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CHEMICAL COMPOSITION AND ENSILING CHARACTERISTICS OF WHOLE CROP WHEAT, OATS, AND BARLEY HARVESTED IN THE MILK AND DOUGH STAGES OF MATURITY

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CHEMICAL COMPOSITION AND ENSILING CHARACTERISTICS OF WHOLE CROP WHEAT, OATS, AND BARLEY HARVESTED IN THE MILK AND DOUGH STAGES OF MATURITY

By

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ABSTRACT

CHEMICAL COMPOSITION AND ENSILING CHARACTERISTICS OF WHOLE CROP WHEAT, OATS, AND BARLEY HARVESTED IN THE MILK AND DOUGH STAGES OF MATURITY

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Wheat, oats, and barley were harvested for silage in the milk and dough stages of maturity to determine whether late maturation has adverse effects on silage quality. Whole plant composition data revealed lower water content, water soluble carbohydrate (WSC), and buffering capacity in the dough stage of maturity ($P<.05$). Fiber fractions were not effected by stage ($P>.05$) in wheat and barley but were lower for dough stage oats ($P<.05$). Total nitrogen was not different ($P>.05$) among species or stage of maturity.

During fermentation in experimental silos, milk stage silages exhibited faster pH declines ($P<.05$), greater WSC utilization ($P<.05$), and greater lactic acid production ($P<.05$), indicating a more significant role of fermentation in the preservation of earlier harvests. Volatile fatty acid analysis indicated all silages were of excellent quality. Protein degradation was more rapid in milk stage silages ($P<.05$), and higher throughout the duration of fermentation.

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1.0 INTRODUCTION

The primary goal in any forage conservation program is to maximize the preservation of nutrients found in the original crop upon harvesting. Haymaking and ensiling are the most widely accepted practices used today. In the past, hay was the principal conserved forage utilized for a number of reasons. The basic skepticisms toward silage were: 1) large dry matter losses involved during ensiling, 2) inconsistency associated with the ensiling process and its endproduct, 3) low production by animals fed silage and 4) due to its immobility, silage is rarely a marketable commodity.

As the understanding of the ensiling process progressed so too did the use of silage as a conservation alternative. Even though silage remains a relatively unmarketable commodity, most other drawbacks have been alleviated through continued research. When harvested properly, silage retains more dry matter than its counterpart hay, and animal performance has not been different when silage is fed in a completely balanced diet. Today, with mechanized feedbunks so prevalent, silage can be fed more efficiently than was possible before. It became evident that when particular attention is paid to

such things as plant maturity, moisture content, and compaction, a consistently high quality feed could be produced with negligible losses from the ensiling process.

These and similar findings have given the livestock producer additional options with respect to forage management. Today it's possible to successfully ensile virtually any crop for use at a later date. Double cropping is an option that enables the midwest farmer to grow and harvest two crops in one year or three silage crops in two years. This type of system makes use of a cool season annual small grain which is harvested as silage by early summer. The increasing use of double, even triple cropping has prompted the greater use of small grains, (wheat, oats, rye, and barley) for ensiling. The optimal stage of maturity at which these grains should be harvested as silage is still under debate however.

Traditionally it has been recommended that small grains be harvested for silage at a stage of growth associated with maximal protein concentrations and minimal fiber content. This situation occurs during early development when water is also present in high concentrations. A great deal of research was directed toward determining which of the earlier stages of growth were more conducive to successful fermentation. Meanwhile, studies by Bishnoi et al. (1978) and Bolsen and Berger (1976) have observed better feedlot performance when animals were fed the more mature milk and dough stage

silage. Higher yields, intakes, protein digestibility, and live weight gains with dough stage silage have prompted the practice of delaying the harvest of these small grains, but accompanying maturation is a loss of nutrients required for efficient fermentation. Ashbell et al. (1985) concluded the greatest loss of nutrients occurred during transition of wheat from the milk to dough stage which provided some insight into the difficulty in ensiling the more mature small grain crops. There have been few studies measuring the fermentation parameters of small grains approaching maturity and the effects of associated nutrient losses on these parameters. This study was developed to assess the chemical composition of late stage wheat, oats, and barley, and to determine how compositional changes that accompany maturation from the milk to the dough stage of maturity effect the subsequent fermentation upon ensiling.

2.0 REVIEW OF LITERATURE

2.1 Silage Microbiology

Silage is the product of anaerobic fermentation of a moist forage. The micro-organisms associated with silage ferment available carbohydrates and amino acids to organic acids which inhibit further microbial activity, hence nutrient destruction. Of most importance to successful ensiling are the lactic acid bacteria. These facultative anaerobes are scarce on intact plants (Stirling and Whittenbury, 1963), and it is believed they arise from farm machinery involved in the harvest (Gibson et al. 1961; Woolford and Wilkins, 1974; Beck, 1978). Obtaining their name from the primary end product they produce, the lactic acid bacteria are divided into two broad categories, homofermentative and heterofermentative.

In silages where lactic acid bacteria dominate the activity, there is a microbial shift from a homolactic to heterolactic dominance (Beck, 1972; cited by McDonald, 1981). On the fourth day of ensiling Beck found the most predominate homofermentative species, Lactobacillus curvatus and Lactobacillus plantarum, comprised 85% of the total lactic acid bacteria. By the tenth day of the

ensiling period, Lactobacillus buchneri and Lactobacillus brevis, major heterolactics, dominated the fermentation. Other genera of lactic acid species found in silage are streptococci and leuconostocs, which are significant in earlier stages of ensiling, and pediococci, more prevalent in the later stages. The most widely accepted theory behind the microbial shift was published by Gibson and Stirling (1959). They attribute the periodical domination by certain micro-organisms to their power of survival; this survival referring to acid tolerance. As the pH decreases, the less acid tolerant homolactics give way to the heterolactics. Beck (1978) supports the tolerance theory, however, believes the shift may be due to the increasing tolerance of acetate in particular, arising from mitochondrial respiration and bacterial fermentation. Another possibility can be explained on the basis of antibiosis. The release of unidentified substances (other than lactate or acetate) which are inhibitory to other organisms cannot be totally excluded (Singh and Laxminarayana, 1973).

Elevated pH, butyrate, and ammonia in silage indicate the activity of bacteria belonging to the genera clostridia. Clostridia are responsible for silage of poor quality, therefore it is of primary interest to minimize their activity. Because of their presence in soil and lack of on green plant material (Gibson et al., 1961), it is generally agreed their presence in silage results from

contamination by soil and farm equipment. Early studies of these spore forming, acid intolerant, facultative anaerobes by Allen and Harrison (1937) led to their division into two main physiological groups. Those which utilize carbohydrates as their main energy source are saccharolytic clostridia while those utilizing proteins were termed proteolytic clostridia.

Gibson (1961) identified the common species of clostridia found in silage. Cl. butyricum, Cl. paraputrificum, and Cl. tyrobutyricum are saccharolytic, but also have the ability to utilize lactate as a substrate. These organisms have a greater acid tolerance than the proteolytic clostridia, and if given a chance, can convert strongly acidic lactate to weaker acids and neutral alcohols (Wood, 1961). This counteraction of acidification provides an environment suitable for proteolytic clostridia, mainly Cl. bifermentans and Cl. sporogenes (Gibson, 1961). These bacteria acquire their energy from amino acids, arising from plant enzyme based protein degradation, through three types of fermentation pathways summarized by Barker (1961) and Mead (1971). Briefly they are deamination, decarboxylation, and Stickland reactions which liberate ammonia and organic acids, carbon dioxide and amines, and fatty acids, respectively. Other clostridia found in silage are Cl. sphenoides and Cl. perfringens which have been shown to be highly saccharolytic and proteolytic.

Another common group of bacteria occurring in silage are from the family enterobacteriaceae. Gibson et al. (1958) identified Klebsiella sp., Escherichia coli, and Bacterium herbicola as those found in the greatest concentrations. Commonly called coliforms or acetic acid bacteria, their existence in silage is limited to the early stages of ensiling provided acidic conditions develop (Langston and Conner, 1962). Considered weakly proteolytic, they ferment mainly carbohydrates to acetate, lactate, and carbon dioxide, and in some situations are known to produce formate, ethanol, 2,3-butanediol, succinate and H_2. For this reason, Stirling (1951) concluded that coliform bacteria were undesirable since they compete with lactic acid bacteria for available carbohydrates.

Fungi are eukaryotic micro-organisms, the majority of which require oxygen in order to grow. With ensiling being a predominantly anaerobic process, their significance was overlooked until 1964 when Beck and Gross found a correlation between the existence of yeasts and silage instability upon feedout. However, Henderson et al. (1972) found 10^5 of these organisms per gram of silage raising the question of their contribution to ensiling itself. It is generally agreed that being very acid tolerant, the fungi, including yeasts and molds, merely survive fermentation and exert their effects by respiring away available nutrients when exposed to air. The more fungi present when the silo

is opened, the greater the extent of aerobic deterioration (Ohyama and Masaki, 1975).

From the time the silo is filled until the time the silage is consumed, definitive changes take place with respect to the microbial population. The majority of microbes found on the original plant are aerobes and their dominance lasts until oxygen is depleted (Gibson and Stirling, 1959). During this stage they compete with plant enzymes for oxygen and nutrients, primarily available carbohydrates. According to Ruxton et al. (1975), rapid achievement of anaerobic condition is crucial. Respiration of carbohydrates produces heat, carbon dioxide and indirectly more aerobes, none of which contribute to successful ensiling. As a result up to 50% of the fermentable sugar can be lost. Once oxygen is depleted, plant and microbial respiration cease and the anaerobes, mainly coliforms, clostridia, and lactic acid bacteria, begin to proliferate. At this time, the forage medium is suitable for all types of anaerobes present and the success or failure of ensiling may be determined in the next few hours.

In a quality silage there was initially enough soluble carbohydrates to sustain a lactic acid fermentation. The coliform bacteria usually predominate until day three at which time the accumulation of lactic acid favors the growth of lactic acid bacteria. The dominate species of lactic acid bacteria thereafter depends on the pH (Beck,

1978). Once a pH of 4.2 has been established, the activity of other microbes is assumed negligible (Gibson and Stirling, 1959).

When a stable pH has not been attained, two pursuant fermentations may develop depending on the carbohydrate supply. Acetate silages have been described by Henderson and McDonald (1975). They found that some silages produced under laboratory conditions had undergone fermentation by coliform bacteria. Due to human handling in these cases, insufficient inoculation with lactic acid bacteria resulted in adequate preservation but by high acetate concentrations. However, when the available carbohydrates are exhausted before a stable pH is reached, clostridia became active.

It has been known for some time that clostridia are extremely sensitive to water availability. The critical pH, below which clostridial growth is inhibited, was observed by Wieringa (1958) when the water availability was adjusted by either wilting or sodium chloride addition. In both cases, the critical pH increased as dry matter increased and when wilted to 30% dry matter, clostridial growth is restricted irrespective of pH. In cases where they do develop, the saccharolytic clostridia are first to appear fermenting lactate to butyrate (Stirling, 1954; as cited by Woolford, 1984). As butyrate replaces strongly acidic lactate, the pH increases to a point where proteolytic clostridia can proliferate depleting the silage

of valuable nitrogen constituents. Thus, the quality of silage is very dependent on the dominating species which is principally governed by the rate and extent of acidification.

2.2 Silage Chemistry

It must be recognized that silage cannot be nutritionally superior to the original forage ensiled. By its very nature fermentation is a destructive process and nutrients in the original crop undergo changes yielding products of equal or diminished value. Some reactions involve the evolution of gases with a consequent loss of dry matter altogether. These reactions are catalyzed by enzymes endogenous to the plant material and those found in the relevant micro-organisms. The majority of reactions during ensiling involve carbohydrates, organic acids, and proteins.

Water-soluble carbohydrates are the primary source of energy for those organisms which are responsible for silage fermentation. The most abundant of these are the readily available sugars (glucose, fructose, and sucrose) and the storage carbohydrates (fructosans and starch). The disaccharide sucrose was found by Raguse and Smith (1966) and Smith (1973) to account for 20-56 g kg^{-1} DM in legumes, nearly twice that observed for the monosaccharides glucose and fructose. Fructose is generally found in greater concentrations than glucose. Fructosans, found primarily in the stem of grasses by Mackenzie and Wylam (1957), are usually in the range of 50-90 g kg^{-1} DM, increasing with age. Starch, used for storage by legumes and grains,

accumulates throughout growth attaining concentrations as high as 280 g kg^{-1} DM in corn (Smith, 1973). Sucrose, fructosans, and starch yield their respective monomers, mainly glucose and fructose, upon acid hydrolysis or enzymatic cleavage. Of the structural carbohydrates, only hemicellulose makes a significant contribution to the fermentation process. The products released from hemicellulose degradation are mainly the pentoses, arabinose and xylose.

The level and proportion of the individual constituents which are collectively regarded as carbohydrates are influenced by numerous factors. Edwards et al. (1968) working with barley cultivars and Waite and Gorrod (1959) with three species of grass, observed peak concentrations of water-soluble carbohydrate in the milk stage of maturity. Accompanying maturation was a steady decline in free monosaccharides and a concomitant increase in both the storage forms of soluble carbohydrates and the insoluble structural carbohydrates with the latter accounting for the greater proportion. Other variables affecting carbohydrate content in green plants include nitrogen fertilization (Jones, 1970), weather conditions (King et al., 1984), plant density, leaf-to-stem ratio and time of day (Smith, 1973). Once harvested, silage is exposed to an initial aerobic phase where plant enzymes continue to oxidize available carbohydrates. The practice of wilting the crop in the field prior to ensiling

potentiates this problem even though photosynthesis is still active (Brady, 1973). The oxidation of monosaccharides through the citric acid cycle via the glycolytic pathway occurs at the expense of sucrose, fructosans, starch, and hemicellulose. It was shown by Wylam (1953) that during a 4 hour wilt, levels of sucrose and fructosan had fallen by 23% and 26%, respectively, while concentrations of glucose and fructose remained constant. Amylase, a starch hydrolysing enzyme, has been identified in various plant families by Gates and Simpson (1968). Carpintero et al. (1979) compared four wilting regimes against the original herbage and found no significant difference in water-soluble carbohydrates. They attributed this to hemicellulase activity on insoluble hemicellulose yielding the soluble sugars, xylose, arabinose, galactose and glucose (Dewar et al. 1963).

Oxidative reactions stop once anaerobic conditions have been established inside the silo and surviving carbohydrates are available to the developing microflora. Wood (1961) found that the homolactic bacteria used the glycolytic pathway to produce two moles of lactate per mole of glucose or fructose. Heterolactics use the hexose monophosphate pathway which yields one mole each of lactate, CO_2, and ethanol when glucose is fermented and lactate, acetate, CO_2 and 2 moles of mannitol when 3 moles of fructose are fermented. The production of carbon dioxide constitutes a loss of dry matter and is one reason

why homolactic fermentation is preferred. Another reason raised by Whittenbury et al. (1967), is the efficiency of lactate production. Since lactate is a stronger acid than acetate, the more efficient homolactic fermentation results in a steeper decline in pH which is beneficial to preservation. The fermentation of pentoses to a mole each of lactate and acetate is similar in both types of lactic acid bacteria (Wood, 1961).

Quantitatively, the two most important organic acids present in herbage are citric and malic acids (Hirst and Ramstad, 1957). Other acids they found in appreciable amounts were malonate, succinate, and glycerate. Interest in organic acids increased when Playne and McDonald (1966) found these acids accounted for 68%-80% of the buffering capacity in herbage. Alfalfa and clover, noted for their difficulty in ensiling, contain twice the concentration of organic acids than ryegrass. Playne et al. (1967) studied the role of plant enzymes in the metabolism of organic acids. When aseptically-grown timothy was ensiled with and without bacterial inoculum, the inoculated silage exhibited a 65% degradation of malic acid while only minimal degradation was observed in microbe-free silage.

The metabolism of organic acids by bacteria during ensiling is rapid and limited to the lactic acid bacteria. The pathways of citrate and malate fermentation, described by Edwards and McDonald (1978), are numerous and involve pyruvate as a common intermediate. A wide range of

products are formed depending on the pH; they include lactate, acetate, formate, 2,3-butanediol, ethanol, CO_2, and alkaline released cations. Gunsalus and Campbell (1944) reported that as the pH was adjusted toward 7, the predominant product changed from lactate to formate and acetate.

An excellent review on the role of nitrogenous compounds in silage has been published by Ohshima and McDonald (1978). Proteins contain 75%-90% of the total nitrogen found in fresh herbage, and their amino acid composition is relatively constant between all forage crops examined (Wilson and Tilley, 1965; Gerloff et al., 1965). The amino acids arginine and glutamate were found in the greatest concentrations, ranging between 12% and 14% of the total protein nitrogen, while glycine, lysine, alanine, and aspartate ranged between 6% and 8%. Lyttleton (1973) identified stage of maturity and fertilization as primary factors influencing forage protein content. The remaining nitrogen found in plant tissue is termed non-protein nitrogen, and relevant constituents include free amino acids, amides, amines, nitrate, and variable length peptides. Low levels of ammonia (1.5% of total nitrogen) have been detected by Brady (1960) and are assumed to occur from nitrate reduction.

Protein degradation to water soluble nitrogen, mainly amino acids, ammonia, and amides, by plant proteinases is considered rapid and extensive (Bergen et al., 1974).

Ohshima and McDonald (1978) reported values of soluble nitrogen as high as 60% of the total nitrogen. Brady (1960) observed a three-fold increase in soluble nitrogen during a 48 hour period of ensiling and limited degradation thereafter. He also examined the fractions of resultant water soluble nitrogen and found a substantial increase in amino nitrogen and minor amounts of ammonia and amide nitrogen. Using microbe free grass, Kemble (1956) confirmed enzymatic activity on amino acids as well, but only to a limited extent. The majority of enzymes involved in protein hydrolysis are known to be acid labile and in silages of sufficiently low pH (4.3), hydrolysis levels off by day 5 of ensiling (Bergen et al., 1974).

Even in silage undergoing vigorous lactic acid fermentation, bacterial degradation of amino acids will occur. Among many accounts of both deamination and decarboxylation of amino acids by lactic acid bacteria, Brady (1966) produced evidence showing deamination of serine, arginine, glutamine, and asparagine by L. plantarum and L. brevis. However, it is generally agreed these activities are minimal compared to clostridial fermentation of amino acids. Clostridial activity results primarily in ammonia which can contain 60% of the total nitrogen in silages where clostridia dominate the microflora (Kemble, 1966). Sources of ammonia are deamination and Stickland reactions along with the reduction of nitrate. Amines, which are products of decarboxylation, are commonly found

in elevated concentrations in butyrate silages and have been associated with toxicity in animals (Dain et al., 1955). Decarboxylation also evolves carbon dioxide again constituting a loss of dry matter. In any extent, the fermentation of amino acids results in a significant loss of nutritive value.

Another undesirable nitrogen transformation is a non-enzymatic browning reaction commonly called the Maillard reaction. These reactions typically involve a condensation between aldose sugars and amino compounds, later polymerizing to form compounds unavailable to enzymatic hydrolysis during rumen fermentation or small intestinal digestion (Hodge, 1953). Prior to polymerization, intermediates in this process, termed premelanoidins, are soluble in water and included in the water soluble nitrogen fraction. Bergen et al. (1974) and Brady (1960) separated the water soluble nitrogen fraction into amino acid nitrogen, including small peptides, ammonia nitrogen, and an undetermined fraction constituting as much as 48% of the total. This later third may contain the premelanoidins but once polymerization has occurred, melanoidins become insoluble accumulating in the fiber fraction. Thomas et al. (1982) identified heat exposure and moisture level as the major factors influencing nitrogen transformation in silage as measured by acid detergent insoluble nitrogen formation. Other factors

included aeration and near neutral pH, characteristic of poor ensiling conditions.

2.3 Fermentation Quality

There have been many attempts to characterize the entire silage fermentation process with quick and easy determinations of single fermentation parameters. This may well be useful to the farmer who has limited access to facilities capable of extensive chemical analysis. However, the effectiveness of preservation is a function of many interrelated factors. Alone or in combination, knowledge of these factors are instrumental in determining what actually occurred in the silo.

The loss of dry matter (DM) during fermentation has been difficult to determine because of the problem of separating these from losses in the initial aerobic phase. McDonald et al. (1973) using calculations based on fermentation pathways, figured a DM loss of 4.2% when heterolactic bacteria dominated the fermentation. This agrees well with actual losses of 2.7% to 10% observed by Anderson and Jackson (1970) for a wide range of dry matters. McDonald et al. (1973) calculated a complete recovery of DM with homolactic carbohydrate fermentation whereas heterolactic fermentation results in losses of 24% and 5% when substrates are glucose and fructose, respectively. Losses associated with clostridial fermentation are much higher. The production of butyrate from lactate liberates 51% of the DM as carbon dioxide, and

amino acid degradation is a significant source of loss no matter what pathway (deamination, decarboxylation, or Stickland) is undertaken. Although the loss of dry matter is a very crude measure of fermentation quality, it can give some insight about the dominant fermentation.

Silage pH has been widely used as a measure of silage quality and it is generally thought that a pH of 4.5 or less is indicative of good fermentation. However, Geasler (1970) found that as the mean dry matter increased from 21% to 43%, mean critical pH increased from 3.52 to 4.65 with one sample achieving stability at pH 5.63. As mentioned earlier, clostridia's acid tolerance is highly dependent on water availability with wetter silages requiring more acid to inhibit clostridial growth. Rather than the amount of acid produced, a more descriptive measure would be the rate of acid production. Responsible for excessive nutrient destruction, undesirable micro-organisms including yeasts, coliforms, and clostridia, are active during the initial stage of ensiling, the extent of which is positively correlated to pH days above 4.00 (Gibson et al., 1958). In addition, most plant enzymes responsible for protein degradation are acid labile and their activity ceases as the pH nears 4.3 (Kemble, 1956; Brady, 1960; MacPherson and Violante, 1966; Bergen et al., 1974). Decreasing the length of time above pH 4.3 will retard protein degradation. Therefore, a rapid decline in pH represents a

lower degree of nutrient destruction compared to more gradual acidification.

Woolford (1984) stated, "Chemical assessments of the principal fermentation products provide an unequivocal basis on which to judge quality." In 1938, Flieg developed a system to evaluate fermentation based on the production of lactic, acetic, and butyric acids. This system was revised by Zimmer (1966) as cited by McCullough (1978) into the most recognized method in use today. Briefly, points are awarded based on each acid's contribution to the total acid concentration, with maximum points indicating quality fermentation. Greater scores are received for elevated proportions of lactic acid and minimal concentrations of acetate and butyrate.

McDonald et al. (1962) produced a clostridial silage yielding a comparatively low level of butyrate, with 29% ammonia nitrogen the only sign of amino acid degradation. These results prompted investigators (Carpintero et al., 1969) to include volatile nitrogen, ammonia being the major constituent, when classifying fermentation quality. The production of ammonia during ensiling is due primarily to proteolytic clostridia (Wood, 1961), and positively correlated to amino acid deamination (Gibson et al., 1958). Well preserved silages have been found to contain up to 12% of the total nitrogen as ammonia, greater amounts are indicative of clostridial activity (Carpintero et al., 1969).

Enhanced formation of acid detergent insoluble nitrogen (ADIN) requires heat and oxygen usually present during the initial aerobic phase after ensiling (Thomas et al., 1982). In the event of an inordinant amount of air entrapped in the silo, heat from plant and microbial respiration can produce temperatures in excess of 60^{0}C. This is evidenced by a dark brown, caramel smelling product and is quantified by ADIN analysis. ADIN values of less than 10% of the total nitrogen are deemed desirable, indicating minimal heat damage to forage proteins (Goering et al., 1972).

Taken together, these factors reveal a great deal about the activities occurring in the silo. Researchers use these parameters to compare the ability of various treatments to influence silage fermentation, hence, silage quality.

2.4 Stage Of Maturity

Annual small grains are generally harvested for silage at four stages of growth. Oltjen and Bolsen (1978) described these stages as follows:

Boot. Head, remaining inside stem, visibly distends sheath of flag leaf. Head of main stem usually enters boot stage first, followed by the tillers. Stage lasts about 10 days.

Flowering. Head is fully emerged with anthems shedding pollen. Fertilization and initial grain development occur. Plant is green but lower leaves have begun to die. Stage lasts about 7 days.

Milk. White, milk-like fluid occupies the kernel, made up of water and many starch granules. More leaves die; embryo develops fully. Stage lasts 10 to 14 days.

Dough. Water content of kernel decreases to a dough consistency. Leaves are dying; plant changes from green to yellow. Stage lasts 10 to 14 days.

The effect of maturity on silage fermentation is related to the content of moisture, water soluble

carbohydrates, and buffering constituents at the time of harvest. Water content being the most important, it has also been directly related to effluent loss (Miller and Cliftin, 1965) and silage intake (Johnston et al., 1970). As with most plant species, the dry matter of cereal grains increases as maturity progresses. While ensiling whole crop barley, Edwards et al., (1968) reported dry matter percentages of 20, 26, 31, and 37 for boot, flowering, milk, and dough stages, respectively. Depending on variety, results of Bolsen and Berger (1976) show similar dry matters at various stages of growth for wheat, oats, and barley.

Lactate, the most beneficial acid with respect to silage production, is the dominant product of fermentation when the moisture content of the ensiled crop is correct. A range of 55% to 70% moisture for cereal crops is recommended, with 60% to 65% the optimum (Oltjen and Bolsen, 1978). As the moisture increases beyond 70%, a given hydrogen ion concentration becomes less inhibitory to undesirable micro-organisms. MacGregor and Edwards (1968) ensiled barley at various growth stages with dry matters ranging from 19.1% to 42%. Silages harvested at boot and flowering stages contained the greatest amount of fermentation acids, yet had the highest pH. Butyrate concentrations reached 1.95% of the DM for the wetter silages while only trace amounts were found at the milk and dough stages. Lactate to acetate ratios were 1.24, 1.50,

3.22, and 1.63 for boot, flowering, milk, and dough stages, respectively. The greater extent of fermentation in the low dry matter silages also suggests higher fermentation losses.

Ensiling excessively dry crops has been reviewed by Gordon (1967). Larger amounts of oxygen are entrapped within the silage mass increasing the possibility of heating and molding. These silages usually exhibit elevated acid detergent insoluble nitrogen and pH due to minimal anaerobic bacterial activity.

Water soluble carbohydrates (WSC) being the primary fermentable substrate are needed in quantity to allow sufficient lactic acid production. Compared to legumes which average 5% to 10% WSC on a dry matter basis and remain relatively constant throughout growth, cereal grain crops have greater WSC concentrations which are very dependent on the stage of maturity. Ashbell et al., (1985) measured the WSC concentration in wheat at the four common stages of maturity. They found WSC concentrations of 20, 19, 28, and 13% in the boot, head, milk, and dough stages, respectively. A mutual peak concentration during the milk stage has also been observed in oats by Sutoh et al., (1972). MacGregor and Edwards (1968) followed the individual components comprising the WSC fraction during the maturation of barley and concluded that varying fructosan levels were responsible for the peak (31.9%) and subsequent decline (14.7%) into the dough stage.

Fructosans are believed to be temporary storage compounds for available carbohydrates before conversion into starch.

It is generally assumed that WSC concentrations of at least 10% will result in satisfactory preservation irrespective of dry matter content. In cereal grain silages, soluble carbohydrates are rarely a limiting factor of preservation (Oltjen and Bolsen, 1978). In fact, they have been extensively used in combination with both legumes and grasses to insure sufficient substrate availability during ensiling. However, in work reported by Sutoh et al., (1972), resultant silage from green oats harvested at boot, flowering, milk, and dough stages were all unsatisfactory as evidenced by Flieg scores of 37, 58, 33, and 27, respectively. The WSC concentrations never reached 9% in that study (Sutoh et al., 1972) and no explanation was given for the unusually low levels of carbohydrates observed.

Organic acids, being the prominent buffering constituent in plants, dictates to some extent the relative ensilability of a crop in terms of the amount of acid necessary to achieve anaerobic stability. MacGregor and Edwards (1968) reported a linear decline in the concentrations of malate and citrate, the most abundant organic acids in barley, during maturation. Total organic acids accounted for 6.6% of the dry matter in the boot stage while only 1.1% was found in the dough stage.

Proteins can contribute to the buffering capacity associated with plant material and their concentration is known to decrease with maturity in corn (Johnson et al., 1966), wheat (Ashbell et al., 1983), oats (Sutoh et al., 1972), and barley (Edwards et al., 1968). However, because of the relatively low crude protein content as compared to legumes, the contribution of proteins to the buffering capacity has been overlooked in cereal grain silage (Oltjen and Bolsen, 1978).

The effect of maturity on proteolysis during ensiling is believed to be related to the plant's dry matter content. Fermentation is known to diminish as the dry matter percentage increases as evidenced by lower concentrations of fermentation acids and high pHs. Hawkins et al., (1970) ensiled freshly chopped and wilted alfalfa of the same maturity and concluded the extent of proteolysis decreases with increasing dry matter content. Geasler (1970) ensiled whole crop corn at various dry matters and reported reductions in water soluble nitrogen and ammonia of 43.7% and 51%, respectively, as the dry matter increased from 22% to 42%. These results were confirmed by Bergen et al., (1974). Reports of proteolysis during the ensiling of small grains are conflicting. When expressed as a percentage of total nitrogen, ammonia production increased with dry matter content in oats (Sutoh et al., 1972), while Ashbell et al., (1985) observed a decrease when ensiling wheat at more mature and dryer

growth stages. Water soluble nitrogen was not presented in either study. However, the effects of moisture on proteolysis in small grain silage would be expected to be similar to those observed for other type silages.

In summary, the earlier stages of growth are associated with high moisture and buffering contents, and comparatively low amounts of water soluble carbohydrates. Taken together, the result is a material with a high probability of protein degradation and clostridial fermentation unless wilted prior to ensiling. Optimal ensiling characteristics are observed during the milk stage of maturity, however, the more mature stages have substantially lower protein concentrations. Although the high dry matter percentage in the dough stage lessens the necessity of fermentation, water soluble carbohydrates may be a limiting factor of successful preservation. Also, diminishing protein contents observed during these latter stages may be offset by lower rates of protein degradation during initial stages of fermentation.

3.0 MATERIALS AND METHODS

Frankenmuth wheat, Corwood oats, and Bowers barley were harvested for silage at the milk and dough stages of maturity. Fermentation was carried out in small experimental silos and opened for analysis at predetermined times after sealing.

3.1 Harvest

In 1986, wheat was harvested on June 24, and July 2, oats and barley on July 2, and July 14, for the milk and dough stages, respectively. Due to greater than average rainfall during the spring, wheat was harvested later than planned. On all harvest dates, a small flail-type harvester was used to cut 5 successive 3' x 25' paths and weights were recorded for average yield analysis. Milk stage wheat was further chopped to .5 inch on a paper cutter in the field. When it appeared that the paper cutter would not suffice for multiple harvestings on July 2, the remaining samples were transferred into burlap bags and transported to the Beef Cattle Research Center where they were hand fed into a typical tractor-drawn forage harvester adjusted for a chop length of .5 inch. After chopping, the samples were immediately packed into silos.

3.2 Fermentation

The experimental silos consisted of 2 quart Mason canning jars equipped with lids modified with automatic gas release valves. Valves were constructed by attaching rubber policemen onto 15 gauge hyperdermic needles which were inserted through rubber stops sealed into the lids. Tiny slits were cut into the policemen with an Exacto knife to allow the escape of gasses without the re-entry of air once the silos were sealed.

Before the silos were filled, 10g of solid carbon dioxide was placed in the bottom of the silo to promote evacuation and anaerobiosis. For each harvest, sixteen silos were filled with approximately 1.5kg of chopped plant material and compressed by hand. Between 4 and 6 hours had elapsed from the time of harvest until the last silo was sealed. Silos were sealed and incubated at 30^0C for 3 days; then allowed to set at room temperature (20^0C) for the remaining time period. In addition to two samples of fresh material taken from each field, two silos of each grain were opened after .5, 1, 2, 4, 8, 16, 32, and 64 days from the average time of ensiling. The contents of the opened silos were mixed thoroughly and a representative sample from each silo was frozen at -20^0C until subsequent analyses.

3.3 Silage Analysis

A Flow diagram of analyses conducted is shown in Figure 1. Immediately after thawing, the silage samples were initially ground through a Hobart chopper for two minutes to reduce the particle size and facilitate mixing. Total nitrogen was directly determined by sulfuric acid digestion in the presence of a copper sulfate based catalyst (Pope Kjeldahl mixtures, P.O. Box 903, Dallas, Texas 75221). The analysis of free ammonia nitrogen was conducted on a Technicon Auto Kjeldahl System calibrated with 100 and 200 ppm nitrogen standards from ammonium sulfate.

Dry matters were determined by drying 100 grams of ground sample at 55^{0}C in a forced air oven (verified by Geasler, 1970). Dried samples were ground in a Wiley mill through a 1 millimeter screen and later analyzed for ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent insoluble nitrogen (ADIN). Ash was determined by heating to 500^{0}C for 3 hours in a muffle furnace and weighing the residue. NDF and ADF was analyzed according to Goering and Van Soest (1970). Amylase digestion according to Goering and Van Soest (1970) was necessary during NDF analysis because of the high concentrations of starch expected in the samples. Hemicellulose was calculated as the difference between NDF

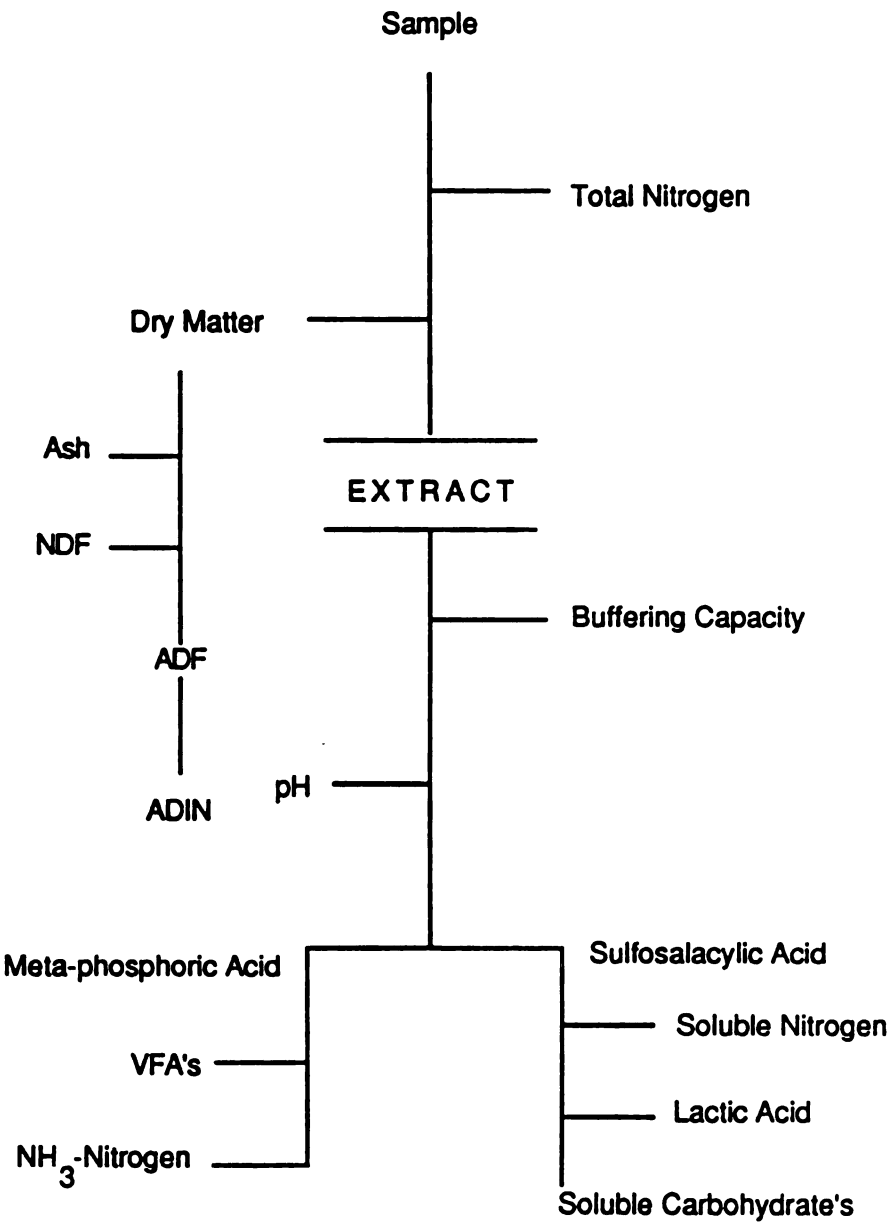

FIGURE 1

Flow Diagram of Laboratory Analysis
Conducted on Silage Samples

and ADF. ADIN was analyzed by micro-Kjeldahl digestion of the ADF residue in concentrated sulfuric acid and Pope Kjeldahl catalyst. Samples were quantified on the Technicon Auto Analyzer.

Silage extracts were prepared by homogenizing a 20 gram aliquot of chopped sample with 200 milliliters of chilled distilled and deionized water. Homogenization was conducted with a Sorvall Omnimixer at maximum speed for two minutes in an ice bath. The resultant slurry was strained through four layers of cheesecloth and portioned into three fractions of 100, 22.5, and 20 milliliters kept chilled until further preparation.

The pH was determined immediately on each 100 milliliter aliquot with an Orion (model 801A) digital ionalyzer equipped with a glass electrode. Time 0 samples were acidified to pH 3 with .1 normal hydrochloric acid and the buffering capacity was calculated as the milliequivalents of sodium hydroxide necessary to raise the pH from 4 to 6. Buffering capacity is expressed as milliequivalents per 100 grams of dry matter.

The 22.5 milliliter portion was deproteinized with 2.5 milliliters of 50% sulfosalicylic acid (SSA) and centrifuged at 15,000 x g for fifteen minutes. The supernatant was poured into a sterilized blood dilution vial and frozen at -20^{0}C for later analysis. Colorimetric procedures of Barker and Summerson (1941) and Dubois et al., (1958) were used for the determination of lactic acid

and water soluble carbohydrates (WSC), respectively. Both were quantified by linear regression on a standard curve constructed by varying the concentrations of lactic acid and glucose around the range expected in the silage samples. Water soluble non-protein nitrogen (WSN) was determined by digesting 5 milliliters of the SSA extract in concentrated sulfuric acid and Pope Kjeldahl catalyst and analyzed on the Technicon Auto Analyzer.

The remaining 20 milliliter portions were vortexed for thirty seconds with 4 milliliters of 25% meta-phosphoric acid and allowed to set at room temperature for 30 minutes. After deproteinization, the samples were centrifuged at 15,000 x g for fifteen minutes. The supernatant was then transferred to sterilized blood dilution vials and frozen at -20^{0}C until further analysis. Volatile fatty acids were analyzed by a Hewlett-Packard gas-liquid chromatograph (Model 5840A) fitted with a 183cm x .32cm stainless steel Chromosorb column (10% SP 1200 and 1% phosphoric acid 80/100 WAW mesh, Supelco Inc., Bellefone, PA.) and with the following conditions programmed into the chromatograph: column temperature, 120^{0}C, injection temperature, 170^{0}C, flame ionization detector temperature, 200^{0}C and carrier gas flow (Helium) 50ml/min. A standard containing the three primary volatile fatty acids was used to determine the concentrations of acetate, propionate, and butyrate present in the silage samples. Ammonia nitrogen was determined with the Technicon Auto Analyzer on the meta-

phosphoric acid extract. The standards (100 and 200 ppm) were prepared from a stock solution of ammonium phosphate at a concentration of one milligram nitrogen per milliliter. It was recognized that free amino acids are not precipitated by meta-phosphoric acid, and certain amino acids are known to react with automated Kjeldahl reagents and recorded by the analyzer. Weber (1983) deammoniated duodenal fluid samples by boiling at 100^{0}C of one hour. After deammoniation, he added 100 and 200 ppm equivalents of ammonium phosphate. When compared against standards prepared from distilled water, the duodenal based standards averaged 2 to 3 ppm more ammonia nitrogen equivalent presumably due to free amino acids. This 1% difference was considered insignificant to warrant the adjustment of standards.

3.4 Statistical Analysis

Statistical analysis was performed on the MSTAT program developed by Michigan State University's Crops and Soil Science Department (East Lansing, Michigan). Fermentation parameters measured over time (dry matter, total nitrogen, ash, pH, lactate, acetate, WSC, WSN, ADIN, ADF, NDF, and hemicellulose) were analyzed as a two factor completely randomized design with forage and stage combinations as one factor (A) and days of fermentation (B) the other. The model statement was as follows:

$$Y_{ijk} = \text{mean} + A_i + B_j + (AB)_{ij} + \text{error}_{(ij)k}$$

Yield and buffering capacity were subjected to one-way analysis of variance in a completely randomized design, again combining forage and stage combinations as treatments. In all sets of analyses, treatment combination means were separated by Tukey's honestly significant difference test (HSD) as described by Gill (1978). Where interaction between factors was not significant, overall means were separated as with treatment combination means.

4.0 RESULTS

Dry matter yield and buffering capacity of the fresh herbage are presented in Table 1. In both the milk and dough stages, wheat yielded approximately twice the dry matter ($P<.05$) as either oats or barley. In general, yields were not different between oats and barley, but harvesting these grains at the dough stage resulted in significantly greater yields ($P<.05$) compared to those obtained in the milk stage harvest. No difference in dry matter yield was observed between these two stages for wheat. At both growth stages, wheat had the lowest buffering capacity ($P<.05$) of the three species evaluated in this study while no difference was found between oats and barley. In each small grain, the buffering capacity decreased with maturation from the milk to the dough stage, however, only the decrease in barley's buffering capacity was significant ($P<.05$).

Values for dry matter, total nitrogen, and ash of the ensiled small grains are presented in Table 2. Since average time effects were not significant ($P>.07$), only initial and final observations are shown here; complete data sets can be found in the appendix. Overall means across time include observations from all sampling days.

Table 1. Dry Matter Yields and Buffering Capacities upon Harvest of Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity.

Species	Stage	DM Yield[1] Ton/acre	Buffering Capacity[2] mE/100g DM
Wheat	Milk	3.97^{A}	17.45^{AD}
	Dough	3.90^{A}	16.60^{A}
Oats	Milk	1.43^{B}	25.10^{BC}
	Dough	1.90^{CD}	22.30^{BE}
Barley	Milk	1.65^{BC}	28.45^{C}
	Dough	2.21^{D}	20.80^{DE}

^{ABCDE}Means within columns with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent averages of five observations and have a standard error of the mean (S.E.M.) of .10.

[2]Tabular entries represent averages of two fresh samples and have a S.E.M. of 1.41. Values are expressed as milliequivalents of base required to raise the pH from 4 to 6.

Table 2. Influence of Stage of Maturity and Time of Ensiling on Dry Matter, Total Nitrogen, and Ash in Wheat, Oats, and Barley.

		Dry Matter (%)			Total Nitrogen (% DM)			Ash (% DM)		
Species	Stage	Day 0[1]	64[1]	Mean[4]	Day 0[2]	64[2]	Mean[5]	Day 0[3]	64[3]	Mean[4]
Wheat	Milk	37.0^{Aa}	34.5^{Ba}	35.5	1.26^{Aa}	1.36^{Aa}	1.30^{a}	6.0^{Aa}	7.4^{Aa}	7.2
	Dough	44.4^{Ab}	43.5^{Ab}	43.6	1.44^{Aab}	1.37^{Aa}	1.35^{a}	6.8^{Aab}	7.8^{Aa}	7.8
Oats	Milk	27.4^{Ac}	25.9^{Ac}	27.2	1.29^{Aa}	1.30^{Aa}	1.30^{a}	8.3^{Ac}	9.6^{Ab}	9.5
	Dough	36.5^{Aa}	34.2^{Aa}	34.5	1.18^{Aa}	1.17^{Aa}	1.17^{b}	8.6^{Ac}	9.6^{Ab}	9.2
Barley	Milk	24.6^{Ad}	25.2^{Ac}	25.7	1.60^{Ab}	1.44^{Aa}	1.46^{c}	8.4^{Ac}	9.1^{Ab}	8.7
	Dough	35.8^{Aa}	34.9^{Aa}	35.6	1.32^{Aa}	1.31^{Aa}	1.28^{a}	7.8^{Abc}	8.4^{Aab}	8.2
	Mean	34.3	33.0		1.35	1.33		7.7	8.7	

^{AB}Stage means within time (rows) with unlike superscripts differ ($P<.05$).

^{abcd}Time means within stage (columns) with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent means of two experimental silos with a S.E.M. of .522.

[2]Tabular entries represent means of two experimental silos with a S.E.M. of .054.

[3]Tabular entries represent means of two experimental silos with a S.E.M. of .298.

[4]Stage means represent means of eighteen silos across all sampling days.

[5]Nonsignificant interaction allowed mean separation with a S.E.M. of .018.

As expected, greater dry matter percentages were observed as maturity progressed from the milk to dough stage ($P<.05$) for all small grain crops. Due to the delayed harvest, the dry matter content was higher in wheat than in oats and barley within both growth stages ($P<.05$). Dry matters were different ($P<.05$) between oats and barley in the milk stage but similar in the dough stage. Except for milk stage wheat, dry matter percentages were unchanged throughout fermentation.

Comparisons of total nitrogen within time and stage of maturity indicate milk stage barley as having the highest crude protein content (total nitrogen x 6.25) on Day 0 ($P<.05$). No differences were observed between the remaining species and stage combinations on Day 0, and all species and stage combinations were similar through Day 64. Nonsignificant interactions between treatments and time allowed the comparison of treatment means across time. Overall, there was a decrease in total nitrogen into the dough stage in oats and barley ($P<.05$) while no change was observed in wheat.

Although not significant, ash, as a percent of dry matter, increased with fermentation in all species and stage combinations. Averaged together, ash increased 13%, from 7.7% of DM at harvest to 8.7% of DM on Day 64. Growth stage effects within species were not significant. The ash content of wheat was significantly lower ($P<.05$) than oats

and barley in the milk stage and oats, but not barley, in the dough stage.

The pH means are reported in Table 3 and shown graphically in Figure 2. At harvest, barley had higher pH values than wheat and oats within the milk stage and oats within the dough stage ($P<.05$). The only difference between stage of maturity at harvest was found in barley ($P<.05$). After .5 days, the pH of milk stage oats and barley had declined to a greater extent than their dough stage counterparts ($P<.05$). This relationship remained throughout fermentation as the pH continually decreased through Day 16. In contrast, dough stage wheat had significantly lower ($P<.05$) pH values during initial fermentation but, by Day 8 and thereafter, the pH of wheat was lower in the milk stage as with oats and barley ($P<.05$). In all silages, the critical pH of 4.5 had been surpassed by the second day of fermentation and oats and barley in the milk stage had reached this value between .5 and 1 days. All species and stage combinations plateaued around Day 16 except for milk stage oats, which continued to decrease throughout the duration of the experiment ($P<.05$). Across species, milk stage silages had lower stable pH values than silages made from the dough stage harvest ($P<.05$).

Initial lactic acid concentrations were not different between treatment combinations (Table 4 and Figure 3), even though small amounts were found initially in dough stage

Table 3. Influence of Stage of Maturity and Time of Ensiling on pH in Wheat, Oats, and Barley.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	5.89^{ABa}	5.93^{AEa}	5.80^{BCa}	5.77^{Ca}	6.09^{Da}	5.99^{Ea}	5.91
.5	5.71^{Ab}	5.02^{Bb}	4.64^{Cb}	5.25^{Db}	4.65^{Cb}	5.87^{Eb}	5.19
1	4.92^{Ac}	4.66^{Bc}	4.40^{Cc}	4.69^{Bc}	4.42^{Cc}	4.95^{Ac}	4.67
2	4.47^{Ad}	4.44^{ABd}	4.22^{Dd}	4.36^{BCd}	4.28^{CDd}	4.47^{Ad}	4.37
4	4.35^{Ae}	4.34^{Ade}	4.08^{Be}	4.27^{Ad}	4.15^{Be}	4.32^{Ae}	4.25
8	4.21^{Af}	4.24^{Ae}	4.09^{Be}	4.15^{ABe}	4.09^{Be}	4.21^{Af}	4.16
16	4.10^{ABg}	4.13^{ABf}	4.04^{Bef}	4.11^{ABe}	4.05^{Bef}	4.15^{Afg}	4.09
32	4.02^{ABg}	4.11^{Af}	3.95^{Bf}	4.08^{Ae}	3.96^{Bf}	4.11^{Ag}	4.04
64	4.01^{ADg}	4.12^{Bf}	3.82^{Cg}	4.08^{ABe}	3.97^{Df}	4.09^{ABg}	4.01
Mean	4.63	4.55	4.34	4.53	4.40	4.68	

^{ABCDE}Species and stage means within time (rows) with unlike superscripts differ ($P<.05$).

^{abcdefg}Time means within species and stage (columns) with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .024.

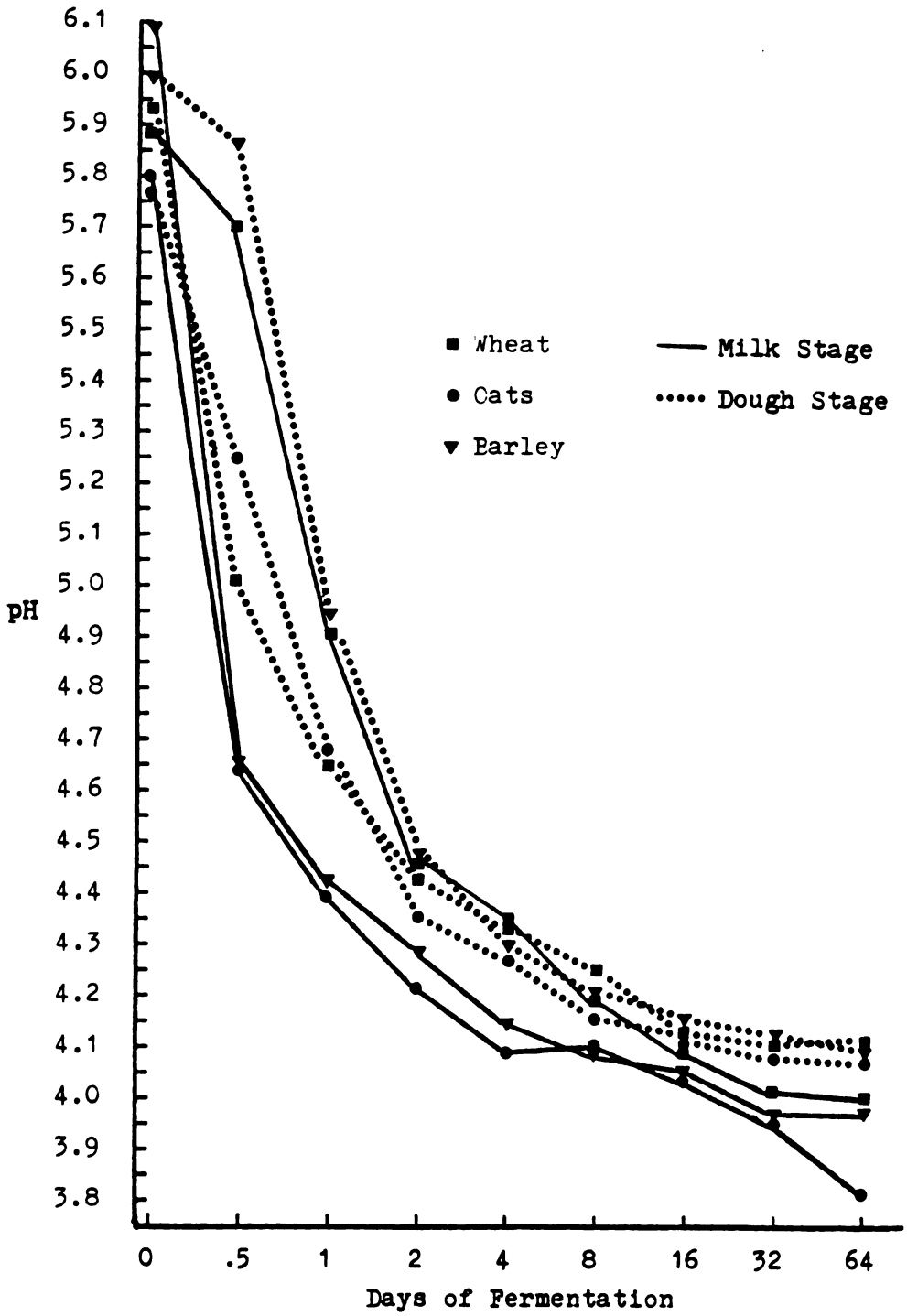

Figure 2. Changes in pH of Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

oats and barley while lactate was absent among the other combinations. Lactate concentrations increased significantly by .5 days of ensiling in milk stage oats and barley ($P<.05$) whereas increases of this same magnitude were not noticed until 1 day in all species at the dough stage and not until 2 days in milk stage wheat silage ($P<.05$). Milk stage oat and barley silages had more lactate than the other silages throughout fermentation but the differences were not always significant ($P>.05$). Mean concentrations across specie and stage combinations increased continually, leveling off around Day 32 attaining a maximum concentration of 7.74% of DM on Day 64. Individually, milk stage silages plateaued later than their dough stage complements ($P<.05$). As can be seen from Figure 3, at any given time dough stage silages generally contained less lactate than milk stage silages except for wheat silage made at the milk stage which did not differ from any of the dough stage silages.

Analyses were performed for the volatile fatty acids, acetate, propionate, and butyrate, but no appreciable amounts of propionate or butyrate were found in any of the samples under study. Acetate means are presented in Table 5 and displayed graphically in Figure 4. Mean acetate concentrations increased sharply within time until Day 4 and gradually thereafter through Day 64. The comparison of individual specie and stage combinations show nonsignificant but higher initial acetate concentrations in

Table 4. Influence of Stage of Maturity and Time of Ensiling on Lactic Acid as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	0.00^{Aa}	0.00^{Aa}	0.00^{Aa}	0.13^{Aa}	0.00^{Aa}	0.21^{Aa}	0.06
.5	0.06^{Aa}	1.60^{ABab}	3.19^{Bb}	1.04^{ABab}	3.09^{Bb}	0.58^{Aab}	1.59
1	1.64^{Aab}	2.62^{ABbc}	4.56^{Bbc}	2.97^{ABbc}	3.86^{ABb}	3.02^{ABbc}	3.11
2	2.97^{Abc}	3.29^{Abcd}	4.57^{Abc}	4.43^{Acde}	5.18^{Abc}	3.54^{Acd}	4.00
4	3.34^{Abc}	4.17^{Abcde}	6.99^{Bcde}	4.10^{Acd}	7.12^{Bcd}	4.56^{Acde}	5.05
8	4.63^{Acd}	4.45^{Acde}	6.77^{ABcd}	5.52^{Acde}	8.14^{Bd}	5.88^{ABdef}	5.90
16	6.25^{ABCd}	5.37^{Ade}	8.53^{Cdef}	6.13^{ABde}	8.26^{BCd}	6.76^{ABCef}	6.88
32	6.35^{Ad}	6.65^{Ae}	9.94^{Bf}	6.89^{ACe}	9.18^{BCde}	7.21^{ACf}	7.70
64	5.96^{Ad}	6.12^{Ae}	9.46^{BCef}	6.22^{Ade}	11.43^{Ce}	7.25^{ABf}	7.74
Mean	3.47	3.81	6.00	4.16	6.25	4.33	

^{ABC}Species and stage means within time (rows) with unlike superscripts differ ($P<.05$).

^{abcdef}Time means within species and stage (columns) with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .563.

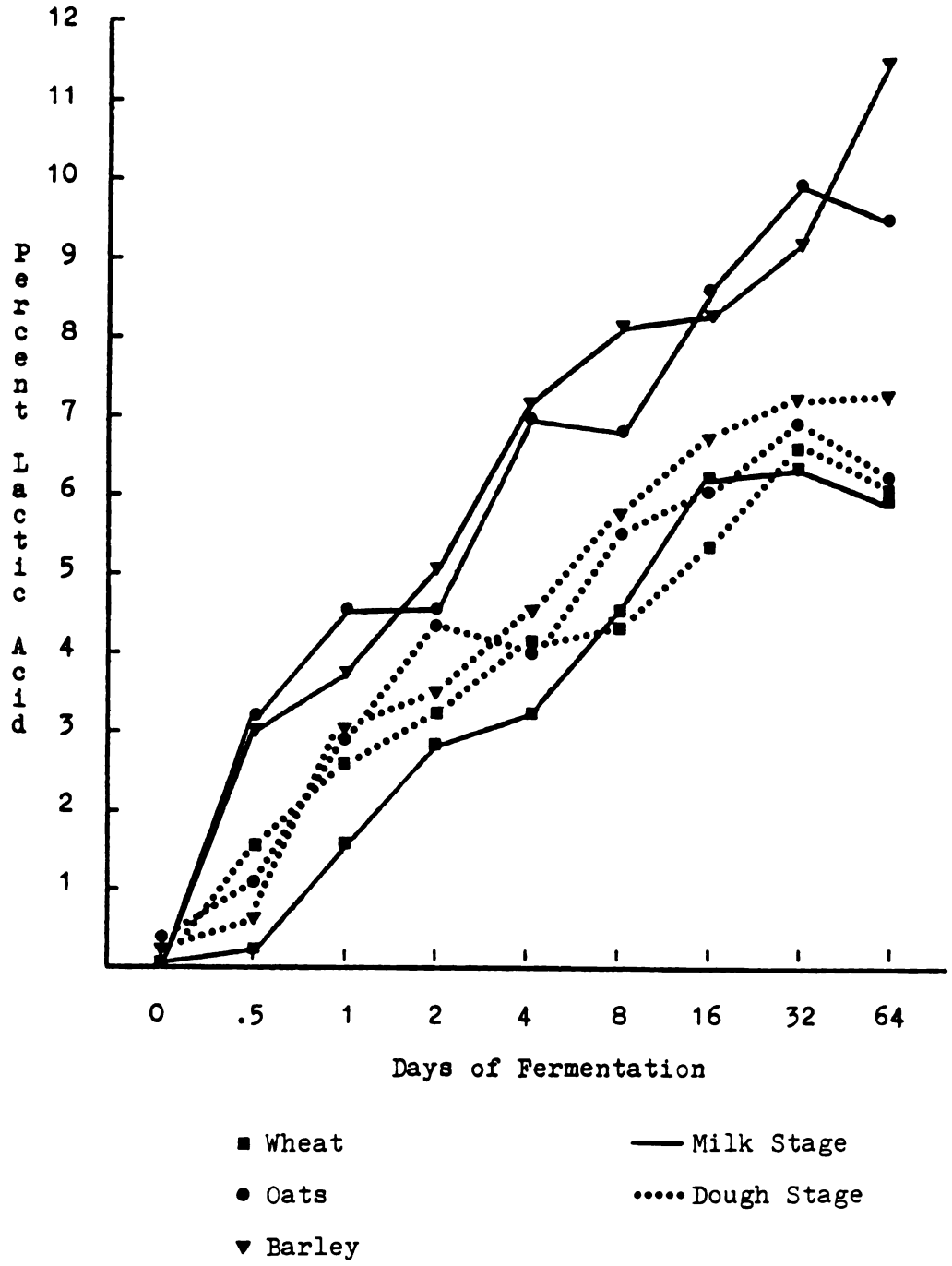

Figure 3. Changes in Lactic Acid (% of DM) of Wheats, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

Table 5. Influence of Stage of Maturity and Time of Ensiling on Acetic Acid as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	0.23^{Aa}	0.41^{Aa}	0.16^{Aa}	0.23^{Aa}	0.09^{Aa}	0.24^{Aa}	0.23
.5	0.25^{Aa}	0.58^{Bab}	0.63^{Bb}	0.42^{ABab}	0.38^{ABab}	0.37^{ABab}	0.44
1	0.68^{Ab}	0.66^{ABab}	0.48^{ABab}	0.51^{ABabcd}	0.34^{Bab}	0.34^{Ba}	0.50
2	0.85^{Abc}	0.66^{ABab}	0.49^{Bab}	0.36^{Bab}	0.45^{Babc}	0.41^{Bab}	0.54
4	0.93^{Abc}	0.73^{ABab}	0.61^{ABb}	0.48^{Babc}	0.59^{Bbcd}	0.45^{Babc}	0.63
8	0.90^{Abc}	0.76^{ABCab}	0.56^{BCb}	0.48^{Cabc}	0.83^{ABde}	0.48^{Cabc}	0.67
16	$0.77A^{b}$	0.70^{Aab}	0.55^{Ab}	0.66^{Abcd}	0.80^{Acde}	0.57^{Aabc}	0.67
32	1.00^{Abc}	0.74^{ABab}	0.61^{Bb}	0.86^{ABd}	0.93^{ABe}	0.78^{ABc}	0.82
64	1.17^{Ac}	0.82^{BCb}	0.64^{Cb}	0.79^{Ccd}	1.12^{ABe}	0.70^{Cbc}	0.87
Mean	0.75	0.67	0.53	0.53	0.61	0.48	

^{ABC}Species and stage means within time (rows) with unlike superscripts differ (P<.05).

^{abcde}Time means within species and stage (columns) with unlike superscripts differ (P<.05).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .077.

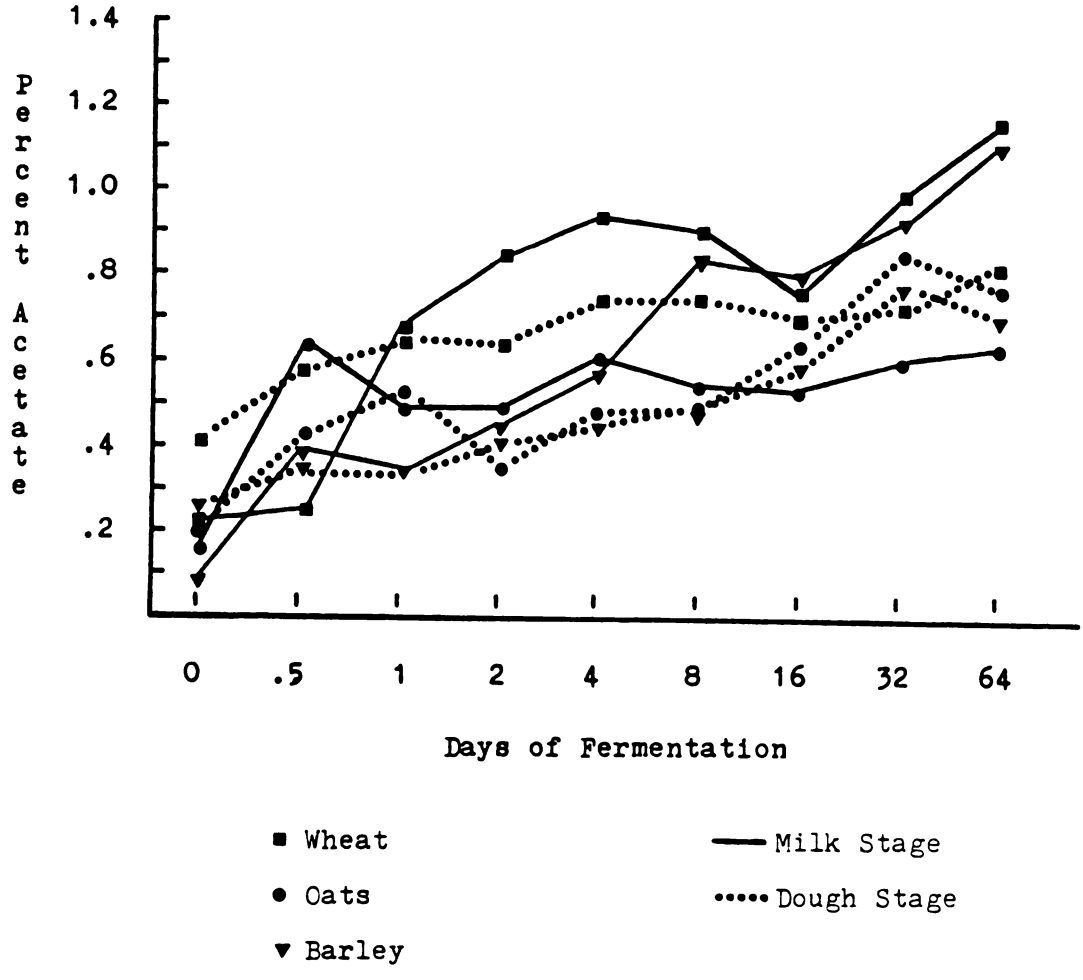

Figure 4. Changes in Acetic Acid (% of DM) in Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

dough stage silages. During fermentation, acetate increased more rapidly in milk stage silages than in their dough stage complements ($P<.05$). By the end of the fermentation period, milk stage wheat and barley silage had the greatest concentration of acetate ($P<.05$) while no differences were observed between the other combinations. Overall means across time show higher average acetate concentrations for milk stage silages in wheat and barley but equal concentrations between these two stages in oats.

Initial water soluble carbohydrate (WSC) concentrations were different ($P<.05$) between milk and dough stages in all cereal grains under investigation (Table 6 and Figure 5). Losses of 39%, 43%, and 54% of the initial WSC were observed with maturation from the milk to the dough stage in wheat, oats, and barley, respectively. Wheat had higher WSC concentrations than oats and barley within both growth stages ($P<.05$) and this relationship persisted through the end of the fermentation period. Figure 5 indicates the relationship between oats and barley within each growth stage was very similar. Dough stage wheat silage responded just as the other dough stage silages except was consistently higher in WSC concentrations ($P<.05$). Excluding wheat, the disappearance of WSC was significant ($P<.05$) throughout fermentation in milk stage silages whereas in dough stage silages extensive WSC utilization occurred only during the first four days of ensiling. WSC concentrations on Day 64 were not different

Table 6. Influence of Stage of Maturity and Time of Ensiling on Water Soluble Carbohydrates as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	18.9^{Aa}	11.5^{Ba}	13.9^{Ba}	7.9^{Ca}	12.5^{Ba}	5.8^{Ca}	11.7
.5	18.7^{Aa}	9.1^{Bab}	10.4^{Bb}	6.1^{CDab}	8.2^{BCb}	5.3^{Dab}	9.6
1	12.1^{Ab}	8.6^{Babc}	7.3^{Bbc}	3.7^{Dbc}	6.6^{BCbc}	4.1^{CDabc}	7.1
2	11.2^{Ab}	7.4^{Bbcd}	6.9^{Bc}	2.1^{Cc}	6.2^{Bbcd}	2.6^{Cbc}	6.1
4	11.8^{Ab}	5.8^{Bcd}	7.4^{Bbc}	2.4^{CDc}	5.0^{BCcde}	2.2^{Dc}	5.8
8	12.1^{Ab}	6.0^{Bbcd}	5.3^{Bcd}	1.6^{Cc}	3.7^{BCcdef}	2.0^{Cc}	5.1
16	11.6^{Ab}	5.9^{Bcd}	4.6^{BCcde}	1.4^{Dc}	2.9^{CDef}	1.4^{Dc}	4.6
32	11.9^{Ab}	5.5^{Bd}	3.8^{BCde}	1.7^{Cc}	3.3^{BCdef}	1.2^{Cd}	4.6
64	11.4^{Ab}	5.1^{Bd}	1.7^{Ce}	0.9^{Cc}	1.6^{Cf}	1.3^{Cc}	3.7
Mean	13.3	7.2	6.8	3.1	5.6	2.9	

^{ABCD}Species and stage means within time (rows) with unlike superscripts differ (P<.05).

^{abcdef}Time means within species and stage (columns) with unlike superscripts differ (P<.05).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .665.

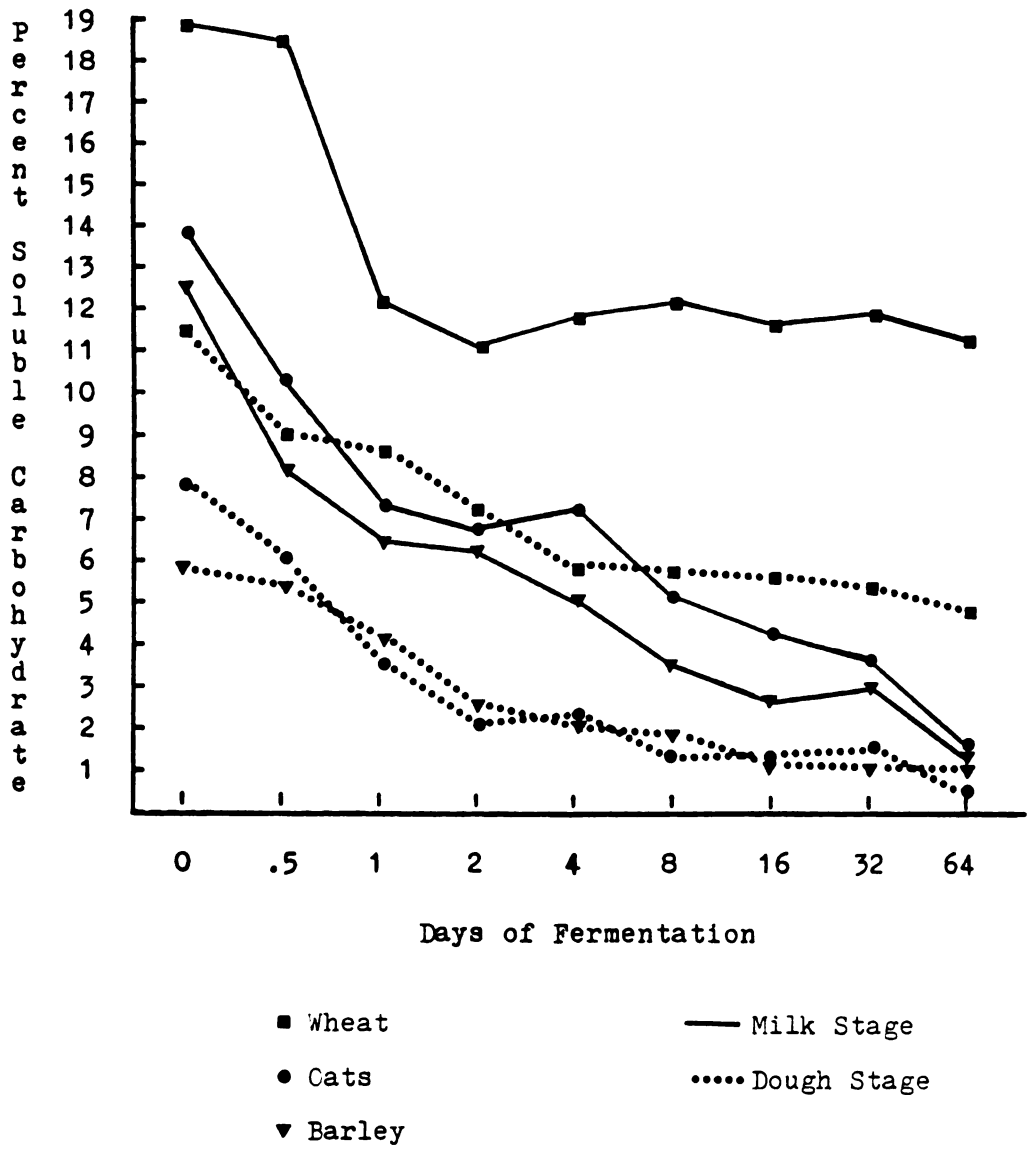

Figure 5. Changes in Water Soluble Carbohydrates (% of DM) in Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

between growth stages in oats and barley. This observation coupled with higher initial concentrations in the milk stage resulted in greater losses of WSC during fermentation in milk stage silages. Milk stage wheat silage responded unlike other silages exhibiting WSC disappearance only between Day .5 and Day 1 ($P<.05$).

Water soluble non-protein nitrogen (WSN) means are presented in Table 7 and displayed graphically in Figure 6. Interactions were found to be nonsignificant ($P>.07$) and permitted the comparison of both treatment and time means. Within species, average WSN was higher in milk stage silages than in dough stage silages ($P<.05$). The comparison of time means show a two-fold increase of WSN within Day 1, no changes between Days 2 and 16, followed by a slight increase thereafter ($P<.05$). Individually there were no differences between specie and stage combinations in the fresh herbage. Compared with their dough stage complements, WSN increased more rapidly ($P<.05$) in oat and barley milk stage silages but concentrations had equilibrated by the sixteenth day of fermentation. No differences were observed between the two growth stages in wheat at all times sampled. Final WSN concentrations were higher for all milk stage silages on Day 64 but the differences were not significant.

Comparison of treatment by time combinations shows few significant differences in ammonia nitrogen between specie and stage combinations within time (Table 8). Differences

Table 7. Influence of Stage of Maturity and Time of Ensiling on Water Soluble Nitrogen as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

Day	Wheat		Oats		Barley		Mean[2]
	Milk	Dough	Milk	Dough	Milk	Dough	
0	27.6^{Aa}	21.2^{Aa}	18.2^{Aa}	21.8^{Aa}	28.2^{Aa}	20.8^{Aa}	23.0^{a}
.5	41.1^{ABb}	36.8^{ABb}	45.8^{ACbc}	30.9^{Bab}	53.5^{Cb}	30.7^{Bab}	39.8^{b}
1	50.8^{ABbc}	44.5^{Abc}	45.0^{ABb}	42.1^{Abc}	56.9^{Bb}	42.6^{Bbc}	47.0^{c}
2	52.7^{ABCbc}	48.9^{ABbcd}	55.1^{ACbcd}	41.6^{Bbc}	62.4^{Cb}	42.6^{Bbc}	50.5^{cd}
4	60.1^{ABcd}	49.2^{BCbcde}	62.0^{Bd}	40.8^{Bbc}	65.4^{Ab}	47.7^{Cc}	54.2^{d}
8	56.6^{ABcd}	49.5^{Bbcde}	58.6^{ABcd}	46.5^{Bcd}	63.0^{Ab}	48.7^{Bc}	53.8^{d}
16	61.7^{Acd}	56.2^{Acde}	54.1^{Abcd}	52.7^{Acd}	58.3^{Ab}	50.1^{Ac}	55.5^{de}
32	68.6^{Ad}	59.9^{ABde}	65.7^{ABd}	55.6^{Bd}	64.6^{ABb}	55.4^{Bc}	61.6^{f}
64	66.0^{Ad}	62.2^{ABe}	61.0^{ABd}	51.5^{Bcd}	63.1^{ABb}	55.4^{ABc}	59.9^{ef}
Mean[3]	53.9^{AB}	47.6^{C}	51.7^{B}	42.6^{D}	57.3^{A}	43.8^{CD}	

^{ABCD}Species and stage means within time (rows) with unlike superscripts differ ($P<.05$).

^{abcdef}Time means within species and stage (columns) with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of 2.86.

[2]Nonsignificant interaction allowed mean separation with a S.E.M. of 1.17.

[3]Nonsignificant interaction allowed mean separation with a S.E.M. of .954.

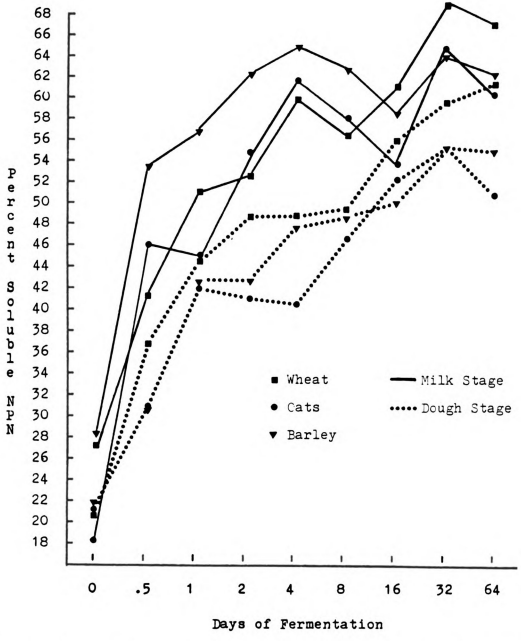

Figure 6. Changes in Water Soluble Nitrogen (% of TN) in Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

(P<.05) were noted only within Days 1, 4, and 64 due primarily to excessive ammonia production in milk stage wheat silage. Examination of Figure 7 points out the close relationship of individual combinations as fermentation proceeded. Insignificant interactions (P>.1) between specie and stage combinations and time allowed general comparisons of aggregate means. Significant increases (P<.05) were noticed after .5, 1, and 4 days of fermentation and not again until Day 64, resulting in a four-fold accretion of ammonia nitrogen. Across time, a significant difference (P<.05) between milk and dough stages was observed only within wheat where higher ammonia concentrations were associated with milk stage wheat silage. Oat silages generally contained the lowest percentage of ammonia nitrogen (P<.05), followed by barley and then wheat.

Changes in acid detergent insoluble nitrogen (ADIN) for each specie and stage combination are depicted in Table 9 and can be visualized in Figure 8. Interactions were not significant (P>.10) which enabled direct comparisons of treatment and time means. Although there was a difference between initial and final means averaged across all silages (P<.05), differences among time means were not consistent and visual interpretation of Figure 8 shows a general straight line trend across time. Averaged over time, dough stage silages contained larger amounts of ADIN than milk stages silages in all three small grains (P<.05). The

Table 8. Influence of Stage of Maturity and Time of Ensiling on Ammonia Nitrogen as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean[2]
0	3.59^{Aa}	3.07^{Aa}	2.76^{Aa}	2.30^{Aa}	3.68^{Aa}	1.98^{Aa}	2.90^{a}
.5	5.93^{Aab}	5.79^{Aab}	3.86^{Aa}	5.40^{Aab}	6.54^{Aab}	4.86^{Aab}	5.39^{b}
1	9.96^{Acd}	7.27^{ABbc}	4.76^{Bab}	5.77^{Bab}	6.42^{Bab}	7.48^{ABbc}	6.94^{c}
2	8.93^{Abc}	8.17^{Abc}	7.75^{Abc}	7.74^{Abcd}	8.30^{Abc}	7.60^{Abc}	8.08^{cd}
4	11.14^{Acd}	8.87^{ABbc}	7.59^{Bbc}	7.14^{Bbc}	11.20^{Acd}	8.84^{ABc}	9.13^{de}
8	10.43^{Acd}	9.61^{Acd}	7.97^{Abc}	8.90^{Abcd}	9.25^{Abcd}	8.92^{Ac}	9.18^{de}
16	11.15^{Acd}	10.01^{Acd}	8.84^{Ac}	8.49^{Abcd}	9.33^{Abcd}	9.23^{Ac}	9.51^{de}
32	12.53^{Ad}	10.22^{Acd}	9.46^{Ac}	9.37^{Acd}	11.11^{Acd}	10.18^{Ac}	10.48^{ef}
64	13.18^{Ad}	13.05^{Ad}	9.71^{Bc}	11.21^{ABd}	12.20^{ABd}	10.18^{ABc}	11.59^{f}
Mean[3]	9.65^{A}	8.45^{B}	6.97^{C}	7.37^{C}	8.67^{AB}	7.70^{BC}	

^{ABC}Species and stage means within time (rows) with unlike superscripts differ (P<.05).

^{abcdef}Time means within species and stage (columns) with unlike superscripts differ (P<.05).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .765.

[2]Nonsignificant interaction allowed mean separation with a S.E.M. of .312.

[3]Nonsignificant interaction allowed mean separation with a S.E.M. of .255.

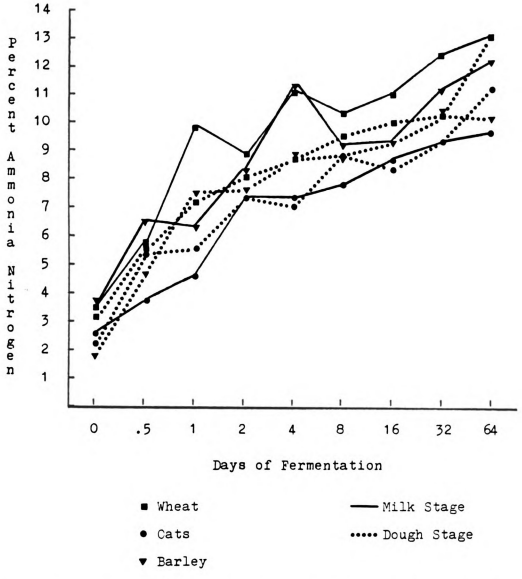

Figure 7. Changes in Ammonia Nitrogen (% of TN) in Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

Table 9. Influence of Stage of Maturity and Time of Ensiling on Acid Detergent Insoluble Nitrogen as a Percent of Dry Matter in Wheat, Oats, and Barley.[1]

Day	Wheat		Oats		Barley		Mean[2]
	Milk	Dough	Milk	Dough	Milk	Dough	
0	3.96^{Aa}	4.92^{Aa}	3.61^{Aa}	4.97^{Aa}	3.55^{Aa}	7.22^{Ba}	4.70^{a}
.5	5.40^{ABab}	5.64^{ABab}	4.30^{Aa}	5.38^{ABa}	4.60^{Aa}	6.62^{Ba}	5.32^{ab}
1	5.74^{ABb}	6.77^{Ab}	4.11^{Ca}	5.73^{ABa}	4.28^{BCa}	7.29^{Aa}	5.65^{b}
2	5.31^{ABab}	6.35^{BCab}	4.63^{Aa}	5.36^{ABa}	4.63^{Aa}	7.34^{Ca}	5.60^{b}
4	5.34^{ABab}	6.64^{BCab}	4.11^{Aa}	4.95^{Aa}	4.22^{Aa}	7.18^{Ca}	5.41^{ab}
8	5.43^{ABab}	5.58^{ABab}	4.16^{Ba}	5.15^{Ba}	4.67^{Ba}	6.85^{Aa}	5.30^{ab}
16	5.12^{ABCab}	6.12^{ABab}	4.43^{Ca}	5.14^{ABCa}	4.56^{BCa}	6.63^{Aa}	5.33^{ab}
32	6.16^{ABb}	6.62^{Aab}	4.32^{Ca}	4.97^{BCa}	4.61^{BCa}	7.56^{Aa}	5.70^{b}
64	6.03^{ABb}	6.42^{ABab}	4.28^{Ca}	5.81^{ABCa}	4.91^{BCa}	7.10^{Aa}	5.76^{b}
Mean[3]	5.39^{A}	6.11^{B}	4.21^{C}	5.27^{A}	4.45^{C}	7.08^{D}	

^{ABCD}Species and stage means within time (rows) with unlike superscripts differ ($P<.05$).

^{ab}Time means within species and stage (columns) with unlike superscripts differ ($P<.05$).

[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .373.

[2]Nonsignificant interaction allowed mean separation with a S.E.M. of .152.

[3]Nonsignificant interaction allowed mean separation with a S.E.M. of .124.

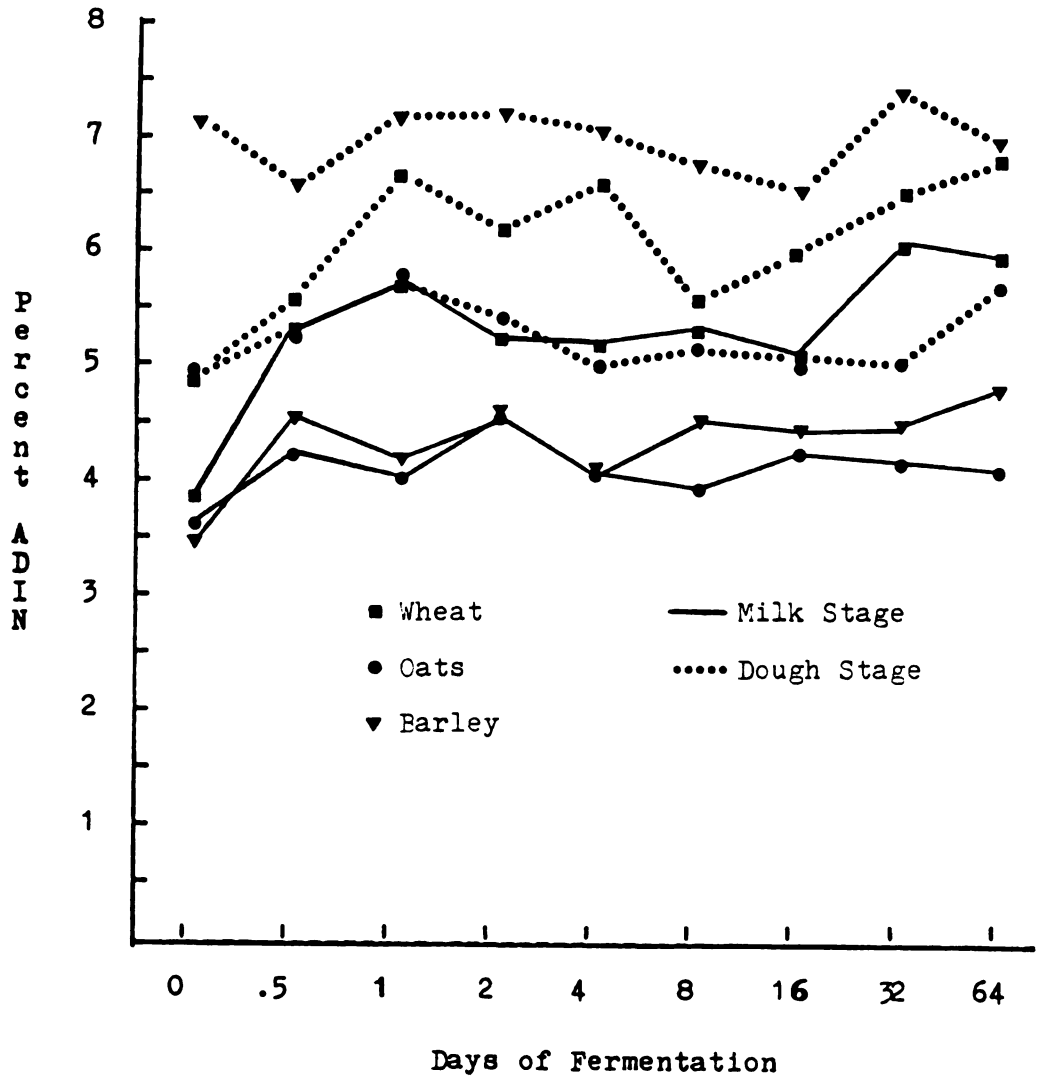

Figure 8. Changes in Acid Detergent Insoluble Nitrogen (% TN) in Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.

largest increase associated with maturation occurred in barley as dough stage barley had the highest initial concentration of ADIN ($P<.05$), nearly 60 %more than in the milk stage. Oats and barley in the milk stage produced silages with the lowest amount of ADIN ($P<.05$). Reviewing individual combination means reveals lower values prior to ensiling although not always significant.

The means for neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose are reported in Table 10. Only initial and final observations are included in this table since the effects of time within specie and stage combinations were inconsistent and often misleading: Complete tables can be found in the appendix. Overall time effects were not significant ($P>.09$) in the analysis of NDF means. Only milk stage wheat silage had different NDF concentrations ($P<.05$) by the end of fermentation. Initially, as maturation proceeded from the milk to dough stage, NDF increased, decreased, and remained the same in wheat, oats, and barley, respectively ($P<.05$). The drop in NDF in barley was consistent but not significant ($P>.05$). NDF concentrations were higher in milk stage oats ($P<.05$) and lower in milk stage wheat, while the other combinations centered around 50% NDF. Interactions in ADF analysis were not significant ($P>.09$) and differences between overall specie by stage means were similar to NDF as anticipated. ADF percentages increased with wheat maturation ($P<.05$) and remained the same in barley. During fermentation,

increased ADF concentrations were observed in three of six forage combinations resulting in a significant ($P<.05$) overall increase of 8.5% over the 64 days of fermentation. As in NDF, higher ADF concentrations were found in milk stage oats ($P<.05$) with the lowest concentration in milk stage wheat. Hemicellulose decreased in all forage combinations during fermentation but significance ($P<.05$) could only be attributed to the difference in milk stage barley silage. Decreases in hemicellulose were associated with the growth stage advancement within oats and barley ($P<.05$) but a nonsignificant increase was observed within wheat. In general, fiber fractions tended to increase as wheat progressed from the milk to dough stage whereas they decreased in oats and barley during the same period. Fermentation had very little affect on these fractions over the 64 days under observation.

Table 10. Influence of Stage of Maturity and Time of Ensiling on Neutral Detergent Fiber, Acid Detergent Fiber, and Hemicellulose in Wheat, Oats, and Barley.

Day	Wheat		Oats		Barley		Mean[5]
	Milk	Dough	Milk	Dough	Milk	Dough	
Neutral Detergent Fiber (% DM)[1]							
0	45.6^{Aa}	49.8^{BCa}	55.7^{Da}	46.6^{ABa}	51.3^{Ca}	48.1^{ABCa}	49.5
64	50.0^{ABb}	51.8^{ACa}	54.7^{Ca}	48.1^{ABa}	49.7^{ABa}	47.2^{Ba}	50.3
Mean[4]	47.6	52.0	53.8	49.2	49.5	48.1	
Acid Detergent Fiber (% DM)[2]							
0	29.7^{Aa}	32.2^{ABa}	37.8^{Ca}	32.6^{ABa}	32.2^{ABa}	32.8^{Ba}	32.9^{a}
64	34.5^{Ab}	35.6^{Ab}	38.6^{Ba}	36.7^{ABb}	35.3^{Aa}	33.9^{Aa}	35.7^{b}
Mean[4,5]	32.6^{A}	35.7^{B}	38.5^{C}	35.7^{B}	33.9^{D}	33.2^{AD}	
Hemicellulose (% DM)[3]							
0	15.9^{ABa}	17.6^{BCa}	17.8^{BCa}	14.0^{Aa}	19.1^{Ba}	15.3^{ACa}	16.6
64	15.6^{Aa}	16.2^{Aa}	16.1^{Aa}	11.4^{Ba}	14.4^{ABb}	13.3^{ABa}	14.5
Mean[4]	14.9	16.3	15.3	13.5	15.6	14.9	

^{ABCD}Species and stage means within time (rows) with unlike superscripts differ (P<.05).
^{ab}Time means within species and stage (columns) with unlike superscripts differ (P<.05).
[1]Tabular entries represent averages of two experimental silos and have a S.E.M. of .899.
[2]Tabular entries represent averages of two experimental silos and have a S.E.M. of .715.
[3]Tabular entries represent averages of two experimental silos and have a S.E.M. of .826.
[4]Means represent averages of eighteen experimental silos sampled across all days.
[5]Nonsignificant interaction allowed mean separation with a S.E.M. of .238 for individual species and stage means and a S.E.M. of .292 for individual time means.

5.0 DISCUSSION

All silages evaluated in this study were of excellent quality according to Flieg scores as modified by Zimmer (1966). With a score of 100 being ideal, values of 98, 100, 100, 100, 100, and 100 were calculated for milk and dough stage wheat, milk and dough stage oats, and milk and dough stage barley silages, respectively. Mason jars equipped with modified lids and the packing scheme employed provided excellent conditions, assuring minimal effects of oxygen inclusion and aerobic microbial activity. Evidence acquired from the fermentation parameters observed suggests small grains harvested at the milk stage undergo a higher degree of both fermentation and nutrient destruction than when harvested at the dough stage. Furthermore, values obtained for the more mature stages in this study are similar to corn silage data alleviating concern over the substitution of these silages for one another in feeding regimes.

Figures 9 thru 14 represent the major fermentation parameters compiled for individual specie and stage combinations. Comparing Figure 9, milk stage wheat, with the others reveals a delayed onset of fermentation in milk stage wheat silage. First cutting wheat (milk stage) was

chopped by hand with a paper cutter, decreasing the extent of laceration and microbial inoculations associated with farm equipment used for the remaining harvests. Diminished release of plant solubles and lower initial bacterial counts may have resulted in an additive effect to postpone bacterial growth and subsequent acid production. Gibson et al. (1961) studied microbial reproduction over numerous degrees of laceration and found a positive correlation between bacterial populations and degree of chopping. Stirling and Whittenbury (1963) followed the changes in bacteria numbers from the field to the silo and found significant numbers of lactic acid bacteria at the silo on herbage lacking these bacteria in the field. They concluded that inoculation must have occurred through the chopper during harvest. Consequently, comparisons between fermentation rates involving milk stage wheat must be done with discretion since differences may be due, in part, to harvesting methods.

Ashbell et al. (1985), working with wheat, reported dry matter yields of 4.9 tons/acre and 5.3 tons/acre for milk and dough stages, respectively. The yield from oats and barley are generally lower than wheat at similar stages of growth, averaging 4 tons/acre as reported by Miller et al. (1967) and Bishnoi et al. (1978). These investigators found little differences in dry matter yields between milk and dough stages with the latter averaging slightly higher yields. Results from this trial show wheat yielding nearly

twice the dry matter as did oats and barley however, all yields were below average and the production of oats and barley was extremely poor when compared to past observations (Miller et al., 1967; Bolsen and Berger, 1976; and Bishnoi et al., 1978). Since yield was not the objective of this study, plots set aside for production were small and their maintenance was unlike those for larger scale production studies. Although production was not comparable to yields observed by other investigators, average increases due to maturity were noted, complying with results of Bishnoi et al. (1978).

Literature on buffering capacities for cereal grain crops is largely limited to corn. Reported as milliequivalents (ME)/100g DM, Wilkinson (1978), as cited by McDonald (1981), observed buffering capacities of 22.5, 18.0, and 14.9 for whole crop corn progressing through the dough, dent, and glaze stages of maturity, respectively. MacGregor and Edwards (1968) reported buffering capacities for barley silages of various maturation stages but these values are irrelevant since these materials had already been ensiled. In the same study, initial organic acids comprising the majority of buffering constituents in fresh herbage accounted for 6.6% of the dry matter in the head stage and continually decreased to 1.1% of the dry matter in the dough stage, implying an inverse relationship between maturity and buffering capacity. In the present experiment, the buffering capacities were similar to values

obtained for corn, averaging 22 ME/100g DM and decreased with growth stage advancement in all three species under investigation. Although wheat was harvested a week late due to weather conditions, this could not account for all the difference observed between wheat, with the lowest capacities, and the other two small grain crops. Depressed buffering capacities have been associated with ease of ensiling observed in grasses and corn when compared to legumes (Playne and McDonald, 1966). Buffering capacity would rarely be a concern when ensiling these small grain crops during later stages of maturity, especially in the dough stage and wheat in particular.

Compositional changes in plants during maturation have been studied in great detail and similar trends during maturation are observed in all crop species (Heath et al., 1973). In a preliminary study by Edwards et al. (1968), the chemical composition of whole-crop barley was analyzed at seven successive stages of growth. During development, dry matter increased steadily, ash decreased until the dough stage, crude protein decreased through the milk stage and increased with seed development and crude fiber increased initially but decreased through the later stages of maturity. Water soluble carbohydrates peaked in the milk stage, decreasing to their lowest levels at the ripe stage. Ashbell et al. (1985) assessed dry matters of wheat in the milk and dough stages of 35% and 43%, respectively. These values are in agreement with those of Bolsen and

Berger (1976) who also reported lower values of 28% and 35% for oats and barley in milk and dough stages, respectively. Oltjen and Bolsen (1978) concluded wheat reached comparable stages of maturity at a higher dry matter percentage than other small grain crops. Dry matters in this experiment are well within the range found for the milk and dough stage by other investigations. The higher dry matters observed for wheat are due in part to the delayed harvest, however, all cereal crops ensiled in this study were assumed to be at comparable growth stages.

Protein concentrations of cereal crops average between 5% and 12% of the dry matter, much lower than forages such as grasses and legumes which may contain up to 22% crude protein. Delaying the harvest of cereal crops until the milk or dough stage reduces crude protein content by as many as 10 percentage units, expanding the differences between forage and cereal crops (Bishnoi et al., 1978). Of wheat, oats, barley, and triticale, they found oats contained higher concentrations of protein at all growth stages investigated. Oltjen and Bolsen (1978) reported protein reductions of 40% as wheat and barley matured from the boot stage (15%) to the dough stage (9%) with the largest reduction occurring between flowering and milk stages. Both Edwards et al. (1968), working with barley and Ashbell et al. (1985) working with wheat concluded that reductions in protein content had plateaued by the milk stage with slight increases observed with seed development

in the dough stage. In contrast to the results of Bishnoi et al. (1978), oats had the lowest crude protein content in the present study (7.8%), compared to wheat (8.3%) and barley (8.5%). Protein concentrations in this experiment are typical for more mature cereal crops, including corn (Geasler, 1970). Slight decreases were noted as maturity progressed from milk to dough stages in oats and barley which might have resulted from poor soil maintenance in these small experimental plots. Wheat dry matter yields were far better than for either oats or barley, suggesting better soil conditions, and no protein reductions were observed in wheat. Although protein reductions were significant, proportionally they accounted for lesser losses compared to those occurring during earlier development.

Reports of crude fiber concentrations in maturing cereal crops are conflicting. Miller et al. (1967) found a direct relationship between maturity and crude fiber in barley. A year later studying eight varieties of barley, Edwards et al. (1968) found an inverse relationship with 32% crude fiber in the head stage and only 19.5% in the dough stage. They also found organic matter digestibilities had increased with maturity, in contrast to results of Miller et al. (1967), supporting their conclusion that seed development dilutes crude fiber percentages. Ashbell et al. (1985) found similar results between milk and dough stages in wheat as did Sutoh et al.

(1972) with oats; however increasing fiber contents were associated with earlier growth stage advancement. Assuming higher digestibilities result from lower fiber concentrations, results of Bolsen and Berger (1976), Bolsen et al. (1976), and Oltjen and Bolsen (1978) show a drop in crude fiber between the milk and dough stages in wheat, oats, and barley. In the present experiment, parallel changes in neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations reveal a similar downward trend in oats and barley. In wheat, fiber concentrations increased into the dough stage which may be the result of grain losses associated with harvesting a dryer cereal crop. Values of NDF, ADF, and hemicellulose obtained in this study were similar across species and comparable to those found in corn (Ensminger and Olentine, 1978).

Waite (1957) believed the concentration of water soluble carbohydrates (WSC) was largely dependent on the rate of protein synthesis; higher rates of protein synthesis would indirectly utilize more of these readily available energy containing compounds, diminishing existent WSC concentrations. This relationship exists in cereal crops as increases in WSC concentrations coincide with decreasing protein concentrations during early growth and visa-versa towards maturation as seed development involves both protein and starch synthesis (Edwards et al. 1968; Geasler, 1970; Sutoh et al., 1972; and Ashbell et al., 1985). In addition, results of MacGregor and Edwards

(1968) and Ashbell et al. (1985) show water soluble carbohydrates comprise between 20% and 30% of the dry matter in wheat and barley during the milk stage, decreasing by as much as 50% in the dough stage. Although WSC concentrations never reached 20% DM in this trial, significant reductions occurred between milk and dough stages as expected. Poor soil conditions may have caused the consistently depressed WSC concentrations, explaining the close relationship between dry matter yield and WSC content in this study. Wheat, which yielded twice the dry matter as oats and barley, also contained significantly higher amounts of soluble carbohydrates, the primary energy substrates during plant development.

Ash concentrations observed during this study were not affected by stage of maturity, agreeing with results of Edwards et al. (1968) for more mature growth stages. Concentrations of ash were higher than those of Edwards et al. (1968), but lower than those of Sutoh et al. (1972) at similar growth stages. In this study higher ash concentrations in oats and barley when compared to wheat are not surprising since WSC were also depressed in these two examples, and a greater percentage of the dry matter would have been assumed by the inorganic ions.

Fermentation patterns for individual grain and stage combinations can be visualized in Figures 9 thru 14. The final pH of silage is largely dependent on moisture content provided available carbohydrate is present (Barnett, 1954).

In low dry matter silages extensive fermentation must take place before a sufficiently low pH value is obtained to preserve the material (Wieringa, 1958). In the present study, milk stage silages had lower pH values in all three species under investigation. Steeper pH declines were also observed in milk stage oat and barley silages while in wheat, delayed acidification was most likely due to the method of chopping. All silages were well preserved as evidenced by low stable pH values attained during fermentation.

Lactic acid accumulation, the primary end-product of lactic acid bacterial metabolism, is mainly responsible for pH declines observed in adequately preserved silages (Whittenbury et al., 1967). This is well illustrated in the present data by noting the close inverse relationship between lactic acid content and pH in Figures 9 thru 14. Further observation of these figures reveals a more rapid rate of fermentation occurring in milk stage silages. The intersection between lactic acid content and pH took place 28 days earlier in milk stage oat and barley silage and 12 days earlier in milk stage wheat despite the delayed onset of fermentation in the latter silage. Edwards et al. (1968) and Ashbell et al. (1985) observed nearly twice the amount of lactic acid in milk stage silages suggesting a greater extent of fermentation in these less mature silages. Similar ratios were observed in this study in oats and barley, however total lactic acid concentrations

far exceeded those of previous researchers for identical growth stage silages. This is hard to explain since WSC concentrations were abnormally low for cereal crops. Lactic acid bacteria must have completely dominated the microbial population since propionate and butyrate, end-products of undesirable microbial activity, were absent from the majority of samples analyzed.

Provided sufficient fermentation by lactic acid bacteria, acetate production arising from enterobacteriaceae activity prior to acidification would be minimal (Langston and Conner, 1962). But, heterolactic bacteria produce acetate when fermenting fructose and excess acetate concentrations have been found in silages undergoing vigorous fermentation (Edwards and McDonald, 1978). Ashbell et al. (1985), when ensiling wheat, found greater concentrations of acetate in wetter silages that also had the highest concentrations of lactate. Geasler (1970) calculated a correlation coefficient of +.70 between lactate and acetate in corn silage implying higher acetate productions are a consequence of normal fermentation in low dry matter silages. Acetate production in this study responded in a similar fashion in wheat and barley resulting in higher acetate concentrations in the milk stage silages. Milk and dough stage silages made from oats contained similar concentrations of acetate throughout the ensiling period which may have been due to limited amounts of fructose available for fermentation. The low overall

levels of acetate found in these silages indicate efficient use of available carbohydrates by the developing lactic acid bacteria.

WSC concentration necessary for adequate preservation are influenced by dry matter content, buffering properties, initial respiration, and bacterial species dominating the fermentation (Woolford, 1984). The fermentation of cereal crops ensiled while in the milk stage utilize a greater percentage of the initial WSC than dough stage silages (MacGregor and Edwards, 1968; Geasler, 1970; Ashbell et al., 1985). This would be expected since larger amounts of volatile fatty acids were produced during the ensiling of milk stage crops. In the present study, milk stage silages lost more WSC through 64 days of fermentation than dough stage silages, presumably due to greater microbial activity. WSC utilization was similar within growth stage for the three species in this study except for milk stage wheat silage where utilization ceased after 1 day of fermentation even though lactic acid accumulated through the sixteenth day of fermentation (Figure 9). Either WSC concentrations were replenished through hydrolysis of storage and structural carbohydrates or the developing microflora preferred organic acids over carbohydrates as their energy source. The actual substrate source of lactic acid fermentation in milk stage wheat silage could not be assessed from the analyses performed in this study. The only specific carbohydrate fraction analyzed was

hemicellulose which was not utilized to any great extent during fermentation.

Bergen et al. (1974) concluded that enzymes endogenous to the ensiled plant material were mainly responsible for protein degradation to water soluble nitrogen (WSN) during ensiling. Enzymes being acid labile prompted investigators to test methods of hastening the reduction of pH to reduce protein degradation (Henderson et al., 1982). Geasler (1970) and Bergen et al. (1974) produced conclusive evidence that increasing dry matter content by wilting or delaying harvest would also substantially decrease WSN concentrations in resultant silages. Results obtained here suggest dry matter content is more influential than pH in reducing the activity of plant proteases. Although milk stage silages exhibited rapid pH declines and lower final pH's, protein conversion to WSN was faster and more extensive in these silages (Figures 9 thru 14). Dough stage silages reached a pH considered inhibitory to protein degradation 12 days later than milk stage silages yet lower WSN concentrations were associated with silages made from the dryer, more mature cereal crops. Lack of moisture may shield proteins from enzymatic degradations explaining how silages exhibiting longer periods of protease activity have less protein degradation. Within growth stage, differences between species were negligible, and WSN concentrations were comparable to concentrations found in corn silage at similar growth stages and dry matters (Geasler, 1970).

Ammonia concentrations obtained by the Technicon Auto Analyzer may be overestimated as evidenced by extreme concentrations found in the fresh cereal samples. Micro-organisms on dead and decaying leaves produce the majority of ammonia in fresh plants, and elevated amounts of ammonia are expected in older plants that have more perishing leaves (Brady, 1960). Even in advanced stages of maturity, the quantity of ammonia nitrogen is usually less than 1.5% of the total nitrogen (Brady, 1960; Bergen et al., 1974). Initial values greater than 2.5% in this study may have been caused by background color in the homogenate which was not subjected to digestion prior to analysis. Relating these concentrations to those obtained through other methods would not be legitimate.

As proteolytic clostridia are assumed to be the major source of ammonia nitrogen in silage, it is surprising that significant amounts of ammonia were found despite evidence against the presence of clostridia in these silages. It is known, however, that some ammonia can come from other sources such as the reduction of nitrates and nitrites, and the action of plant enzymes and Enterobacteriaceae (Brady, 1960; Seale et al., 1986). In accordance, observations here show the rate of ammonia production was greatest during the first four days of ensiling, but gradual increases in ammonia-N concentrations were noted thereafter. This secondary accumulation of ammonia may arise from lactic acid bacteria which are capable of amino

acid fermentation (Brady, 1966). There were no conclusive differences in ammonia production between milk and dough stage silages which agreed with findings of Sutoh et al. (1972) and Ashbell et al. (1985). Lower ammonia concentrations observed in oat silages are of minor practical importance since all silages in this trial contained ammonia concentrations typical of quality silages.

Rates of acid detergent insoluble nitrogen (ADIN) accumulation were negligible and not different between specie or stage combinations investigated here. Slight increases in ADIN concentrations were noted in initial stages of fermentation, undoubtedly the result of incubation at 30^0C for three days. Maillard reactions responsible for ADIN production during ensiling are nonenzymatic, spontaneous and occur to some degree in well made unheated silages (Yu and Veira, 1977). Maturation of the three cereal species in this study increased the initial content of ADIN which persisted throughout fermentation. Both Thomas et al. (1982) and Yu and Veira (1977) consistently found highly negative correlations between nitrogen digestibilities and ADIN. The lower nitrogen digestibilities assumed in these dough stage cereal silages do not agree with results of Bolsen and Berger (1976) who reported higher nitrogen digestibilities in lambs fed silages made from dough stage cereal crops. ADIN was not assessed by Bolsen and Berger (1978) and,

contrary to the present study, silages made from early cut crops generally produce more ADIN due to higher levels of moisture at earlier growth stages (Thomas et al., 1982).

Dry matter losses could not be assessed with any appreciable accuracy since differences were rarely significant throughout fermentation. Losses greater than 7% would have shown significance but taking into account that lactic acid bacteria dominated the microflora, losses would rarely exceed 7% in such cases (McDonald et al., 1973).

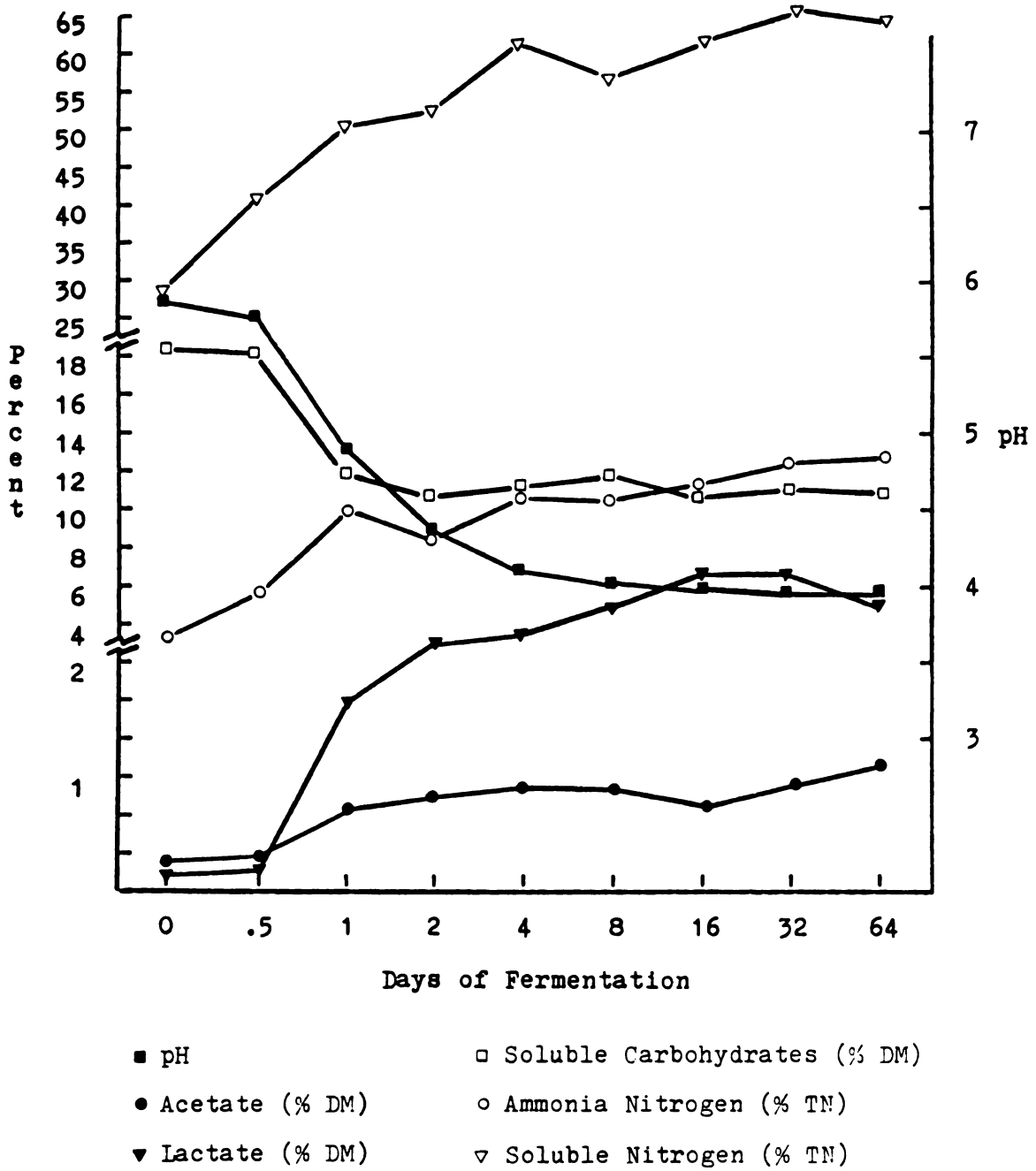

Figure 9. Changes of Major Fermentation Parameters During Ensiling of Milk Stage Wheat.

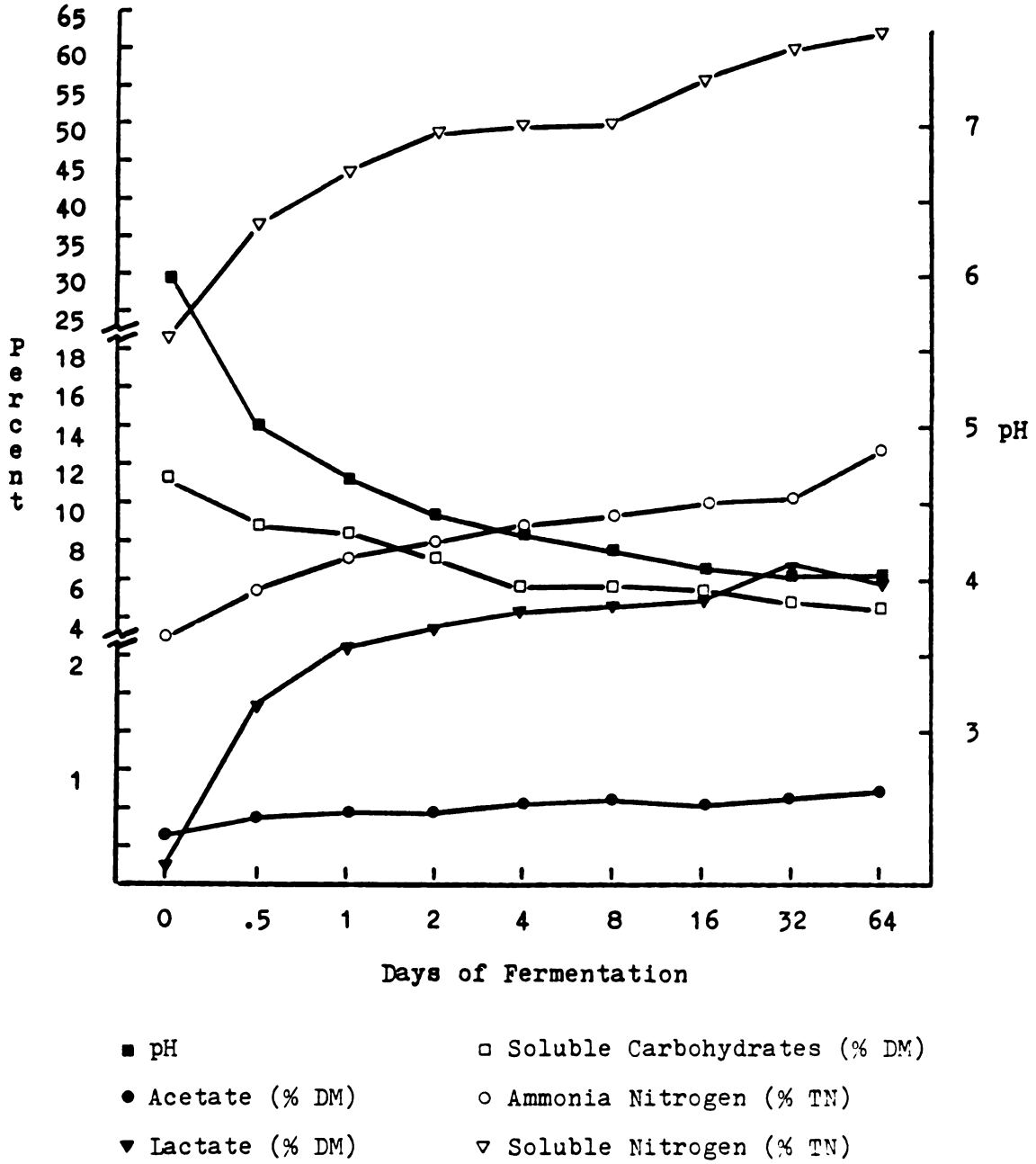

Figure 10. Changes of Major Fermentation Parameters During Ensiling of Dough Stage Wheat.

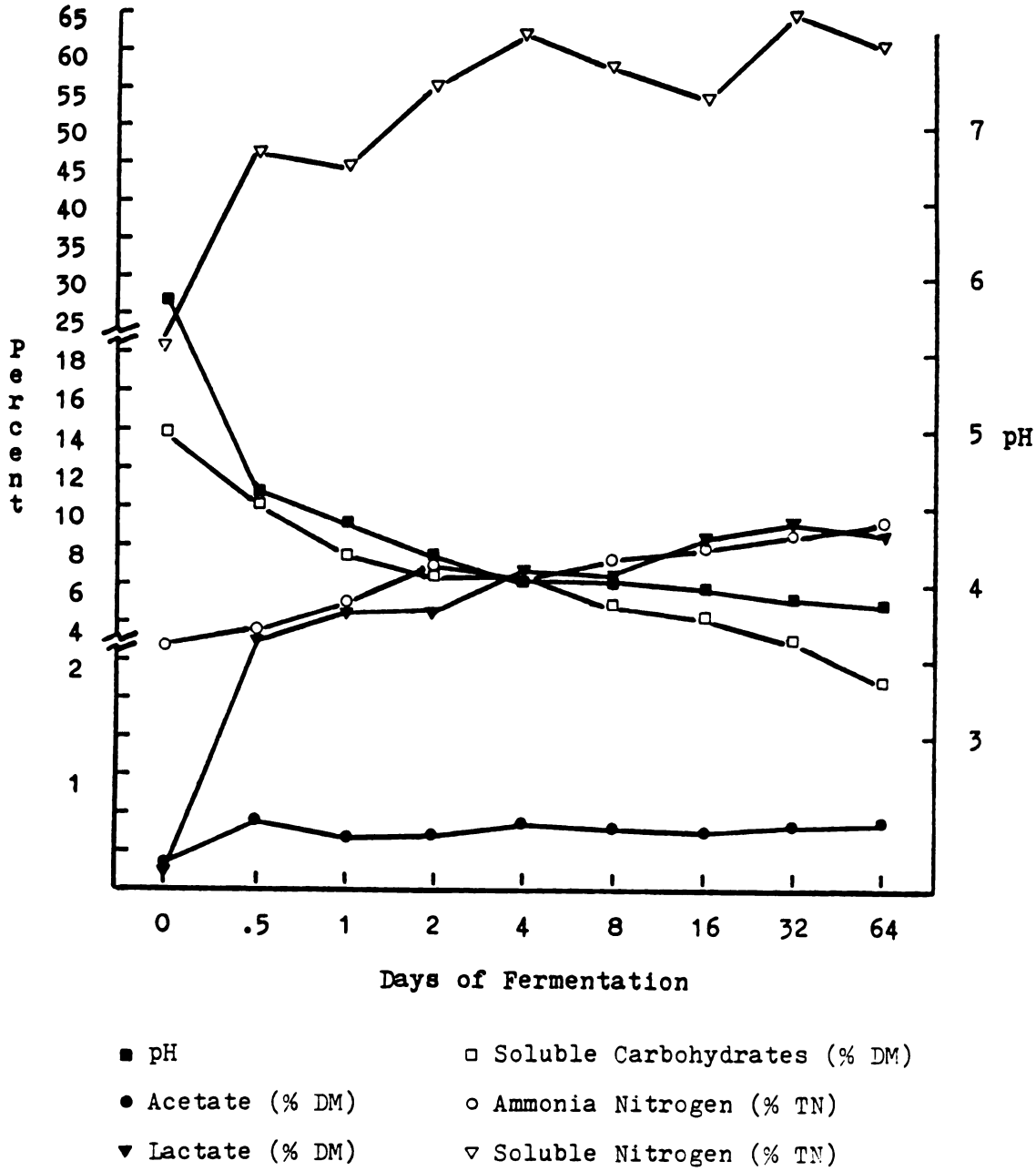

Figure 11. Changes of Major Fermentation Parameters During Ensiling of Milk Stage Oats.

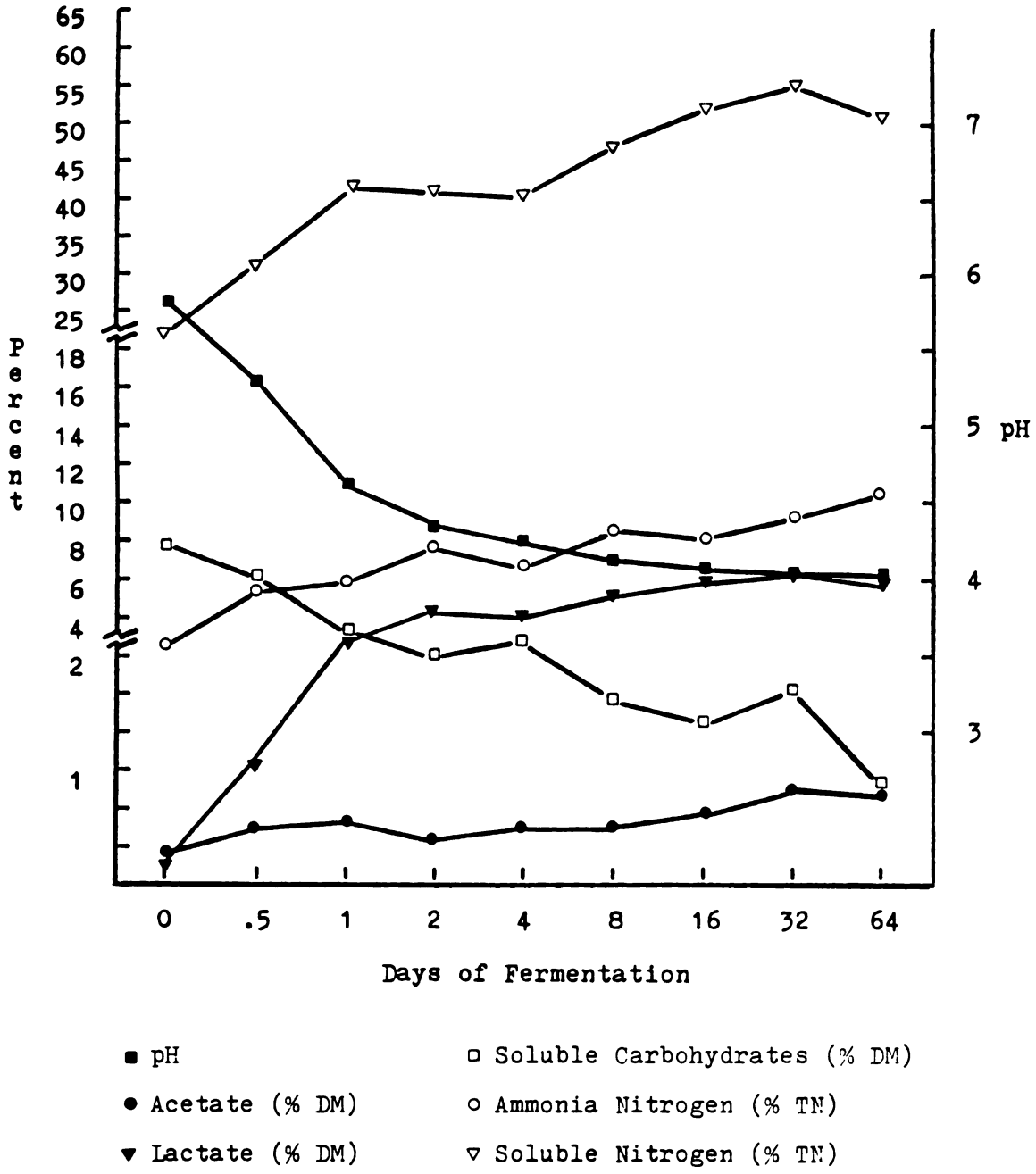

Figure 12. Changes of Major Fermentation Parameters During Ensiling of Dough Stage Oats.

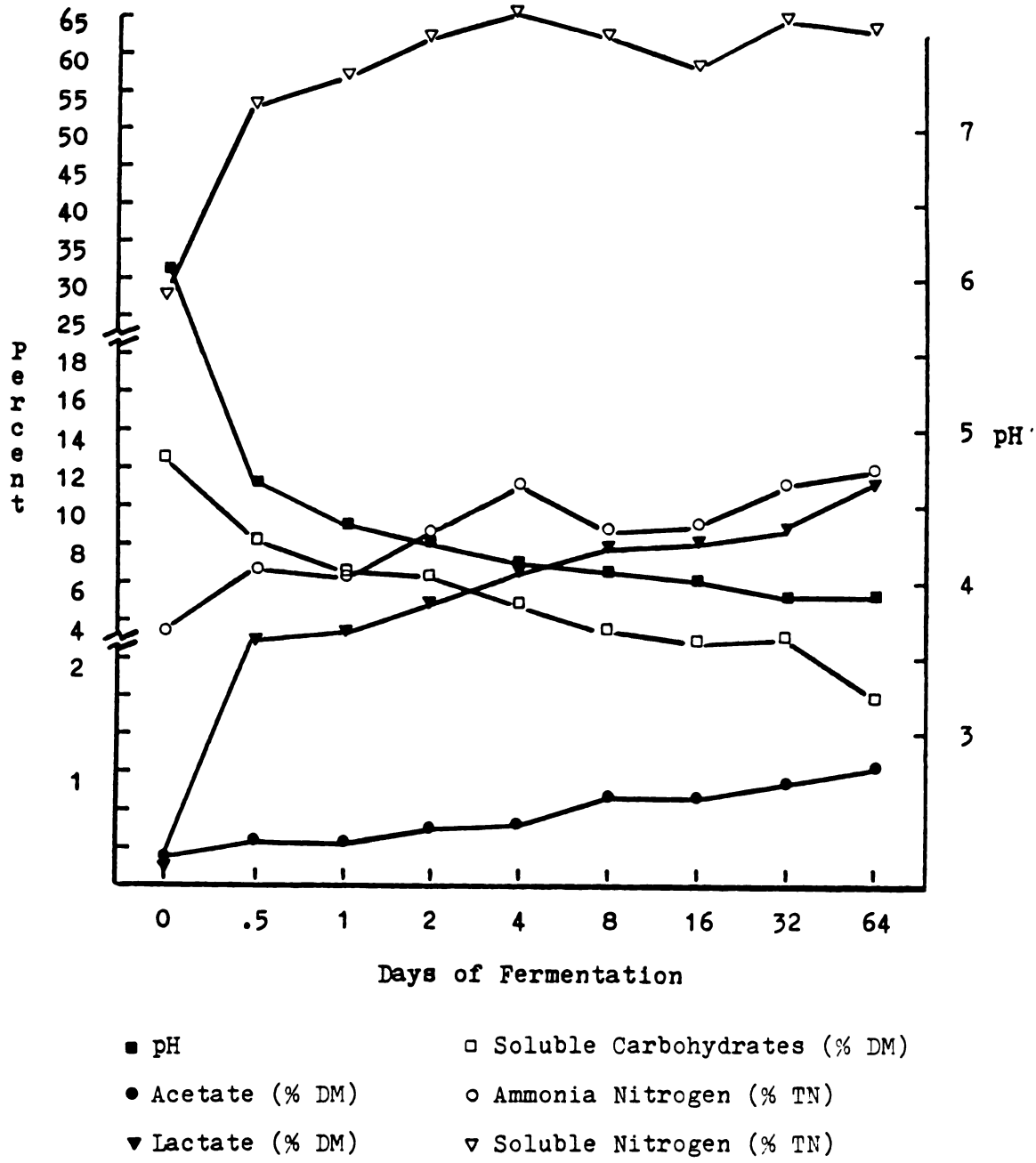

Figure 13. Changes of Major Fermentation Parameters During Ensiling of Milk Stage Barley.

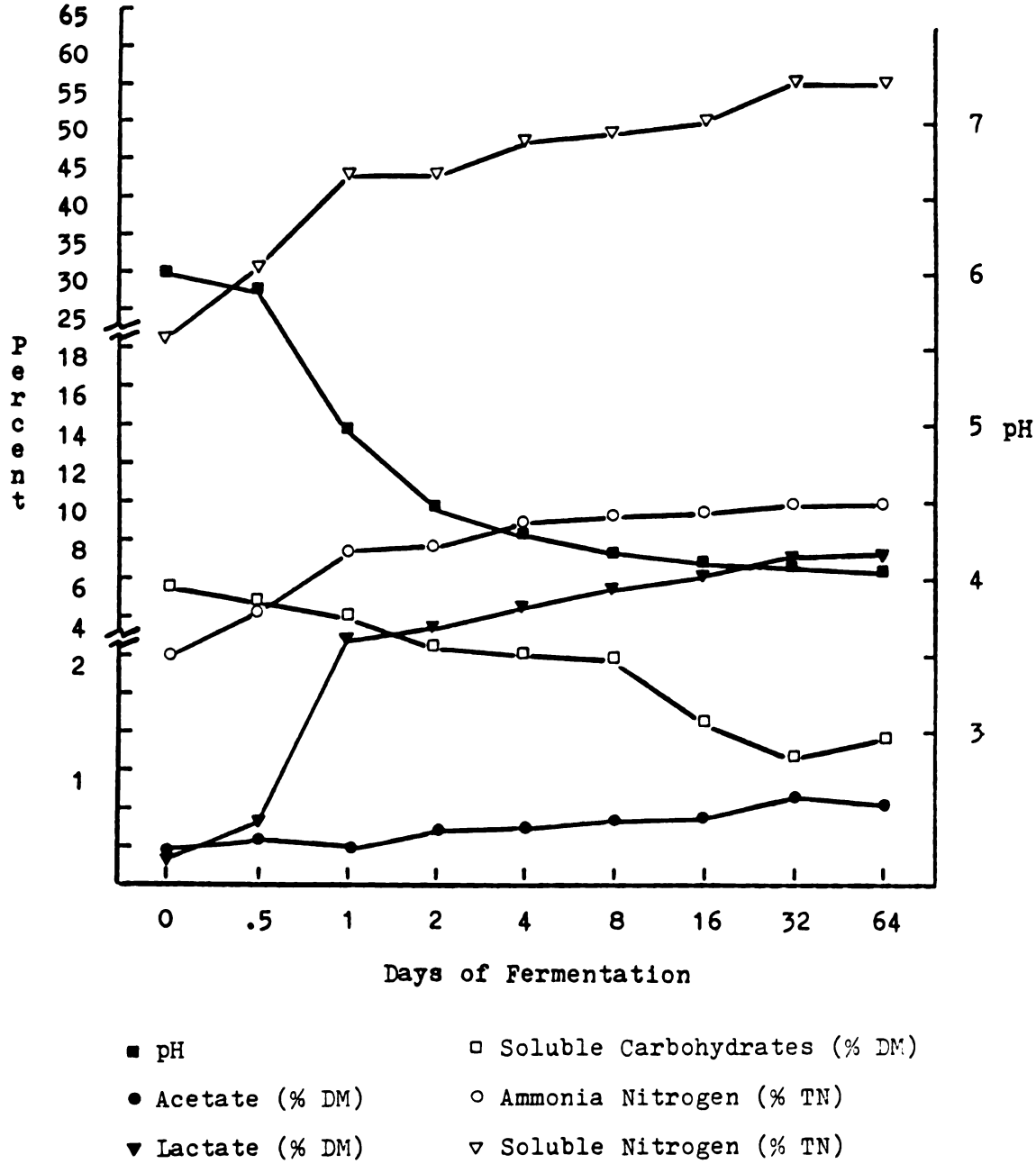

Figure 14. Changes of Major Fermentation Parameters During Ensiling of Dough Stage Barley.

6.0 SUMMARY

Whole plant wheat, oats, and barley were harvested, chopped, and ensiled at the milk and dough stages of maturity. Equipped with gas release valves, Mason jars combined with the addition of solid carbon dioxide prior to packing provided ideal ensiling conditions as all silages produced were of superior quality. Chemical analyses prior to and during fermentation of these small grains revealed compositional changes upon development and their effects on fermentation.

Dry matter yields were below those established for small grain crops in this area, however a truly representative estimation could not be obtained due to the size and location of the experimental plots. Dry matter percentages were higher during the dough stage which resulted in greater yields for oats and barley, but no change was observed for wheat.

Chemical analyses prior to ensiling revealed only minor differences in ash, protein, and fiber contents between the small grains investigated here. Developmental changes of these constituents are more pronounced during early rather than late growth and their concentrations would be expected to remain relatively stable over the two

weeks between harvests. Acid detergent insoluble nitrogen (ADIN) concentrations were similar among species in the milk stage and rose slightly in the dough stage in wheat and oats but doubled in barley. Substantial differences were noted in buffering capacities and water soluble carbohydrates (WSC) with wheat having the lowest buffering capacity and highest concentration of WSC. Although WSC concentrations fell sharply in the dough stage, the buffering capacity decreased as well and existing carbohydrates appeared sufficient for acid production.

During ensiling a classic example of inoculation by farm equipment was observed. In milk stage silages, lactic acid appearance (production) was faster except in milk stage wheat which was chopped by hand on a paper cutter while the remaining harvests were chopped through a forage harvester. WSC were utilized to a greater extent in milk stage silages reflecting the higher concentrations of acids found. Hemicellulose did not contribute to acid production as few differences were found in its concentration during ensiling. Acetate production was negligible in all silages, however higher concentrations were noted in milk stage wheat and barley.

Milk stage silages exhibited more rapid declines in pH but also had more protein degradation. Results of this investigation show protein degradation in silages to be more responsive to moisture content than acidification. Ammonia production was significant but similar in all

silages despite the apparent domination by lactic acid bacteria providing some evidence of amino acid deamination (utilization) by these bacteria. ADIN concentrations were not different as a result of fermentation.

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Appendix Table 1. Dry Matter Means for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	37.0	44.4	27.4	36.5	24.6	35.8	34.3
.5	35.0	44.0	28.6	35.4	25.8	35.7	34.1
1	35.0	42.9	28.5	34.1	26.0	34.3	33.5
2	35.6	42.8	28.4	33.3	26.5	36.1	33.8
4	36.5	43.7	26.1	35.2	25.8	35.6	33.8
8	36.0	43.9	25.4	34.3	26.1	36.7	33.7
16	35.5	43.5	27.2	33.6	25.7	36.1	33.6
32	34.5	43.5	27.6	34.3	26.1	35.6	33.6
64	34.5	43.5	25.9	34.2	25.2	34.9	33.0
Mean	35.5	43.6	27.2	34.5	25.7	35.6	

[1]Tabular entries represent averages of two experimental silos.

Appendix Table 2. Total Nitrogen Means for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	1.26	1.44	1.29	1.18	1.60	1.32	1.35
.5	1.30	1.42	1.29	1.20	1.43	1.33	1.33
1	1.26	1.22	1.35	1.15	1.43	1.25	1.28
2	1.28	1.29	1.19	1.16	1.44	1.22	1.26
4	1.30	1.29	1.31	1.17	1.45	1.22	1.29
8	1.29	1.40	1.34	1.15	1.42	1.31	1.32
16	1.36	1.31	1.35	1.12	1.48	1.29	1.32
32	1.32	1.36	1.28	1.19	1.46	1.25	1.31
64	1.36	1.37	1.30	1.17	1.44	1.31	1.33
Mean	1.30	1.35	1.30	1.17	1.46	1.28	

[1]Tabular entries represent averages of two experimental silos.

Appendix Table 3. Ash Means (% DM) for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	6.05	6.78	8.32	8.63	8.37	7.83	7.66
.5	7.39	8.12	9.32	8.64	9.00	8.34	8.47
1	7.92	7.74	9.41	10.03	8.90	9.09	8.85
2	7.42	8.56	9.27	9.62	8.48	7.71	8.51
4	7.19	7.83	10.15	8.72	8.27	8.08	8.37
8	7.27	7.81	9.96	9.01	8.81	7.75	8.43
16	6.89	7.62	10.33	9.34	8.86	8.01	8.51
32	7.52	8.12	8.95	9.00	8.86	8.55	8.50
64	7.40	7.80	9.61	9.62	9.12	8.40	8.66
Mean	7.23	7.82	9.48	9.18	8.74	8.19	

[1]Tabular entries represent averages of two experimental silos.

Appendix Table 4. Neutral Detergent Fiber Means (% DM) for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	45.6	49.8	55.7	46.6	51.3	48.1	49.5
.5	47.2	53.3	53.5	49.9	49.7	48.2	50.3
1	47.7	54.3	52.1	48.7	50.6	47.6	50.2
2	45.8	52.2	54.0	53.5	48.9	50.2	50.8
4	47.4	53.6	54.8	48.7	48.9	49.1	50.4
8	47.6	50.3	53.5	48.3	48.6	47.0	49.2
16	48.1	51.8	51.9	49.7	50.0	46.6	49.7
32	48.8	50.5	53.7	48.9	48.3	48.8	49.8
64	50.0	51.8	54.7	48.1	49.7	47.2	50.3
Mean	47.6	52.0	53.8	49.2	49.5	48.1	

[1]Tabular entries represent averages of two experimental silos.

Appendix Table 5. Acid Detergent Fiber Means (% DM) for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	29.7	32.2	37.8	32.6	32.2	32.8	32.9
.5	32.9	36.2	38.7	34.7	33.6	32.9	34.8
1	33.4	37.6	38.0	36.1	34.4	34.6	35.7
2	33.2	36.5	38.0	37.1	33.9	33.8	35.4
4	32.2	36.9	39.0	34.8	33.6	32.9	34.9
8	32.1	34.6	38.5	36.5	33.9	32.0	34.6
16	32.1	35.6	39.2	36.9	34.7	31.8	35.1
32	33.6	36.1	38.4	36.0	33.9	34.1	35.3
64	34.5	35.6	38.6	36.7	35.3	33.9	35.7
Mean	32.6	35.7	38.5	35.7	33.9	33.2	

[1]Tabular entries represent averages of two experimental silos.

Appendix Table 6. Hemicellulose Means (% DM) for Wheat, Oats, and Barley at the Milk and Dough Stages of Maturity During Ensiling.[1]

	Wheat		Oats		Barley		
Day	Milk	Dough	Milk	Dough	Milk	Dough	Mean
0	15.9	17.6	17.8	14.0	19.1	15.3	16.6
.5	14.2	17.1	14.8	15.3	16.1	15.3	15.5
1	14.4	16.8	14.1	12.7	16.2	12.9	14.5
2	12.6	15.7	15.9	16.4	15.0	16.4	15.3
4	15.2	16.8	15.8	13.9	15.3	16.2	15.5
8	15.5	15.7	15.0	11.9	14.7	15.0	14.6
16	15.9	16.2	12.7	12.8	15.3	14.9	14.6
32	15.2	14.4	15.3	12.9	14.4	14.7	14.5
64	15.6	16.2	16.1	11.4	14.4	13.3	14.5
Mean	14.9	16.3	15.3	13.5	15.6	14.9	

[1]Tabular entries represent averages of two experimental silos.