	1.95(0.00)

^aMean with standard deviation in brackets.

Table 4. TBA Values for Retail Over-Wrap Samples Stored at 4°C.

Meat Type	Fat %	TBA Number (mg malonaldehyde/kg meat)		
		Storage Time (days)		
		0	4	8
Beef-Beef	20	1.75(0.03) ^a	1.86(0.01)	4.21(0.01)
	25	1.77(0.01)	1.88(0.04)	4.22(0.03)
	30	1.82(0.01)	1.93(0.08)	4.26(0.04)
Beef-Pork	20	1.90(0.04)	2.16(0.10)	4.90(0.04)
	25	1.96(0.08)	2.26(0.03)	4.90(0.04)
	30	1.98(0.05)	2.31(0.06)	4.96(0.03)
Regular	20	1.76(0.01)	1.87(0.06)	4.72(0.02)
	25	1.78(0.04)	1.88(0.06)	4.70(0.02)
	30	1.81(0.08)	1.90(0.06)	4.71(0.01)
Grass	20	1.69(0.02)	1.80(0.07)	4.10(0.04)
	25	1.69(0.02)	1.83(0.05)	4.14(0.04)
	30	1.74(0.03)	1.85(0.06)	4.22(0.02)

^aMean with standard deviation in brackets.

total fatty acids in pork were unsaturated with linoleic acid predominant. Beef adipose tissue lipids are less susceptible to oxidation due to a high concentration of saturated fatty acids. The lean tissue lipids (2-4% of the total fat) of beef, however, could be a site of lipid oxidation due to high concentrations of phospholipids containing nearly 50% polyunsaturated fatty acids (Hornstein et al., 1961).

Because of their close proximity to the heme catalysts of the mitochondria, the fatty acids of these phospholipids are primarily responsible for the development of rancidity. (The lipid forms a complex with heme pigment and free radicals are formed.)

TBA values obtained for the retail over-wrap samples (Table 4) over time appear comparable to results in previous investigations. Keskinel et al. (1964) reported an increase in TBA values for ground beef stored at 5°C. A value of 0.52 for 1 day storage was obtained while a value of 7.21 was found after 10 days refrigeration. In another study on ground meat samples (4°C), Greene (1969) found a TBA value of 5.7 at day 2 and a value of 12.5 at day 8. Abo-gnah (1978) recorded TBA values of 1.2 at day 0, 3.8 at day 6 and 4.2 at day 9 for raw ground beef stored at 4°C.

TBA numbers for the beef-pork mixes were somewhat different than those obtained in a study by Lopez-Lorenzo et al. (1980). They determined that TBA values of pure ground pork increased during a 20 day (1°C) storage period from 1.0 to 13.0. In the present study, beef-pork TBA numbers ranged from approximately 1.95 at day 0 to 4.92 at day 8 (Table 4). Composition of the mixes could have accounted for the differences in TBA numbers. Since the beef-pork mix was formulated with grass-fed beef, it had a lower level of unsaturated fatty acids (more

susceptibility to oxidation than saturated fatty acids) than the pure ground pork samples. Keskinel et al. (1964) found contrasting results to both the above studies reporting that lipid extracted pork muscle had lower TBA numbers than beef due to a higher phospholipid content (phospholipids generally contain a high level of unsaturated fatty acids).

Meat type and fat level did not have a significant effect on TBA values for freezer wrap meat (Table 5). These samples had TBA values which were approximately 1/2 that of the refrigerated retail over-wrap samples. It appeared that oxidation was delayed by as much as 4 days in the freezer wrap meat. Other researchers have found that TBA values for frozen meat (-18°C) were lower than those of refrigerated meat due to a reduced rate of autoxidation (Keskinel et al., 1964; Love and Pearson, 1971). It is not known why the TBA values increased at a slower rate for the freeze-thawed meat than the retail over-wrap samples.

From analysis of the Duncan Mean Separation test, it was found that the mean TBA value over time for each of the 4 meat formulations were significantly different from each other in the retail over-wrap and freezer wrap packaging systems. The beef-pork mix had the highest mean value (1.98/3.03) followed by the regular (1.92/2.79), beef-beef mix (1.90/2.63) and grass-fed mix (1.85/2.56). (The 1st TBA number in brackets represents the value for the freezer wrap meat and the 2nd figure represents the TBA number for the retail over-wrap samples.)

In a recent study, Brown et al. (1979) observed lower TBA values for grass-fed beef than grain-fed beef. Samples were frozen at -34°C and stored at -4°C for 30 days. Prior to analysis, the meat was thawed

Table 5. TBA Values for Freezer Wrap Samples Stored at 4°C.

Meat Type	Fat %	TBA Number (mg malonaldehyde/kg meat)				
		Storage Time (days)				
		0	1	3	5	7
Beef-Beef	20	1.73(0.15) ^a	1.79(0.13)	1.86(0.21)	1.96(0.15)	2.07(0.21)
	25	1.73(0.15)	1.75(0.18)	1.88(0.25)	1.99(0.17)	2.10(0.20)
	30	1.78(0.18)	1.81(0.16)	1.91(0.21)	2.03(0.23)	2.12(0.17)
Beef-Pork	20	1.82(0.21)	1.81(0.19)	1.89(0.18)	2.02(0.13)	2.17(0.10)
	25	1.83(0.20)	1.83(0.18)	1.96(0.21)	2.06(0.14)	2.19(0.10)
	30	1.85(0.23)	1.85(0.18)	2.02(0.21)	2.12(0.14)	2.23(0.15)
Regular	20	1.74(0.21)	1.77(0.25)	1.89(0.21)	1.96(0.16)	2.11(0.12)
	25	1.76(0.18)	1.79(0.23)	1.94(0.18)	2.00(0.18)	2.11(0.12)
	30	1.78(0.16)	1.81(0.20)	1.98(0.20)	2.04(0.21)	2.16(0.13)
Grass	20	1.68(0.07)	1.65(0.07)	1.83(0.19)	1.93(0.17)	2.00(0.16)
	25	1.71(0.14)	1.71(0.15)	1.84(0.19)	1.93(0.12)	2.02(0.21)
	30	1.76(0.23)	1.82(0.18)	1.86(0.16)	1.99(0.17)	2.06(0.18)

^aMean with standard deviation in brackets.

for 7 hours. A heavier load of microbial growth on the grass-fed beef samples was also found. Pseudomonas fragi, reported as the predominant psychrotrophic species on ground meat, metabolizes the peroxides and aldehydes produced in autoxidation (Smith and Alford, 1968). This could result in a lower concentration of autoxidation end-products and thus, decreased TBA values with increased microbial growth. Such a relationship was not noted in the present investigation. This was possibly due to the good condition of the packaging films and relatively superior quality of the grass-fed beef which resulted in low microbial numbers and low TBA values.

Increased TBA values over time for ground meat samples stored in a vacuum packaged environment at 4°C is depicted in Table 6. Meat type and fat percentages did not affect lipid oxidation significantly. While the approximate mean TBA number for retail over-wrap samples at day 8 was 4.50, the mean TBA value for the vacuum packaged samples was 3.50 by day 14. This data indicates at least an additional week of shelf life for the vacuum packaged samples based solely on the TBA value, in comparison to the meat mixes refrigerated at 4°C in the retail over-wrap film. It was suggested by Greene (1969) that anaerobic packaging of meat is a useful technique in extending shelf life as oxygen levels are decreased. This occurrence often results in reduced psychrotrophic growth and lower lipolytic activity of the Pseudomonas species (Forrest et al., 1975). The genus Pseudomonas possesses lipases which break down fat to further the oxidation process.

In the present study, the packaging system began to lose its integrity between day 21 and day 28, allowing air to enter into the package. This probably led to a faster rate of lipid oxidation. Roth

Table 6. TBA Values for Vacuum Samples Stored at 4°C.

Meat Type	Fat %	TBA Number (mg malonaldehyde/kg meat)		
		Storage Time (days)		
		0	14	28 ^b
Beef-Beef	20	1.75(0.04) ^a	3.30(0.01)	5.02(0.13)
	25	1.77(0.01)	3.35(0.03)	5.06(0.13)
	30	1.82(0.01)	3.36(0.02)	5.10(0.14)
Beef-Pork	20	1.90(0.04)	4.08(0.22)	5.14(0.18)
	25	1.96(0.08)	4.08(0.22)	5.21(0.15)
	30	1.98(0.05)	4.15(0.26)	5.27(0.14)
Regular	20	1.76(0.01)	3.33(0.02)	5.02(0.10)
	25	1.78(0.04)	3.35(0.03)	5.07(0.09)
	30	1.81(0.08)	3.36(0.05)	5.09(0.06)
Grass	20	1.69(0.02)	3.18(0.04)	5.26(0.55)
	25	1.69(0.02)	3.19(0.06)	5.29(0.52)
	30	1.74(0.03)	3.26(0.04)	5.30(0.50)

^aMean with standard deviation in brackets.^bVacuum was lost.

and Clark (1972) encountered a similar problem with a vacuum packaging system where noticeable changes in odor and/or color occurred after 32 days of storage at 5°C.

C. Sensory Evaluation

The purpose of the initial difference test was to determine if an experienced panel could detect differences in the meat mixes due to fat levels or meat types. No significant difference was detected among the meat samples due to the fat level (20-30%). However, type of meat in the mix significantly affected ($P < 0.05$) flavor, juiciness and texture scores (Table 7). The mean flavor scores for the regular and beef-beef mixes were in the "slightly intense (beefy)" range while the beef-pork and grass mixes were in the "moderately bland" to "slightly bland-intense" region. A lack of typical beefy flavor in the grass and beef-pork mixes corresponds to previous studies. Bowling et al. (1977) found forage-finished beef to be of limited acceptability at the retail and wholesale level due to a lack of palatability. Differences in beef fat flavor due to fatty acid composition has been suggested as a major difference between grass-fed and grain-fed beef (Amiri, 1980; Melton et al., 1982). It was proposed that linolenic acid contributed to the low flavor score of grass-fed beef. Characteristic flavor differences in pork and beef also are related to fat (Hornstein and Crowe, 1960). Beef has higher levels of palmitoleic, palmitic, myristic, heptadecenoic and tetradecenoic acids, while pork has higher levels of stearic, linoleic, linolenic and oleic acids.

Scores for juiciness were found "slightly juicy" to "moderately

Table 7. Difference Test: Means^a and Standard Deviations of Scores for the Evaluation of Sensory Attributes of Meat Patties.

Meat Type ^b	Flavor Score ^c	Juiciness Score ^c	Texture Score ^c
Beef-Beef	4.9(1.4) ^d	5.3(1.1) ^{de}	5.6(0.8) ^e
Beef Pork	3.9(1.2) ^e	5.7(1.3) ^d	6.2(1.2) ^d
Regular	5.2(1.3) ^d	5.1(1.1) ^e	5.6(0.86) ^e
Grass	4.1(1.4) ^e	4.8(1.2) ^e	4.7(1.1) ^f

^aMeans of 10 judges.

^bMeat type is the mean for the 3 fat levels.

^cFlavor, juiciness and texture scored on an 8-point scale; 8 = extremely intense (beefy), extremely juicy and extremely tender; 1 = extremely bland, extremely dry and extremely tough, respectively.

^{d-f}Means in column groups followed by different letters are significantly different ($P < 0.05$).

juicy" for the beef-beef and beef-pork blends, respectively. Grass-fed beef and regular beef mixes had a juiciness attribute of "slightly dry" to "slightly juicy." Texture value for the beef-pork mix was in the "moderately tender" range. This value compared well with the tenderness scores reported for beef/pork mixes (Parizek et al., 1981). Pork fat, with its lower melting point, had a tendency to smear more than beef fat during grinding, mixing and patty formation. The beef-beef and regular blends were described as "slightly tender" to "moderately tender." Grass-fed beef had the lowest score in the region of "slightly tough" to "slightly tender." Grass-fed beef is known to have higher Warner-Bratzler shear values than grain-fed beef (Meyer et al., 1960; Bowling et al., 1977) due to its extremely thin fat layer (Schroeder et al., 1980) which results in a greater susceptibility to cold shortening of the sarcomeres.

Based on the difference test, a consumer taste panel was conducted using all of the meat mixes at a constant fat level, 25% (Table 8 and Appendix B). A total of 53 participants took part in the consumer panel. The results showed that regular ground beef ($\bar{X} = 6.9$) and the beef-beef mix ($\bar{X} = 6.0$) had mean scores which were in the "like moderately" range. The grass-fed beef mix ($\bar{X} = 5.6$) had a score in the "neither like nor dislike" to the "like slightly" range. Beef-pork ($\bar{X} = 5.1$) had the lowest mean score in the "neither like nor dislike" range. A frequency distribution of the scores is found in Table 9. A higher percentage of the regular ground beef samples were in the like region (86.7%). Sixty-four percent of the beef-beef samples were in this region, followed by grass-fed beef (56.7%) and beef-pork with the

Table 8. Consumer Taste Panel Test: Means^a and Standard Deviations of Sensory Acceptability Scores of Meat Patties.

Meat Type	Sensory Score ^b
	Acceptability Score
Beef-Beef	6.0(1.7) ^d
Beef-Pork	5.1(2.1) ^{de}
Regular	6.9(1.4) ^c
Grass	5.6(1.7) ^{de}

^aMeans of 53 judges.

^bAcceptability scored on a 9-point scale; 9 = like extremely and 1 = dislike extremely.

^{c-e}Means in column groups followed by different letters are significantly different ($P < 0.05$).

Table 9. Consumer Taste Panel Test: Percentage of Scores Within Acceptability Ranges.

Meat Type	Percentage of Scores		
	% Acceptable	% Neither Like Nor Dislike	% Unacceptable
Beef-Beef	64.2	9.4	26.4
Beef-Pork	45.2	11.3	43.5
Regular	86.7	5.6	7.7
Grass	56.7	9.4	33.9

lowest (45.2%). The regular and beef-beef mixes had the highest sensory scores which could be attributed to the more popular appeal of a beef fat flavor. Grass-fed beef was described as being "less beefy," "sour," "bland," "dry," and possessed a "metallic after-taste" and therefore, sensory scores were lower. Brown et al. (1979) postulated that the low quantity of free sugars, important in the production of a desirable beef flavor in grass-fed beef, contributed to lower flavor scores. The beef-pork mixes proved the least desirable of all the meat formulations. Pale color, greasiness and crumbly texture accounted for the low sensory ratings.

In a sensory study of beef/pork hamburger mixes, Parizek et al. (1981) found a score of 5.6-5.7 for the blends. This compares to the scores obtained in the present study for the grass-fed beef alone ($\bar{X} = 5.6$), but exceeds the grass-fed beef-pork mix score ($\bar{X} = 5.1$). This information may imply that the type of beef (grain vs. grass) may have an effect on the sensory evaluation of blends with pork. Parizek et al. (1981); however, did conclude that these mixes were a palatable alternative to all-beef patties and were equivalent in juiciness and overall acceptability.

The results from the sensory analysis indicated a difference in flavor, juiciness, texture and overall acceptability between the type of meat patties. The regular ground beef usually had the highest scores for the sensory attributes, and beef-pork often was in the lower end of the scoring. The findings of this study indicated a possible improvement of grass-fed beef when combined with grain-fed fat beef trim. In contrast, consumer acceptability of beef-pork samples was

poor, and the mixing of these meat types could not be recommended as an alternative to improving the marketability of forage-fed beef.

CHAPTER V

CONCLUSIONS

Numerous investigations have reported superior flavor characteristics of grain-fed beef when compared to grass-fed beef. However, higher costs of grain and concern over excessive amounts of fat in the human diet have led to an increased interest in improving the sensory qualities of the more economical and lean forage-fed beef. Microbial numbers in this present study were similar to previously reported levels for regular grain-fed beef. Psychrotrophic growth rates on the grass-fed beef packaged in retail over-wrap were significantly lower than the other 3 meat types. However, the addition of the fat beef and/or pork trim to the forage-fed beef had comparable microbial levels as the regular grain-fed beef which indicate that grass-fed beef mixes may be used safely as ground meat products. Differences in degree of lipid deterioration in the meat formulations were influenced by variation in fatty acid composition of muscle and fat sources. TBA values of the beef-pork mixes were significantly higher than the other 3 meat mixes which indicate increased susceptibility to rancidity. Sensory evaluation data showed average scores to be in the acceptable range for each meat mix. However, beef-pork blends presented a possible problem with its lower sensory flavor attributes as described by the taste panel. On the basis of this investigation, the addition of fat beef to grass-fed beef shows potential as an alternative to grain-fed beef. In contrast, possible rancidity problems and low palatability scores of the beef-pork mix showed it to be an unacceptable alternative for increasing the utilization of grass-fed beef.

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APPENDICES

RECEIVED BY BOARD

APPENDIX A

SCORING SHEET FOR GROUND BEEF

DATE: _____

JUDGE: _____

DIRECTIONS:

You will be given samples of ground beef to test for flavor intensity, tenderness and juiciness. Sniff each sample before tasting. You do not have to swallow samples, expectorate in empty cup provided. Rinse mouth with water between each sample.

Use the scale on this scorecard for each attribute. Do not use fractional numbers.

You will be given a total of 12 samples. Please do not leave until all samples have been tasted.

KEY:

FLAVOR INTENSITY (Beefy)

- 8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

TENDERNESS

- 8 Extremely tender
7 Very tender
6 Moderately tender
5 Slightly tender
4 Slightly tough
3 Moderately tough
2 Very tough
1 Extremely tough

JUICINESS

- 8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

[illegible]

THANK YOU FOR YOUR COOPERATION

APPENDIX B

Date: _____

Taster: _____

Product: Ground Beef

Taste test these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude.

<u>Code</u>	<u>Code</u>	<u>Code</u>	<u>Code</u>
_____	_____	_____	_____
____ Like extremely	____ Like extremely	____ Like extremely	____ Like extremely
____ Like very much	____ Like very much	____ Like very much	____ Like very much
____ Like moderately	____ Like moderately	____ Like moderately	____ Like moderately
____ Like slightly	____ Like slightly	____ Like slightly	____ Like slightly
____ Neither like or dislike	____ Neither like or dislike	____ Neither like or dislike	____ Neither like or dislike
____ Dislike slightly	____ Dislike slightly	____ Dislike slightly	____ Dislike slightly
____ Dislike moderately	____ Dislike moderately	____ Dislike moderately	____ Dislike moderately
____ Dislike very much	____ Dislike very much	____ Dislike very much	____ Dislike very much
____ Dislike extremely	____ Dislike extremely	____ Dislike extremely	____ Dislike extremely
<u>Reason</u>	<u>Reason</u>	<u>Reason</u>	<u>Reason</u>
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Thank you for your cooperation.

APPENDIX C

Table 10. Log Counts of Retail Over-Wrap Ground Meat with Fat Contents of 20 and 30%.

Media ^b	Log Number (colony forming units/g)									
	Beef-Beef		Beef-Pork		Regular		Grass			
	20	30	20	30	20	30	20	30	20	30
SMA										
Day 0	4.01(0.12) ^a	4.24(0.29)	3.41(1.24)	4.36(0.91)	5.54(0.52)	4.24(0.57)	4.46(0.77)	3.29(0.41)		
2	5.12(0.38)	4.90(0.58)	5.10(1.22)	4.60(0.11)	5.39(0.59)	5.29(0.76)	5.15(1.14)	4.07(0.62)		
4	6.01(0.19)	6.68(0.69)	5.85(1.66)	6.69(0.31)	7.10(1.05)	6.74(0.26)	6.90(1.14)	6.35(1.11)		
6	7.83(0.84)	7.10(0.01)	6.12(1.61)	7.87(0.26)	8.18(0.30)	7.85(0.12)	7.96(0.09)	7.49(0.51)		
8	7.58(0.63)	7.26(0.13)	6.63(1.56)	7.67(0.02)	8.02(0.37)	7.61(0.11)	7.94(0.36)	7.36(0.26)		
VRB										
Day 0	2.25(0.38)	2.71(0.11)	2.30(0.41)	1.72(1.01)	3.07(0.31)	2.71(0.11)	2.28(1.14)	1.00(0.00)		
2	2.77(0.26)	2.79(0.19)	3.18(0.06)	2.80(0.28)	3.55(0.35)	3.51(0.66)	2.77(0.14)	2.84(0.33)		
4	3.17(0.50)	2.89(0.61)	3.21(0.02)	3.60(0.08)	3.49(0.15)	3.58(0.14)	3.16(0.36)	3.54(0.08)		
6	3.98(0.37)	4.70(0.08)	3.39(0.09)	5.15(0.61)	3.82(0.46)	3.91(0.85)	4.06(1.33)	5.44(0.11)		
8	4.89(0.81)	4.81(0.03)	5.56(0.03)	5.28(0.28)	5.10(0.85)	5.98(0.14)	5.02(0.19)	5.12(0.40)		
PDA										
Day 0	1.64(1.10)	2.15(1.00)	1.58(0.01)	1.81(0.15)	2.07(0.93)	2.66(0.93)	2.00(0.88)	2.46(0.64)		
2	2.21(1.05)	2.52(1.35)	2.85(0.05)	2.41(0.57)	2.43(0.37)	3.70(0.40)	2.58(0.52)	3.16(0.22)		
4	2.81(0.29)	3.25(0.59)	2.96(0.45)	3.42(0.92)	2.77(0.19)	3.52(0.40)	2.80(0.24)	4.07(0.83)		
6	3.51(0.03)	4.58(0.93)	3.46(0.53)	4.17(0.93)	3.39(0.33)	4.29(0.77)	3.17(0.33)	4.81(0.08)		
8	3.82(0.10)	4.96(0.20)	4.03(0.41)	4.26(0.81)	3.66(0.20)	4.34(0.59)	3.79(0.16)	5.26(0.95)		

^aMean with standard deviation in brackets.

^bSMA = Standard Methods Agar; VRB = Violet Red Bile Agar; PDA = Potato Dextrose Agar.

Table 11. Log Counts of Freezer Wrap Ground Meat with Fat Contents of 20 and 30%.

Media ^b	Log Number (colony forming units/g)																					
	Beef-Beef						Beef-Pork						Regular		Grass							
	20		30		20		30		20		30		20		30							
															% fat							
															</							

Table 12. Log Counts of Vacuum Ground Meat with Fat Contents of 20 and 30% Fat.

Media ^b	Day	Log Number (colony forming units/g)													
		Beef-Beef				Beef-Pork				Regular				Grass	
		20	30	20	30	20	30	20	30	20	30	20	30		
fat %															

SMA															
0	4.01(0.12) ^a	4.24(0.29)	3.75(0.71)	4.36(0.91)	5.88(0.94)	4.24(0.57)	4.92(0.06)	3.29(0.41)							
7	5.25(0.15)	5.52(0.33)	4.82(0.19)	4.84(0.14)	6.05(0.18)	5.94(0.04)	5.37(0.14)	4.36(0.37)							
14	5.55(0.20)	6.27(0.48)	6.32(0.31)	6.26(0.81)	5.55(0.18)	6.13(0.54)	5.99(0.05)	6.16(0.54)							
21	6.07(0.21)	6.42(0.55)	6.50(0.20)	6.24(0.64)	6.48(0.57)	6.23(0.47)	6.38(0.38)	6.17(0.51)							
28	6.82(0.32)	6.56(0.05)	6.95(0.30)	6.70(0.03)	6.74(0.11)	6.57(0.08)	6.79(0.06)	6.61(0.09)							
CVT															
0	3.29(0.51)	4.40(0.37)	4.52(0.35)	3.84(0.03)	4.39(0.39)	4.31(0.43)	2.82(0.31)	3.99(0.01)							
7	5.79(0.23)	5.76(0.16)	5.86(0.20)	5.03(0.06)	5.91(0.23)	5.74(0.04)	5.96(0.10)	5.21(0.59)							
14	6.01(0.16)	5.89(0.06)	6.63(0.08)	5.82(0.01)	5.98(0.13)	6.29(0.57)	6.29(0.14)	5.94(0.18)							
21	6.38(0.39)	6.35(0.38)	6.62(0.02)	5.84(0.02)	6.02(0.11)	6.25(0.49)	6.36(0.12)	6.21(0.59)							
28	6.72(0.13)	6.58(0.05)	7.18(0.21)	6.62(0.01)	6.60(0.54)	6.59(0.18)	6.58(0.07)	6.60(0.03)							
VRB															
0	2.25(0.38)	2.71(0.11)	2.30(0.41)	3.22(0.30)	3.40(0.34)	2.71(0.11)	2.82(0.31)	3.99(0.01)							
7	3.88(0.53)	4.58(0.68)	3.90(0.46)	3.11(0.82)	3.81(0.43)	4.78(0.85)	3.57(0.24)	5.21(0.59)							
14	4.73(0.87)	5.72(0.04)	4.72(0.54)	5.17(0.28)	5.34(0.44)	5.87(0.01)	4.79(0.07)	5.94(0.18)							
21	5.31(0.19)	6.18(0.02)	6.46(0.42)	6.14(0.17)	6.32(0.19)	6.73(0.17)	5.91(0.00)	6.21(0.59)							
28	5.88(0.34)	6.53(0.09)	6.36(0.21)	6.12(0.81)	6.32(0.19)	6.60(0.04)	5.90(0.02)	6.60(0.03)							
APT															
0	2.68(0.77)	3.03(0.89)	3.01(0.54)	3.26(0.37)	2.64(0.11)	3.59(0.15)	2.53(0.46)	3.24(0.34)							
7	5.89(0.21)	5.58(0.07)	5.80(0.25)	4.98(0.94)	5.81(0.19)	5.60(0.09)	5.81(0.19)	5.50(0.02)							
14	6.26(0.24)	5.67(0.14)	6.04(0.06)	5.67(0.22)	5.79(0.25)	5.63(0.13)	5.83(0.18)	5.64(0.12)							
21	6.60(0.56)	6.20(0.54)	6.72(0.10)	6.10(0.71)	6.50(0.78)	6.09(0.63)	5.90(0.17)	6.11(0.58)							
28	6.60(0.10)	6.54(0.00)	6.81(0.02)	6.62(0.04)	6.85(0.25)	6.52(0.03)	6.32(0.28)	6.49(0.01)							

^aMean with standard deviation in brackets.

^bSMA = Standard Methods Agar; CVT = Crystal Violet Tetrazolium Agar; VRB = Violet Red Bile Agar; APT = All Purpose Tween Agar.

VITA

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