THE UNIVERSITY OF MANITOBA

IN VITRO RUMEN DIGESTION OF PROCESSED FEED GRAINS AND FABA BEAN CULTIVARS

BY

KATHERINE ELAINE BUCKLEY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

RUMINANT NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

WINNIPEG, MANITOBA

FALL, 1978

IN VITRO RUMEN DIGESTION OF PROCESSED FEED GRAINS AND FABA BEAN CULTIVARS

BY

KATHERINE ELAINE BUCKLEY

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Two separate investigations of substrate digestion by a culture of rumen microorganisms were conducted. The effect of processing method viz. dry ground, dry heat, reconstituted ground, reconstituted whole, steamed (0 kg/cm^2 and 6.8 kg/cm^2) and cooked, on dry matter digestibility and volatile fatty acid production of common feed grains was evaluated using a closed in vitro technique. In addition, in vitro dry matter digestibility of faba bean (Vicia faba) cultivars containing various amounts of condensed tannin was studied.

There appeared to be little advantage in processing rye, high-fat oats, normal oats, waxy barley and normal barley either by "hot" or "cold" processing methods. Cooking increased ($P < 0.05$) in vitro digestibility of waxy barley compared to the dry ground treatment, possibly by increasing the digestibility of components other than starch. Heat treatments including popping and roasting decreased in vitro dry matter digestibility of corn compared to the dry ground treatment, while reconstituting whole and ground corn increased in vitro dry matter digestibility. Heat treatment did not always produce a low acetate:propionate ratio in the in vitro fermentation system for all grain species. In addition, the lowest acetate:propionate ratio was not always concomitant with the highest in vitro dry matter digestibility. A determination of extent of gelatinization of heat treated cereal grains revealed that cooking altered all cereal starches to the greatest degree ($P < 0.05$). Steaming at 0 or 6.8 kg/cm^2 and dry heating did not appear to alter rye starch.

Whole bean *in vitro* dry matter digestibility was significantly ($P < 0.05$) greater in tannin-free faba bean cultivars than in tannin-containing cultivars. In subsequent trials where the cotyledons and hulls of some of the cultivars were examined separately, this difference was shown to be largely due to the higher digestibility of hulls from the tannin-free cultivar. Regression analysis indicated that *in vitro* digestibility of the whole bean may be equally related to hull tannin content and hull lignin content. Autoclaving various faba bean fractions decreased ($P < 0.05$) *in vitro* dry matter digestibility of protein concentrate, whole beans and cotyledons, but, had no effect on digestibility of hulls and starch. Adding condensed tannin, isolated from faba bean hulls (cultivar, Diana), to the fermentation media decreased ($P < 0.05$) *in vitro* dry matter digestibility and *in vitro* protein digestibility of faba bean protein concentrate. It is unknown whether the effect of added tannin was mediated through binding of sample protein or binding of the apo-protein part of microbial enzymes. Preliminary experiments indicate that condensed tannin added to the fermentation media had no effect on *in vitro* dry matter digestibility of corn starch, but, further experimentation is necessary to confirm this observation.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. M. E. Seale, Chairman of the Department of Animal Science, for placing the facilities of the Department at my disposal. Sincere thanks are extended to Dr. T. J. Devlin for his support and advice during preparation of this manuscript and the studies described within it. I am indebted to Dr. R. R. Marquardt for supplying the faba bean samples and tannin extracts used in this study, and for his encouragement and advice which was invaluable in the completion of this thesis.

Thanks are due to Dr. R. J. Parker and Mrs. K. Wittenberg for their assistance and advice pertaining to statistical evaluation of the data.

Technical assistance from Mr. J. McKirdy, Mr. L. Campbell and Mr. D. Wong are gratefully acknowledged.

I also wish to thank Mr. J. Woodhouse for his assistance with maintenance and handling of the experimental animals.

I am deeply indebted to my husband, Wayne, for his support during this study.

Financial support was obtained from the Manitoba Department of Agriculture.

TABLE OF CONTENTS

LIST OF TABLES

***

LIST OF FIGURES

SECTION I

IN VITRO

RUMEN DIGESTION

OF

PROCESSED FEED GRAINS

INTRODUCTION

In order to maximize the growth rate of feedlot cattle, large amounts of feed grains are being included in the finishing ration. Since feed costs constitute roughly two-thirds of the total cost of gain, there is a sustained interest in obtaining maximum performance with the feedstuffs. As a result, preparation and processing of feed grains is receiving considerable attention.

To determine which grain processing methods produce beneficial results in terms of efficiency of feed conversion, livestock performance and digestibility trials must be carried out. In attempting to circumvent these costly and time consuming feeding trials and establish reliable indicators of utilization, *in vitro* techniques have been developed. The present investigation made use of such a technique to screen a large number of samples of rye, corn, barley and oats processed under various conditions ranging from mild to very extreme. Research evaluating processed oats and rye is limited therefore, it was thought that *in vitro* information on these two species may be useful. It was anticipated that a study of *in vitro* dry matter disappearance and *in vitro* volatile fatty acid production would contribute to the general knowledge of utilization of processed cereals by rumen microbiota.

LITERATURE REVIEW

Cereal processing methods are aimed primarily at increasing the efficiency with which nutrients are utilized. Other objectives of processing are the improvement of grain handling and storage characteristics. Since the optimum degree of processing for each feed grain is not yet known, evaluation of grain treatments have been the subject of numerous investigations. Grain processing techniques and their effect on *in vitro* and *in vitro* digestibility, and cattle performance are reviewed in this thesis with emphasis on physical and chemical alterations of grain components which affect digestion by ruminants.

A. Methods of Processing

Production aspects of feed grain processing have been extensively reviewed in the literature (6, 13, 18, 21, 46, 51, 127).

Processing methods can be designated as either "hot" or "cold" processes. Addition of moisture is essential in some of these methods but not required in others.

a. Cold Processes

Cold processing methods involving only mechanical alteration of feed grains are Dry Rolling, Cracking, Pelleting, Crimping and Grinding. These methods are believed to improve digestion by exposing the endosperm to hydrolytic enzymes in the rumen (7).

Reconstitution refers to the addition of moisture to grain to a level of 25-30% followed by fermentation in an oxygen limited atmosphere for approximately twenty-one days (48). The grain may be

subjected to rolling or grinding prior to or following reconstitution. During fermentation it appears that partial disruption of the protein matrix surrounding starch granules releases free starch granules and protein bodies (110). It has been suggested that protein matrix disruption is the primary reason starch utilization is improved by reconstitution (75).

Soaking grain twelve to twenty-four hours in water prior to rolling or grinding has been shown to be beneficial (48). This process softens the endosperm and waxy coating and results in a feed that is very palatable to the animal, thus increasing consumption and subsequent performance.

High-moisture harvested grains with a moisture content of 25-30% are ensiled in oxygen limited structures and usually ground or rolled when fed. Organic acids, usually propionate or combinations of propionate and acetate can be applied to high-moisture grain to prevent mold and fungal growth during storage (6). It has been suggested (39) that extensive protein solubilization may occur during storage of the moist grain. A readily available source of nitrogen may allow microflora in the rumen to utilize high-moisture grain starch at a faster rate than dry grain starch.

b. Hot Processes

In the Extrusion process heat is generated from friction developed as the grain is forced through a tapered screw or die, with temperatures approaching 190 C (96) at extrusion point. The heat vaporizes moisture in the grain expanding the starch granules.

Popping, Micronizing and Roasting require application of direct

heat. During the popping process, air-dry grain is exposed to air temperatures between 178 C (98) and 230 C (124) for 30 seconds. This treatment pops the seed coat and results in a low density feed. The grain is usually rolled to reduce bulk before feeding. Micronization is similar to popping, except that infa-red radiation is the source of heat. Following a short exposure (0.5-1.0 min) to the radiation at temperatures of 140-180 C (54) grain is then flaked by passing through rollers. The last mentioned dry heat processing technique is roasting. Roasting is generally carried out in a revolving cylinder equipped with fins to lift the grain through the jets of a gas flame. An exit temperature of 137 C for roasted grain has been found to be satisfactory (36). In these dry heat processes as in extrusion, natural moisture in the grain is vaporized to steam, expanding the starch granules of the endosperm.

Steam processing, Pressure Cooking and Expansion are hydrothermal processing methods requiring the addition of both moisture and heat. Steam processing at atmospheric pressure for 8-30 min (27, 41) or pressure cooking at pressures of 1.4-5.6 kg/cm^2 for 1-10 min (40, 67, 96, 99) are usually followed by rolling or flaking of the still hot product. The production of expanded grain is similar to production of extruded grain except that the grain is tempered with steam heat prior to passage through the die (6). The objective of hydrothermal processing methods is to alter physical characteristics of the starch granules, rendering them more susceptible to degradation. Heat and moisture cause starch granules to swell and mechanical forces rupture the granules (48). Harbers (49) has observed that hydrothermal processing also disrupts the protein matrix surrounding the starch granules.

B. Properties of Starch Affecting Digestibility

Starch contained in the endosperm of cereal grains constitutes the largest proportion of a cereal grain (see Table 1).

Table 1. Starch concentration in grain dry matter

grain	range	mean
corn	63.7-78.4	71.9
wheat	54.2-71.1	63.8
sorghum	60.4-76.6	70.2
oats	34.4-70.0	44.7
barley	52.2-71.7	64.6

From NRC (84)

Not all cereal starches are equally digestible by ruminants. As noted by Hale (46) sorghum starch appears to be most resistant to digestion in the rumen of any common feed starch. Varietal and type differences also affect the extent of starch degradation (54, 123). Differences in digestibility probably reflect variations in chemical composition and physical properties such as granular organization and swelling ability.

a. Chemical Composition

Starch comprises a mixture of amylose and amylopectin and relative amounts of these two fractions differ according to the type of cereal. Table 2 gives the proportions of amylose in whole granular starches from a number of cereals. In the majority, values range from 22 to 28% with the exception of flint maize where amylose is virtually

absent.

Table 2. The amylose contents and gelatinization temperature range of whole granular cereal starches

Cereal	Amylose % in starch	Gelatinization temperature range (C)
Barley	22	59-64
Maize	28	62-72
Oats	27	-
Wheat	26	65-67
Maize (flint)	1	66-69
Sorghum	25	67-77
Amylomaize	61	92

From Armstrong (7)

Banks and Greenwood (11) noted that starches with high amylose content are very resistant to alfa-amylolysis, and are limited in their swelling ability. However, work by Sandstedt et al (104) provides evidence that amylose content in itself, is not the major factor determining digestibility. They (Sandstedt et al, 104) propose that differences in susceptibility of high-amylose and normal starches to enzyme action are probably due to differences in bonding between starch molecules and possibly anomalous linkages within the molecule.

It has been reported that amylopectin is more readily digested than amylose (103). The greater digestibility of amylopectin may not be related to its higher solubility in water. An investigation by Goering et al (43) with high-amylopectin starch has shown this starch to be less soluble than high-amylose starch yet more susceptible to enzymatic hydrolysis than normal starch. This has lead to speculation that non-waxy starches contain an enzyme-resistant fraction which

differs in magnitude among grain varieties (43).

b. Granular Organization

Starch accumulates in discrete particles in the endosperm and may be tightly packed together in a continuous protein matrix (hard endosperm) or loosely packed in a thin film of protein (floury endosperm) (50). From histological studies of Sullins and Rooney (110), Hale (46) inferred that the nature of the protein matrix may affect accessibility of starch granules to animal enzymes.

Hellman, Boesch and Melvin (52) reported that most starch granules consist of approximately equal parts of highly organized crystalline regions (crystallites) and amorphous or gel-like regions. In cereal starches the amorphous regions account for about 75% of the granule (107). The texture of the gel phase is too fine to admit large amylase molecules therefore amylase acts at fissures or structural imperfections on the granule surface (38). It is possible that processing methods such as steam rolling, alter the gel-like regions or create flaws in the granule surface allowing easier enzyme access.

c. Optical Anisotrophy

Due to the presence of crystalline regions in the predominantly amorphous mass, the starch granule is optically anisotropic i.e. the index of refraction is not the same in all directions resulting in a phenomenon termed double refraction or birefringence. Under the polarizing microscope, unaltered starch granules exhibit a "maltese cross" formed by two zones of light extinction (107). Birefringence is lost when crystallinity is destroyed.

d. Swelling Ability

When starch is placed in water, the amorphous regions of the granules swell as water is absorbed. Below 55 C birefringence is retained and the process of swelling is reversible. However, above 55 C irreversible swelling or gelatinization occurs and crystallinity of the granules is destroyed (38). According to Banks and Greenwood (11) factors such as granule size, amylose content and degree of association between molecules in the amorphous region influence gelatinization temperature. As a result there is a range of temperatures (see Table 2) over which loss of birefringence occurs.

Changes due to gelatinization are generally believed to make starch more available to rumen organisms and postruminal digestive enzymes, consequently the extent of gelatinization has been used to estimate the efficacy of grain processing (48). Although the level of gelatinization of some cereal starches has correlated well with efficiency of gain, gelatinization of starch as the only evaluation of processed grains has been shown to have limitations (31, 81, 118, 128).

Although heat and moisture cause gelatinization, subsequent treatment of the hot material influences the extent of gelatinization. The studies of Pfost (94) and Johnson et al (62) revealed that flaking steam processed grain significantly increased gelatinization.

If over-cooking occurs during hydrothermal processing, starch granules rupture and soluble amylose leaks out. On slow cooling a gradual alignment of glucose chains of soluble amylose into tightly bound aggregates takes place. This phenomenon is referred to as

retrogradation and is irreversible. Retrograded amylose is quite resistant to the action of alfa-amylase (11).

The extent of gelatinization of a starch can be estimated by the technique of Sung (111). A more accurate measurement of gelatinization is easily achieved by suspending a small sample of starch in water and counting the number of granules that still retain birefringence using a microscope and polarized light (106).

C. In Vitro Techniques for Estimating the Effect of Processing on Grains

According to Hale (46), in vitro assay techniques which measure parameters such as gas production, enzymatic starch digestion, dry matter disappearance or volatile fatty acid production appear to be good indicators of in vivo digestibility and utilization of starch. These techniques involve incubation of the grain samples with a pure enzyme preparation or inoculum obtained from the rumen. Electron microscopy has also been used in combination with in vitro techniques to observe the effects of processing on digestion of feed grains.

a. Incubation with Pure Enzyme

Purified enzyme extracts may be used to determine dry matter disappearance or starch availability. Starch availability is usually expressed in terms of amount of reducing sugar released from the sample upon incubation with the enzyme. The procedures used by Walker et al (124) for starch availability and White et al (126) for dry matter disappearance are typical of the basic technique.

White et al (126) measured in vitro dry matter disappearance

(IVDMD) of raw, roasted, volatile fatty acid (VFA) treated, high-moisture ensiled and reconstituted corn incubated with amyloglucosidase. IVDMD of VFA treated corn was significantly higher than other treatments except high-moisture corn. The lowest IVDMD values were obtained with roasted and raw corn and these were similar.

Tonroy and Perry (117) investigated starch digestion by determining IVDMD of processed corn incubated with alfa-1,4-glucan glucohydrolase. No significant difference was observed between raw and roasted corn however, pressure flaking corn resulted in a significant increase in IVDMD over raw corn. In another study Tonroy and Perry (116) observed starch digestion by amyloglucosidase of ensiled high-moisture (HM), air-dry (DRY), ensiled reconstituted (RECON) and volatile fatty acid (VFA) treated corn. In vitro starch digestion by the amyloglucosidase indicated that DRY contained the same amount of digestible starch as HM and RECON corn and more digestible starch than VFA. The small difference between treatments suggest only slight if any differences in starch structure or content between the four treatments.

The susceptibility of corn starch to enzymatic degradation was studied by Felsman et al (36) who also used an amyloglucosidic enzyme (Diazyme L-100). Results indicated that enzymatic degradation, measured as glucose production increased with increasing roasting temperature, with the optimum temperature at 127-137 C.

Walker et al (124), estimating starch digestion of sorghum grain by release of reducing sugar upon incubation with amyloglucosidase, found pressure cooking and rolling to increase starch availability to a greater degree than popping and steam rolling, which, in turn, were more digestible than raw grain.

McNeill et al (82) reported that carbohydrate susceptibility to amloglucosidase was greater for steam-flaked and micronized than dry or reconstituted sorghum grain. McNeill et al (83) suggest that disruption of the protein matrix around starch is the primary reason starch utilization is improved by micronization and steam-flaking.

Using an amyloglucosidase procedure, Liang et al (67) reported an increase in susceptibility of sorghum starch to enzymatic attack as pressure for pressure-cooking was increased by increments from 1.8 to 6.0 kg/cm^2. Lengthening cooking time from one to ten minutes was also beneficial.

Prasad et al (96) estimated in vitro starch availability in processed sorghum and starch availability as well as IVDMD in processed wheat using Diazyme-100, an amyloglucosidase. Their results indicated that expanded sorghum contained more available starch than sorghum treated by dry rolling, steam-flaking, pressure-cooking or extruding. IVDMD of wheat was not significantly affected by processing but starch availability of extruded and steam-flaked wheat was significantly greater than dry-rolled wheat.

Croka and Wagner (29) and Aimone and Wagner (3) used amyloglucosidase digestion coupled with yeast fermentation to evaluate processed grain samples. Gas production from yeast fermentation indicated that micronization increased starch availability in sorghum grain (29) and wheat (3).

In vitro studies by Osman et al (87) utilized incubation of processed barley and sorghum with porcine pancreatin or a homogenate of lyophilized bovine pancreas. Steaming or pressure-cooking at 1.4 kg/cm^2 for one minute without further processing decreased in vitro

starch digestion of barley and sorghum as compared to untreated grains. Digestibility of both grains was improved with increased steam pressure and increasing flake flatness. They suggested that degree of flaking was the principal factor influencing susceptibility of barley and sorghum starch to enzyme attack (87).

The effects of all processing methods on enzymatic digestion of cereal starches have not been fully elucidated, however, experimental evidence to date indicates that moist heat treatments improve the in vitro digestibility of starch in common feed grains to a greater degree than other treatments.

b. Incubation with Rumen Inoculum

Most of the in vitro methods used for determining the effects of grain processing are modifications of a technique described by Tilley and Terry (115) which was developed for in vitro digestion of forage crops. Methods used by Salsbury et al (101, 102), Baumgardt et al (12) and Johnson et al (61) for cellulose digestion have also been modified by a number of researchers for evaluation of cereal processing methods. Some of the in vitro techniques involve two stages; digestion with rumen inoculum followed by incubation with pepsin. The peptic digestion of the two-stage procedures is omitted when an estimate of ruminal digestion is desired.

Salsbury, Hoefer and Luecke (100) carried out in vitro fermentations to determine the effect of heat treatments on corn starch. Digestion by rumen microoganisms was followed by hydrolysis in 0.5 M HCl for one hour at 121 C to estimate "readily hydrolyzable dry matter" (RHDM). Corn starch suspended in water and boiled for three minutes

was digested more rapidly by rumen microorganisms than untreated starch. Application of dry heat to corn starch resulted in a decrease in rate of digestion of RHDM compared to untreated starch.

The influence of roasting, VFA treatment, reconstituting and high-moisture storage on IVDMD of corn was studied by White et al (126). IVDMD was significantly greater for raw and VFA treated corn than for roasted corn when inoculum was taken from a high concentrate fed steer. In vitro starch digestibility was 98.6, 98.8, 98.9, 98.9 and 94.5% for raw, fatty acid treated, high-moisture, reconstituted and roasted corn, respectively. White et al (126) suggested that gelatinization by dry heat lowered the digestibility of starch by rumen microorganisms. However, the possibility also exists that roasting rendered the corn protein unavailable to rumen microorganisms, thereby decreasing IVDMD.

Based on a 48 hour incubation period, Tonroy and Perry (117) found that processing raw corn either by roasting or pressure flaking increased starch digestibility but decreased dry matter digestibility indicating that the utilization of other components in corn was impaired by processing. The fact that both dry matter and starch digestion at shorter incubation periods were enhanced by processing indicated that processing increased the rate at which total dry matter and starch were utilized.

In another study Tonroy and Perry (116) compared IVDMD of four types of cold processed corn viz. dry (DRY), ensiled high-moisture (HM), ensiled reconstituted (RECON) and volatile fatty acid treated (VFA). They found that HM and VFA had the greatest percent dry matter digestibility, DRY had the lowest and RECON was intermediate.

Croka and Wagner (29) investigated the effects of micronizing on

IVDMD and gelatinization of sorghum grain. Micronization appeared to increase sorghum starch availability to rumen microorganisms and produce a greater degree of gelatinization compared to dry-rolling.

Micronization has been found (3) to lower 12- and 24-hour IVDMD values of wheat although at 6 hours there was no significant difference between micronized and dry-rolled wheat. Aimone and Wagner (3) suggested that micronization may reduce protein solubility in wheat thus reducing IVDMD.

In their experiments, Newhaus and Totusek (85) found that the greatest increase in IVDMD of whole-reconstituted and high-moisture harvested sorghum grain were achieved at moisture levels between 23 and 26% at temperatures of 43 C. Storage time had little effect on high-moisture sorghum grain but significantly affected whole-reconstituted grain; digestibility increasing with each successive day of storage to 32 days. Drying whole grain following the reconstitution process appeared to decrease dry matter digestibility (85).

An in vitro screening technique employing gas production by rumen microorganisms was developed (119) to evaluate the effect of grain processing methods on digestion. High positive correlations were found between gas production and in vitro starch digestion. Steam processing and flaking of either milo or barley significantly increased gas production (119). Either steam processing and flaking or pressure-cooking and flaking resulted in similar gas production values for sorghum (119). According to Trei et al (119) both disruption of the protein matrix and alteration of the starch granule may be implicated in increased gas production.

Christiansen and Wagner (24) investigated the effect of

reconstitution and physical form of wheat on IVDMD. Their data suggested that dry-rolled wheat was superior to whole-reconstituted wheat and wheat reconstituted then rolled, and similar to wheat rolled before being reconstituted. In addition, dry-rolled sorghum was found to be less digestible than wet or dry processed wheat (24). They ventured the possibility that the proteinaceous matrix surrounding starch granules of wheat was less resistant to attack by digestive enzymes than that surrounding sorghum starch (24). Thus, special processing techniques may not enhance starch availability as much in wheat as in sorghum.

c. Electron Microscopy

In a unique investigation, Harbers (49) employed purified porcine pancreatic alfa-amylase to hydrolyze steam-flaked, micronized and popped sorghum grain. He (49) then studied starch granule structural changes due to processing and amylolysis by scanning electron microscopy. Steam-flaking altered starch granules such that they resembled erythrocytes or became shapeless conglomerates. Popping sorghum grain changed the starch granules into thin lattices of interconnecting sheets. Micronizing had much the same effect as popping but granules near the surface of the kernel resembled those processed by steam-flaking. Protein bodies remained intact but the matrix protein was disrupted. Although the rate of hydrolysis was not determined in the individual samples, Harbers (49) observed that amylolysis first starts on gelatinized starch and indentations of damaged granules. Outer edges of damaged granules appeared to be more resistant than indentations, but less resistant than raw sorghum grain starch granules.

D. The Processing of Grain and Livestock Performance

A true indication of the efficacy of feed grain processing is obtained through livestock performance trials. The following discussion of grain processing and livestock performance will be restricted to cattle since sheep appear to be unique in their ability to digest and utilize grain (46, 88). Due to the importance of sorghum as a feed grain in south-west U.S.A. it has been included along with grains of more significance in Canada.

a. Processing Sorghum and Livestock Performance

In fattening trials (Hale et al, 47), steam processing and flaking of milo resulted in a 9% increase in rate of growth, 4% in feed intake and a 5% improvement in feed conversion efficiency compared with the dry-rolled grain. Similar results with steam processed and dry-rolled sorghum were also obtained by others (41, 44, 122, 127). However, Franks et al (37) and Schake et al (105) found no advantage in gain and feed efficiency of steam-flaked sorghum over the dry-rolled product.

The importance of producing a flat flake after steaming has been illustrated in a number of studies (44, 45, 87, 112, 118, 127) and conflicting results may be due to variations in physical characteristics of experimental steam-flaked rations. Simply steaming the sorghum without flaking has been shown (44, 127) to reduce feed conversion efficiency compared to the raw rolled product.

In a performance trial comparing rations containing pressure-cooked (60 psi for 1.5 min), flaked and steam-flaked (20 min at 100 C)

sorghum, Erwin (33) reported non-significant differences in average daily gains but a 6% increase in feed efficiency with the ration containing pressure-cooked sorghum. A similar study by California researchers (127) indicated that feeding pressure-cooked sorghum processed at 20 psi for 1.5 min improved average daily gain and feed efficiency compared to sorghum processed by steaming, dry-rolling or pressure-cooking at 60 psi for 1.5 min. Garrett et al (40, 41) demonstrated that steers were better able to utilize steam-pressure processed sorghum than sorghum steamed at atmospheric pressure.

In a finishing trial, Prasad and coworkers (96) found extruded sorghum to be utilized as well as steam-flaked and better than dry-rolled sorghum but there was no significant effect on average daily gain.

In a number of studies (37, 79, 90, 105) cattle fed reconstituted grain gained at the same rate as cattle fed dry-rolled sorghum but consumed less feed resulting in a more desirable feed conversion. Early-harvested high-moisture grain has been shown to be comparable to reconstituted grain in terms of efficiency of utilization (79, 90). Steam-flaking may (37) or may not be (105) as beneficial as reconstituting sorghum grain.

Feeding popped sorghum grain to finishing steers has been reported (98, 32) to significantly reduce feed intake as compared to dry-rolled sorghum. The reduced feed intake was accompanied by increased efficiency of feed utilization and a non-significant decrease in rate of gain.

b. Processing Barley and Livestock Performance

In two out of three feeding trials Thomas and Myers (113) obtained no response to heat treatment of barley compared to dry-rolled barley when fed to steers in a high concentrate ration. Average daily gains reported for the third trial were 1.18 kg/day for steam-rolled barley and 1.04 kg/day for dry-rolled. No feed conversion efficiency data were presented in this study.

Comparing dry-rolled barley with steam-flaked barley in high concentrate rations, Hale et al (47) and Hale (44) found a significant increase in rate of weight gain and feed intake due to steam processing with no difference in feed conversion efficiency. These findings are in agreement with results obtained by Christensen, Duck and Nicholson (22) who incorporated dry-rolled, pelleted, steam-rolled and steam-rolled pelleted, barley into four (97.5% barley) rations for Holstein steers. Treatment did not affect feed efficiency but the feeding of steam-rolled diets resulted in significantly faster rate of daily gain and superior dressing percentage. No significant response was obtained from pelleting the barley compared to rolling.

Feedlot performance of cattle fed dry-rolled and pressure-cooked, flaked barley was significantly improved by the latter process as indicated by gain and feed utilization (127). In the same study (127) steaming barley 8 min at atmospheric pressure reduced average daily gain and feed efficiency compared to dry-rolling.

c. Processing Corn and Livestock Performance

The value of cracking or grinding corn compared to other types of processing for cattle has been investigated in a number of studies (17, 26, 42, 53, 62, 71, 125, 128). The degree of grind (or particle

size) appears to critical. Finely ground grain has been criticized because of its association with reduced palatability (48), digestive upsets and liver abcesses (125). Fine grinding has also been shown to decrease the concentration of protozoa in rumen fluid (128).

Gerken et al (42) and White and Hembry (125) reported a reduced rate of gain and feed efficiency in steers fed ground corn vs. steers fed whole shelled corn. However, Burkhardt, Embry and Luther (17) found no difference in feedlot response of steers fed unprocessed corn and corn rolled to a medium degree of fineness. Feeding finely ground corn and cracked corn resulted in similar average daily gain and feed efficiency in yearling heifers (53, 71).

Clanton and Woods (26) found that steers fed a ration containing 75% corn had significantly slower rates of gain and consumed significantly less corn when the grain was offered ground and pelleted rather than cracked.

It has been observed (31, 128) that feeding high levels of gelatinized grain starch in high concentrate rations had adverse effects on livestock performance. DeBie and Woods (31) found that steers recieving 80% expanded corn in a mixed ration gained less and consumed less feed than those fed cracked corn. Performance of steers fed rations containing 5, 10, 20 or 30% expanded corn was not significantly different from the cracked corn ration (31). Woods and Wilson (128) noted a marked decline in livestock performance when unheated corn was replaced with 50% and 100% gelatinized corn. In contrast, experiments by Mudd and Perry (75) showed that substituting raw cracked corn with expanded corn in a high concentrate steer ration was possible without reducing daily gain. An improvement in feed

conversion was associated with the heat processed grain (75).

Reconstituted (17, 71) and high-moisture (71, 93) corn has been found to reduce rate of gain when fed to steers. However, Perry et al (93) reported a significant increase in feed efficiency for those steers fed high-moisture corn compared to those fed raw rolled corn. Studies by Burkhardt et al (17) and Matsushima and Stenquist (71) indicated that no advantage in feed efficiency was to be gained from feeding reconstituted and high-moisture corn rather than dry-rolled corn. According to Armbruster (6) the expected improvement in feed efficiency when feeding reconstituted and high-moisture corn should be 3% and 5% respectively over dry-rolled corn. In a recent investigation, Utley and McCormick (121) found that steers fed high-moisture corn gained about 8% faster and were 6% more efficient in converting concentrate dry matter to body weight gain than steers fed dry corn. Inconsistent results among trials may be due to physical form and moisture content of grain, time in storage and temperature during reconstitution and the kind of facility used to store the high-moisture corn.

Heat processing corn by steam-flaking (53, 71), steaming and cracking (47), roasting (92, 93) and pressure-cooking (127) has been found to enhance feed conversion efficiency. Feeding corn pressure-cooked 1.5 min at 20 psi (127) or roasted at 148 C (92, 93) has been reported to result in higher average daily gains compared to dry-rolled corn.

In summary, therefore, it appears likely that performance of cattle fed corn rations can be improved to the greatest extent by steam heat and dry heat treatments and to a lesser extent by high-moisture

harvesting and reconstitution.

d. Processing Wheat and Livestock Performance

Limited comparisons with dry-rolled wheat have shown that processing wheat by reconstitution (23) or various heat (both wet and dry) treatments (9, 15, 41) in order to increase feed efficiency and average daily gain appear to be of little or no value. However, the results of Christiansen and Wagner (23) indicated that intake of cattle fed reconstituted wheat tended to be higher thus increasing average daily gain. It has been suggested that reconstitution decreases dustiness of rolled or ground wheat resulting in increased feed consumption (6). In recent experiments with micronized wheat, Aimone and Wagner (2) reported a 9% increase in rate of gain and an 8% increase in feed consumption in steers fed micronized wheat compared to those fed dry-rolled wheat. No significant differences existed in feed efficiency. Roasted wheat substituted for raw rolled wheat in a steer finishing ration has been shown to improve feed efficiency by 7% (14). Roasting had no effect on average daily gain in one out of two trials (14).

E. Effect of Processing Grain on its Digestibility

One of the aims of processing is to increase digestibility of the starch component of feed grains by modification of the chemical and physical properties of starch. Processing may also affect (i) site of digestion of starch, (ii) proportions and concentrations of individual volatile fatty acids produced in the rumen and (iii) digestibility of protein in the rumen. These latter aspects must be considered when evaluating processing methods.

a. Processing of Sorghum and Digestibility by Cattle

Fine grinding of sorghum did not increase the digestibility of dry matter or nitrogen compared to dry-rolling or coarse grinding (16, 58, 72). However, Hinman and Johnson (55) found that starch digestion was greater for ground than dry-rolled sorghum in finishing rations.

There is overwhelming evidence that cold processing by reconstitution greatly increases digestibility of various components of sorghum grain. Potter, McNeill and Riggs (95), McGinty, Breuer and Riggs (78) and McGinty and Riggs (79) found an increase in protein digestibility of reconstituted grain compared to dry ground sorghum when fed to finishing cattle. Starch digestibility has also been shown to be improved by reconstituting rather than dry grinding (81). The digestibility of other fractions such as dry matter (16, 78, 79), organic matter and non-protein organic matter (78, 79) has been reported as significantly greater for reconstituted than for dry ground sorghum.

Digestibility of wet and dry heat processed sorghum has been the subject of numerous studies (16, 30, 33, 41, 47, 55, 56, 57, 58, 64, 65, 72, 81, 95, 96, 98). Heat processing of sorghum grain improves digestibility of dry matter without significantly altering the digestibility of crude protein (Table 3). Although pressure-cooking appears to increase digestibility of gross energy (41), there is disagreement between studies of Garrett et al (41) and Husted et al (58) as to whether steam-flaking influences digestibility of gross energy. From a comparison of the different groups of results presented in Table 3, it can be seen that apparently similar physical forms of sorghum show

Table 3. Digestibility coefficients determined for cattle fed diets high in sorghum

Sorghum in diet (%)	Description of grain	Processing conditions: Steam time (min)	Processing conditions: Steam pressure (kg/cm^2) & temperature (C)	Digestibility (%): DM †	Digestibility (%): N ‡	Digestibility (%): GE §	Digestibility (%): Starch: Rumen	Digestibility (%): Starch: Total	Reference
78	dry-rolled	Nil	Nil	61.6a	49.6				47
78	steam-flaked	25	0 kg/cm^2 (99 C)	69.7b	51.4				
80	dry-rolled	Nil	Nil	73.9b	67.9				30
80	micronized	Nil	Nil	81.4c	72.3				
79	coarse ground	Nil	Nil	76.0a	66.4				16
79	fine ground	Nil	Nil	75.7a	64.7				
79	steam-flaked	20	0 kg/cm^2 (93 C)	79.9ab	65.6				
77	dry-rolled	Nil	Nil	68.8a	58.8				58
77	fine ground	Nil	Nil	70.7a	59.4				
77	steam-flaked	25	0 kg/cm^2 (99 C)	76.3b	55.3				
77	pressure-flaked	1	2.8 kg/cm^2	75.2b	53.5				
77	soaked & cut	Nil	Nil	66.2a	54.9a	64.9a			58
77	dry-rolled	Nil	Nil	65.2a	52.7a	63.7a			
77	steam-flaked	25	0 kg/cm^2 (99 C)	74.9b	56.0a	75.5b			
77	steam cut	25	0 kg/cm^2 (99 C)	64.4a	45.2b	63.2a			

Table 3. (cont.)

Sorghum in diet (%)	Description of grain	Processing conditions: Steam time (min)	Processing conditions: Steam pressure (kg/cm^2) & temperature (C)	Digestibility (%): DM	N	GE	Starch: Rumen	Starch: Total	Reference
72	ground	Nil	Nil		59.4	71.4a			41
72	steam-flaked	8	0 kg/cm^2		54.8	72.0a			
72	steam-flaked	20	0 kg/cm^2		55.5	71.6a			
72	pressure-cooked	1	1.8 kg/cm^2		57.7	74.3ab			
72	& flaked	1	3.5 kg/cm^2		59.4	75.4b			
72		1	5.3 kg/cm^2		59.0	76.1b			
55	dry-rolled	Nil	Nil		51.8	62.4			96
55	expanded	-	-		48.3	63.6			
55	pressure-cooked	10	5.3 kg/cm^2		44.8	60.0			
83	dry ground	Nil	Nil				42.03a	96.76a	81
83	steam-flaked	20	0 kg/cm^2				83.41c	99.74b	
83	micronized	Nil	Nil				42.99a	97.14a	
80	steam-rolled	8	0 kg/cm^2				90	97.3	57
80	pressure-cooked	1.5	3.5 kg/cm^2				95	97.6	
80	dry-rolled	Nil	Nil				52a	96a	64
80	steam-flaked	-	-				74b	99b	

Table 3. (cont.)

Sorghum in diet (%)	Description of grain	Processing conditions: Steam time (min)	Processing conditions: Steam pressure (kg/cm^2) & temperature (C)	Digestibility (%): DM	Digestibility (%): N	Digestibility (%): GE	Digestibility (%): Starch: Rumen	Digestibility (%): Starch: Total	Reference
84	dry ground	Nil	Nil				86	97.3a	57
84	dry-rolled	Nil	Nil				77	92b	
84	steam-flaked	-	-				81	100a	
84	micronized	Nil	Nil				84	99a	
84	dry-rolled	Nil	Nil				60	81a	56
84	micronized low	Nil	Nil				61	98b	
84	micronized med.	Nil	Nil				64	98b	
84	micronized high	Nil	Nil				68	98b	

a,b,c Means in the same column bearing different letters differ significantly ($P < 0.05$).
†values relate to digestibility of dry matter.
‡values relate to digestibility of nitrogen.
§values relate to digestibility of gross energy.

widely differing ruminal starch digestibilities when fed to cattle. These differences may be partially due to differences in sorghum type (54). Variation in processing conditions and their interaction with grain source may account for differences in response to dry heat and hydrothermal processing.

b. Processing of Barley and Digestibility by Cattle

It is generally accepted that barley should be coarsely ground or rolled prior to feeding to beef cattle. Extremely fine grinding not only requires more power and time, but reduces feeding value compared to coarsely ground barley (48). Finely ground barley has also been associated with increased tendency to bloat (97).

In digestibility trials, Parrott et al (91) found that steam processing and flaking of barley does not improve digestibility of the proximate fractions or the availability of total digestible nutrients (TDN), except when the TDN of the barley is low. Similarly, Garrett et al (41) found no significant effects due to various steam and pressure conditions on gross energy or nitrogen digestibility of barley.

c. Processing of Corn and Digestibility by Cattle

Since eighteen to thirty-five percent of whole shelled corn consumed by dairy cattle was observed unaltered in the feces, Morrison (74) concluded that grinding of shelled corn was necessary for more complete digestion.

No significant differences in digestibility of dry or organic matter, of starch or of protein was found in steers fed rations of dry whole shelled corn or dry crimped corn (7). In contrast to these

Table 4. Digestibility coefficients determined for cattle fed diets high in corn

Corn in diet (%)	Description of grain	Processing conditions: Steam time (min)	Processing conditions: Steam pressure (kg/cm^2) & temperature (C)	Digestibility (%): DM	Digestibility (%): N	Digestibility (%): Starch: Rumen	Digestibility (%): Starch: Total	Reference
95	shelled whole	Nil	Nil	87.41b	74.48b			120
95	high-moisture	Nil	Nil	89.44b	76.83b			
70-75	steam-flaked	12	0 kg/cm^2 (95 C)	74.8	64.5			62
70-75	cracked	Nil	Nil	70.1	59.6			
76-80	steam-flaked	12	0 kg/cm^2 (95 C)	77.5b	66.8b			62
76-80	steam-flaked & cracked†	12	0 kg/cm^2 (95 C)	77.1b	65.6b			
76-80	steam cracked	12	0 kg/cm^2 (95 C)	72.4a	62.5a			
80	dry-rolled	Nil	Nil	78.62	64.83			93
80	roast rolled	Nil	132 C	78.61	63.31			
80	pressure flaked	-	3.52 kg/cm^2 (110 C)	80.81	69.15			
90	dry-rolled	Nil	Nil	75.8D		71.7D	93.6D	27
90	steam-flaked	20-30	0 kg/cm^2 (100 C)	82.6C		91.6C	99.0C	
95	whole	Nil	Nil			75.18a	98.59	77
95	steam-flaked	-	-			76.95b	98.79	

Table 4. (cont.)

Corn in diet (%)	Description of grain	Processing conditions: Steam time (min)	Processing conditions: Steam pressure (kg/cm^2) & temperature (C)	Digestibility (%): DM	Digestibility (%): N	Digestibility (%): Starch: Rumen	Digestibility (%): Starch: Total	Reference
78	dry-rolled	Nil	Nil	79.9		77.8b	96.3b	39
78	steam-flaked	20	0 kg/cm^2	80.5		83.0c	99.1c	
78	high-moisture	Nil	Nil	80.4		89.3c	99.1c	
78	high-moisture VFA treated	Nil	Nil	78.5		62.8a	95.8b	

a,b,c,d, Means in the same column bearing different letters differ significantly ($P < 0.05$).
C,D Means in the same column bearing different capital letters differ significantly ($P < 0.01$).
†Flaked product was run back through same roller mill to break up flakes.

findings, Adeeb, Wilson and Campling (1) reported that grinding increased digestibility of corn in cattle.

Heat processing of corn has little effect on dry matter and crude protein digestibility (Table 4). However, Johnson et al (62) reported that steaming followed by cracking significantly reduced dry matter and crude protein digestion of corn compared to steam-flaked corn thus demonstrating the importance of flaking after steaming. In three different investigations (27, 39, 77), steam-flaking significantly increased starch digestion in the rumen. There is evidence (27, 39) that steam-flaking also increases starch digestion postruminally. High-moisture ensiling was ineffective in increasing dry matter and protein digestibility but may have increased starch digestibility (39, 120).

When the ratio of gelatinized corn to raw corn was increased in an 86% corn ration, linear decreases in TDN, crude protein digestibility and dry matter digestibility were observed but could not be repeated (75). McLaren and Matsushima (80) found that ruminal digestion of corn starch in cattle fed an 85% concentrate finishing ration was more complete if starch was 90 or 45% gelatinized compared to 10% gelatinized. Virtually all of the starch escaping the rumen intact was digested in the small intestine regardless of the level of gelatized starch in the ration.

d. Processing of Wheat and Digestibility by Cattle

Garrett et al (41) found no significant effects on digestibility of energy or nitrogen due to dry heat or hydrothermal processing of wheat, which comprised 71-84% of rations for steers. A digestibility

study conducted by Cornett, Sherrod and Albin (28) indicated that dry matter, organic matter and crude protein digestibilities were not significantly different for dry-rolled, steam-flaked and micronized, flaked wheat. Digestibility of the energy component, however, was highest for steam-flaked wheat (28).

Prasad et al (96) compared the effects of pressure-cooking, steam-compacting, dry-rolling, dry grinding and dry compacting wheat on digestibility of the proximate fractions by yearling steers fed a ration containing 78% wheat. Compacting was effected by a Komarek-Greaves Briquet press. Processing the wheat by these various methods did not affect digestibility of dry matter, nitrogen, ether extract or nitrogen-free extract.

In a recent investigation, Aimone and Wagner (2) observed the influence of micronizing wheat on digestibility of the proximate fractions and starch by steers fed 85% wheat rations. No significant differences existed between dry-rolled and micronized wheat for any of the components measured. Aimone and Wagner (2) suggested that wheat is very digestible and is not improved by processing to the extent noted for corn and sorghum.

F. Sites of Digestion of Starch and Grain Processing

Factors such as the type of cereal starch, level of dietary starch and grain processing influence the site of starch digestion. Barley starch is almost completely fermented in the rumen while appreciable amounts of corn and sorghum starch may pass intact into the lower digestive tract (8, 46, 123). Karr et al (63) found that as the starch content of steer rations increased, the percent digested in the rumen

decreased. Their data and that of Little et al (69) also suggest that there is a biological maximum to starch utilization in the small intestine.

Starch escaping digestion in the small intestine is subjected to further fermentation in the large intestine and caecum. The nutritional significance of caecal starch fermentation is not clearly defined but it is known that starch is acted upon by caecal microbiota producing VFA's which are then absorbed into the bloodstream (35, 76).

Losses due to methane production and heat of fermentation should be lower if some starch from cereal grains is digested in the intestinal tract. However, there is no evidence that feed utilization is improved by increasing the postruminal digestion of starch. On the other hand, there is evidence that utilization may be improved by increasing ruminal digestion. Feedlot performance studies with steers fed sorghum (44, 47, 122) and corn (53, 71) have shown that steam-flaking improves feed conversion of these feed grains. At the same time, digestibility experiments with sorghum (64, 81) and corn (27, 39) in steer rations have indicated that steam-flaking substantially increases starch digestion in the rumen thus decreasing the amount of starch entering the small intestine. Increased ruminal digestion of starch may result in increased microbial growth and, therefore, entry of microbial protein into the duodenum.

G. Grain Processing and Volatile Fatty Acids in Rumen Liquor

Ruminal volatile fatty acids (VFA) serve as the major source of energy for ruminants rather than glucose (5). The relatively small amounts of glucose absorbed from the alimentary tract of ruminants in

relation to known rates of glucose entry into the total body pool shows that gluconeogenesis is a major metabolic activity (68). Propionate, the only VFA which makes a net contribution to glucose synthesis, is quantitatively the most important single precursor of glucose (5). Since acetate is used less efficiently than propionate, efforts have been made to alter VFA production by rumen microbiota.

In 1960, Shaw et al (108) reported that grain processing method affected the acetate:propionate (A/P) ratio, which was associated with rate of gain and feed efficiency. The influence of processing cereal grains on in vivo VFA production has since been widely investigated but to date reports appear to be inconclusive.

In an in vitro investigation, Theurer, Trei and Hale (112) found that steam-flaking sorghum and barley significantly increased total VFA production by 42 and 46% over the untreated sorghum and barley, respectively. Although molar percents of individual acids were not markedly altered by steam-flaking, increasing flake flatness resulted in an increase in propionic and butyric acid concentrations.

In vivo studies by Hinman and Johnson (55, 56) comparing heat processed and dry ground sorghum indicated that feeding heat processed sorghum tended to increase total VFA concentrations and decrease the A/P ratio in rumen fluid from fistulated steers. However, Franks et al (37) found that feeding reconstituted or steam-flaked sorghum rather than dry ground sorghum did not significantly affect VFA patterns or levels in the rumen fluid of steers.

Woods and Wilson (128) observed that substituting gelatinized corn for raw ground corn resulted in decreased molar proportions of acetate and butyrate and increased propionate. Similarly, Hentges

et al (53) reported that steam-flaking raw ground corn increased the molar proportion of propionate and total VFA concentration in rumen fluid. However, other research groups have found no significant differences in molar proportions of individual fatty acids (27, 39, 86, 114) or total VFA concentrations (27, 75) when either ground or heat processed corn was fed to steers. There have also been reports of decreased molar proportions of propionate and butyrate (75), propionate alone (31) and decreased total VFA concentrations (114) when heated instead of raw ground or cracked corn was included in the steer ration.

Another investigation (116) showed that feeding rations containing high-moisture and reconstituted corn rather than raw ground corn resulted in higher levels of acetate and lower levels of propionate in rumen fluid. In contrast, Galyean et al (39) found that steers fed a ration containing 78% ground high-moisture corn had significantly higher proportions of propionic and butyric acid and significantly lower proportions of acetic acid in the rumen liquor than those fed rolled corn.

Papsolomontos and Wilkinson (89) commented that animal variation, the nature and physical form of other components of the diet, the ratio of concentrate to roughage and level of feeding as well as the degree of processing are probably factors contributing to the inconsistent effect of processing methods on VFA production.

MATERIALS AND METHODS

A. Animals

Two steers, one Angus (364 kg) and one Hereford (436 kg) fitted with permanent rumen cannulae, were maintained as sources of inoculum. They were housed in an unheated barn with access to an outdoor pen and fed simple hay and grain maintenance diets.

Twenty-one days were allowed for the steers to adapt to the experimental diets. The animals were confined in a stanchion while consuming their diet.

The Angus received a 65% concentrate diet consisting of 4.5 kg of a 50:50 mixture of locally grown barley and oats and 2.3 kg of good quality alfalfa-grass hay. Rumen fluid from this animal was used for *in vitro* digestibility determinations of barley, oats and rye. The Hereford consumed 2.7 kg of coarsely ground corn daily, which was the maximum that would be consumed voluntarily, and 2.3 kg of good quality alfalfa-grass hay. Rumen fluid obtained from the Hereford was used for *in vitro* digestibility determinations of corn. A salt block and fresh water were available at all times. Half of the designated grain diet was fed to each animal at 8:30 AM and at 4:00 PM.

B. Grain Processing Methods

Waxy barley (high amylopectin) and normal barley grown at Indian Head, Saskatchewan and locally grown rye, high-fat oats and normal oats were processed by seven different methods. Locally grown dent corn was processed in the same manner as the other grains and also by

a popping and roasting process. Following processing, the grains were dried in a forced air dryer at 30 C for 48 h then ground in a Wiley mill through a 1 mm mesh screen. Details of the processing methods are as follows:

Dry ground - The dried, but otherwise unprocessed whole grain, was ground through a 1 mm mesh screen of a Wiley Mill.

Reconstituted whole - Water (22 C) was added to the grain to raise the moisture level to approximately 30%, the grain was mixed and the container covered with parafilm. The grain was left at room temperature (22 C) for 8 h with periodic mixing. At the end of this time period, the grain was tightly compacted in 100 ml boiling tubes and sealed with parafin wax. A sample was reserved for dry matter analysis. The tubes were stored for twenty-one days in the dark at 22 C.

Reconstituted ground - The procedure was the same as the reconstituted whole method except that the grains were ground through a 5 mm mesh screen prior to reconstitution.

Dry heat - Whole grains were spread in a single layer in a large metal pan and heated in an oven at 92 C for 8 min.

Steam processed - Steaming was accomplished in a Retort Steam Processor, Sprague Sells, Model 29. A sample of whole grain was processed for 8 min at 95 C at a pressure gauge reading of 0 kg/cm^2. Another sample of whole grain was processed in the same processor for 1.5 min at 115 C and a pressure gauge reading of 6.8 kg/cm^2.

Cooked - The cooking procedure was carried out as described by Keating et al (65). One part whole grain was combined with two parts water in a shallow metal pan, the pan was covered tightly with aluminum foil and the grain cooked in an oven at 70 C for 14 h.

Popped - Corn was heated in an oven at 176 C for 10 min with occassional shaking of the cooking pan. In 40% of the grain, the expanding endosperm split the testa.

Roasted - Whole corn was roasted in a coffee bean roaster, Jabez Burns and Sons, Model No. 12X514, for 6 min at a temperature of 134 C.

C. In Vitro Technique

The in vitro dry matter disappearance technique employed in this investigation was a method described by Ingalls (59) for forage evaluation, with minor modifications.

a. Fermentation Vessels

The fermentation vessels consisted of 80 ml flanged boiling tubes fitted with one-hole rubber stoppers (No. 4) and bunsen valves. The valve was made from a 5 cm piece of glass tubing inserted into a 7.5 cm piece of rubber tubing which had a 1.5 cm slit to allow fermentation gases to escape.

b. Buffer Solution

Approximately 12 h prior to collection of the rumen fluid, 1000 ml of a 0.1 M phosphate buffer was prepared by diluting 4.08 g of KH_2PO_4 and 8.73 g Na_2HPO_4 with distilled water. The buffer was warmed to 39 C over this 12 h period. Stock solutions of urea and sodium carbonate were prepared by diluting 8.00 g of urea and 18.40 g of sodium carbonate ($Na_2CO_3 \cdot H_2O$) to 100 ml with distilled water. One hour before collection of rumen fluid 20 ml of the sodium carbonate stock solution and 25 ml of the urea stock solution were added to

the prewarmed phosphate buffer. Concentrated H_3PO_4 was added to bring the pH of the buffer to 6.8.

Samples (0.5000 g) of the ground processed grain were weighed into the boiling tubes and 20 ml of the prepared buffer was added to the samples just before rumen fluid collection.

c. Rumen Inoculum Preparation

Rumen fluid was collected prior to the 8:30 AM feeding. Water was withheld at least ½ h before the collection. Ingesta was obtained by hand from the central region of the rumen and the liquor was expressed through four layers of cheescloth into a prewarmed thermos jug and transported quickly to the laboratory. The rumen fluid was transferred to 1000 ml Erlynmeyer flasks and maintained at 39 C for ½ h to allow particulate matter to form a layer on the surface of the fluid. Most of the particulate matter was then removed by aspiration and the rumen fluid was filtered through four layers of cheescloth into a 1000 ml beaker. A gas mixture of 90% N_2 and 10% CO_2 was bubbled through the rumen fluid for 2 min while stirring gently with a magnetic stirrer.

d. Inoculation of Fermentation Vessels

Following rumen inoculum preparation, 20 ml of rumen fluid were added to each boiling tube with a volumetric pipette and the beaker was stirred continuously. A gas mixture of 90% N_2 and 10% CO_2 was bubbled through the rumen fluid during inoculation of the tubes. Four to six blanks containing buffer and rumen fluid only were included in each digestibility trial to determine residual nonfilterable

dry matter originating from the inoculum. The entire inoculation procedure was carried out in a walk-in temperature controlled incubator (Canlab. Model 1280HP) maintained at 39 C.

When all fermentation tubes had been inoculated, they were stoppered, swirled and incubated for 24 h in a converted refrigerator unit with an attached temperature control (Model 805, Incubator, Cat. No. 31213) set at 39 C. The contents of the fermentation tubes were swirled after 4 and 8 h of incubation. At the end of the fermentation period, the tubes were placed in a freezer until thoroughly cooled.

Dry matter disappearance was determined by filtering the fermented material through a tared Pyrex crucible (see Appendix A) with a coarse sintered glass filter. To aid filtering a pad of Celite 545 Diatomite Filtering Agent was prepared in the crucible. Supernatant was removed by light vacuum. Two to three washings with 60 ml of distilled water were required to transfer all of the material from the tube to the filter. The filtering crucible was then dried in a forced air oven at 80 C for 24 h, placed in a dessicator for at least 1.5 h then weighed. Percent <u>in vitro</u> dry matter disappearance (IVDMD) was calculated according to the following formula (4):

$$\text{IVDMD} = \frac{\text{g DM in substrate} - \left(\text{residual DM} - \text{g DM in 20 ml of inoculum}\right)}{\text{g DM in substrate}} \times 100\%$$

Four digestion trials with each trial including three replicates of each treatment were conducted for each species of grain. The trials were carried out on four different days using a fresh inoculum preparation from the same steer for each day. Further details are provided in the Experimental Design.

D. Volatile Fatty Acid Analysis

In one of the four digestion trials described for the IVDMD determinations, duplicate samples for each processing method were included for volatile fatty acid analysis. At the end of the 24 h fermentation period the contents of the duplicate tubes were combined and filtered through three layers of cheesecloth into a plastic container. One milliliter of concentrated $HgCl_2$ was added to each container to stop fermentation and the samples were frozen until analysis could be carried out.

On the day of analysis the samples were thawed and prepared as described by Erwin (34). One milliliter of metaphosphoric acid (25%, w:v) was added to 5 ml of rumen fluid in a 20 ml capped centrifuge tube. After standing for 30 min, the contents were centrifuged at 3000 rpm for 10 min. The supernatant was removed immediately and stored in screw cap vials. Analysis by gas-liquid chromatography was carried out in duplicate.

The instrument used was a Burrell gas-liquid chromatograph (Model JG, Burrell Corp., Pittsburgh, Pa.) fitted with a flame ionization detector. The flash vaporizer temperature was maintained at 220 C to provide rapid vaporization of the sample. The 2 m stainless steel column with a 0.6 cm diameter was packed with 20% MPGS on Firebrick 60:80 with 2% H_3PO_4. Oven and detector temperatures were 150 and 220 C respectively. The helium carrier gas flow rate was 50 ml/min, hydrogen flow to the flame jet was 20 ml/min and air flow to the detector chamber was 300 ml/min. Under these conditions free fatty acids and their isomers were eluted in approximately 16 min. Molar

proportions of the volatile fatty acids were determined by comparing peak areas with those of a standard solution of known concentration.

E. Feed Grain Analysis

Dry matter, crude protein (N × 6.25), fat (ether extract), and crude fiber were determined by methods according to the Association of Official Agricultural Chemists (10). Starch content was determined by an unpublished method (R. A. MacGregor, Barley Lab., Canadian Grain Commission, Winnipeg, Manitoba) in which a ground (0.05 mm screen) grain sample was dispersed in 25 ml of distilled water, boiled for approximately 3 min, autoclaved at 121 C for 4 h, then digested with amyloglucosidase (genus Rhizopus, Sigma Chemical Co.) at 55 C, pH 4.8. Total glucose released was determined by adding 2 ml of enzyme-buffer-chromogen mixture (Glucostat Special, Worthington Biochemical Corp., Freehold, N.J.) to the digested sample and allowing color to develop in the dark at 37 C for 30 min. Absorbance was measured at 540 nm and compared to a standard D-glucose curve. The extent of gelatinization of heat treated grains was determined as mg maltose/g sample released after incubation with beta-amylase (111).

F. Experimental Design

A randomized complete block statistical design was used to analyze in vitro dry matter digestibility data of rye and corn (109). Each of the feed grains studied was blocked on four rumen inocula, each block representing a separate in vitro digestion trial of three replications of each treatment (Table 5).

The means of in vitro dry matter disappearance determinations

were compared for statistically significant differences among treatments using Student-Newman-Keul's multiple range test (66).

Table 5. Statisical design for rye and corn experimental trials

Trial	Treatments†						
1	DG	DH	RGr	RWh	SA	SP	Cook
2	DH	Cook	DG	RGr	SP	RWh	SA
3	SA	RWh	SP	Cook	DG	DH	RGr
4	SP	RGr	RWh	DH	Cook	SA	DG

†DG - Dry ground
DH - Dry heat
RGr - Reconstituted ground
RWh - Reconstituted whole
SA - Steamed at 0 kg/cm^2
SP - Steamed at 6.8 kg/cm^2
Cook - Moist cooking for 14 h

A split-plot randomized complete block design (70) was used to analyze data from in vitro dry matter disappearance trials involving barley and oat cultivars. Again, results were blocked on four rumen inocula, each block representing a separate in vitro digestion trial of three replicates of each treatment (Table 6). Main plot treatments were variety and the sub-plot treatments were processing method. This design was used because it is very sensitive in determining sub-plot treatment differences and indicating any interaction between main plot and sub-plot treatments. Following the appropriate F tests for main and sub-plot treatments and interaction, treatment means were subjected to Student-Newman-Keul's multiple range test.

Table 6. Statistical design for oat and barley experimental trials

Trial 1		Trial 2		Trial 3		Trial 4	
V1†	V2‡	V1	V2	V1	V2	V1	V2
DG	RGr	DH	Cook	RGr	RWh	SP	SA
DH	SA	Cook	SA	SP	Cook	RGr	RWh
Cook	RWh	DG	RGr	SA	DH	Cook	SP
RGr	Cook	RWh	SP	DH	SA	SA	DG
RWh	SP	RGr	DH	DG	RGr	DH	Cook
SA	DH	SP	DG	Cook	SP	RWh	RGr
SP	DG	SA	RWh	RWh	DG	DG	DH

†V1 - variety 1
‡V2 - variety 2

In vitro volatile fatty acid data and gelatinization data were analyzed for statistical significance using a completely randomized design and significance among treatment means was determined by Tukey's test (109).

RESULTS

A. In Vitro Dry Matter Disappearance (IVDMD)

The data for rye, corn, oats and barley are tabulated separately since treatment differences among the cereals were not compared. The values given are the means of observations from four digestion trials. A summary of the mean squares from the analysis of variance is presented in Appendix B.

a. Rye

Dry matter disappearance data for rye are arranged in order of increasing digestibility (Table 7).

Table 7. Average IVDMD of rye processed by various methods

Treatment	IVDMD (%)
Cooked	62.41a
Steamed 6.8 kg/cm^2	64.75ab
Steamed 0 kg/cm^2	67.50b
Dry ground	68.46b
Reconstituted ground	68.63b
Dry heat	69.98b
Reconstituted whole	70.65b

a,b Treatment means bearing different letters differ significantly ($P < 0.05$).
SE = 1.36

Cooked rye had a significantly ($P < 0.05$) lower IVDMD than other treatments except for rye steam heated at a pressure of 6.8 kg/cm^2. Although reconstituted whole rye had the greatest IVDMD value, it was similar to all treatments other than cooked rye.

b. Corn

Reconstituted ground corn had a significantly ($P < 0.05$) greater IVDMD than any other treatment (Table 8). Corn which was popped or steamed at 6.8 kg/cm^2 had a significantly ($P < 0.05$) lower IVDMD than the other treatments. Small though significant ($P < 0.05$)

Table 8. Average IVDMD of corn processed by various methods

Treatment	IVDMD (%)
Popped	45.33a
Steamed 6.8 kg/cm^2	47.21ab
Roasted	49.48b
Steamed 0 kg/cm^2	52.29c
Cooked	53.15cd
Dry heat	55.42de
Dry ground	56.75e
Reconstituted whole	58.42e
Reconstituted ground	62.12f

a,b,c,d,e,f Treatment means bearing different letters differ significantly ($P < 0.05$).
SE = 0.87

differences existed between values obtained for cooked, steamed 0 kg/cm^2 and roasted corn. Dry matter digestibilities of whole reconstituted corn, dry ground and dry heated corn were not statistically ($P > 0.05$) different.

The IVDMD of dry ground corn was found to be 15% higher than that obtained by Tonroy and Perry (116) and 2.5% higher than that of another publication by Tonroy and Perry (117).

c. Barley

For both cultivars of barley, cooking improved IVDMD over all

Table 9. Average IVDMD of normal and waxy barley processed by various methods

Treatment	IVDMD (%)	
	Normal	Waxy
Dry ground	60.33a	65.55a
Reconstituted ground	61.56a	64.76a
Reconstituted whole	61.85a	63.74a
Dry heat	62.06ab	63.10a
Steamed 6.8 kg/cm^2	62.45ab	61.43a
Steamed 0 kg/cm^2	62.96ab	63.21a
Cooked	65.85b	69.41b
SE	1.02	1.02

a,b Treatment means in the same column bearing different letters differ significantly ($P < 0.05$).
SE = 1.56 for subplot (processing) treatments for different main (variety) plots.

other treatments but this was significant ($P < 0.05$) only for waxy barley (Table 9). There was an interaction ($P < 0.05$) between variety of barley and two processing methods, dry grinding and steam pressure (6.8 kg/cm^2) processing.

Processing normal barley beyond a simple grinding process increased IVDMD although non-significantly ($P > 0.05$) for most treatments. In contrast, subjecting waxy barley to various treatments except for the cooking process, reduced ($P < 0.05$) IVDMD slightly compared to dry ground waxy barley. Among those processing treatments where there was no variety-processing method interaction, there were no significant ($P > 0.05$) differences due to variety.

d. Oats

Analysis of data by split-plot randomized complete block revealed that interaction between variety and processing treatment was not significant ($P > 0.05$), nevertheless, confounding of variety and blocks was suspected (Table 10). The effect of variety on IVDMD just approached significance so the average of the varieties could be taken to determine overall processing treatment effect. In general, high-fat oats had an average advantage of 4.73% in dry matter disappearance compared to normal oats.

As with the barley cultivars, cooking substantially increased the IVDMD of oats compared to all other treatments. The average IVDMD for all treatments except cooked oats were similar. Steaming at 0 kg/cm^2 or a pressure of 6.8 kg/cm^2 appeared to be detrimental to IVDMD of both high-fat and normal oats.

Table 10. Average IVDMD of normal and high-fat oats processed by various methods

Treatment	IVDMD (%)		
	Normal	High-fat	Average
Steamed 0 kg/cm^2	39.05a	43.26a	41.16a
Steamed 6.8 kg/cm^2	40.14a	43.06a	41.60a
Dry heat	40.55ab	46.37ab	43.46a
Reconstituted whole	41.55ab	46.81ab	44.18a
Dry ground	41.79ab	45.97ab	43.88a
Reconstituted ground	41.85ab	47.47ab	44.66a
Cooked	45.36b	50.75b	48.06b
SE	1.22	1.22	0.86

a,b Treatment means in the same column bearing different letters differ significantly ($P < 0.05$).

B. In Vitro Production of Volatile Fatty Acids (VFA)

VFA concentrations were calculated on the basis of the quantities of acetic, propionic, isobutyric, butyric, isovaleric and valeric acids present in rumen fluid, but only values for acetic, propionic and butyric acids are reported because isobutyric, isovaleric and valeric acids varied only slightly among treatments. The molar proportions of acetic, propionic and butyric acids and the acetate:propionate (A/P) ratio are tabulated separately for each cereal; rye, corn, oats and barley. No statistical comparisons were made between cereals since the in vitro digestibility trials were carried out on different days.

a. Rye

Generally the heat treated rye had lower levels of acetate and higher levels of butyrate than non-heat treated rye (Table 11). Although non-significant ($P > 0.05$), two heat processing methods, dry heat and cooked, resulted in higher *in vitro* propionate production. Steaming rye at pressures of 6.8 kg/cm^2 significantly ($P < 0.05$) lowered propionate levels compared to cooking or dry heating rye. The A/P ratio was somewhat lower for the heat processed grain than the "cold" processed grain with the exception of rye steamed at 6.8 kg/cm^2 which had a significantly ($P < 0.01$) higher A/P ratio than cooked or dry heated rye.

b. Corn

Generally "cold" processing resulted in lower levels of acetate and higher levels of butyrate than corn processed with dry or moist heat (Table 12). Acetate was significantly ($P < 0.01$) lower and propionate significantly ($P < 0.01$) higher for reconstituted ground corn than for any other treatment. Popping corn resulted in significantly ($P < 0.01$) higher levels of acetate than all treatments except corn steamed at 6.8 kg/cm^2 and significantly ($P < 0.01$) lower levels of propionate. Heat treated corn tended to have a higher A/P ratio than reconstituted ground and dry ground corn but not reconstituted whole grain. Cooked corn produced the most favorable A/P ratio of the heat processed grain.

c. Barley

Molar proportions of VFA and the A/P ratios for normal and waxy

Table 11. Effect of rye processing method on *in vitro* VFA production†

Treatment	VFA			A/P ratio
	Acetic	Propionic	Butyric	
Cooked	54.62a	24.43a	17.38a	2.23A
Dry heat	55.50a	23.61a	16.77ab	2.35AB
Steamed 0 kg/cm^2	57.34b	22.68ab	16.44ab	2.53ABC
Steamed 6.8 kg/cm^2	57.37b	20.41b	18.31a	2.81C
Dry ground	58.52bc	22.79ab	14.90bc	2.57ABC
Reconstituted whole	59.45c	22.54ab	13.90c	2.64BC
Reconstituted ground	61.38d	21.61ab	13.01c	2.84C
SE	0.28	0.54	0.41	0.05

a,b,c,d; A,B,C Treatment means in the same column with different lower case or capital letters are significantly different at $P < 0.05$ and $P < 0.01$ respectively.

†Volatile fatty acid concentrations are expressed as molar percent.

Table 12. Effect of corn processing method on *in vitro* VFA production

Treatment	VFA			A/P ratio
	Acetic	Propionic	Butyric	
Reconstituted ground	57.14a	26.04a	13.00ab	2.19a
Dry ground	59.28b	23.99b	13.19ab	2.47b
Reconstituted whole	60.54bc	22.29c	13.97a	2.72cd
Roasted	61.08bcd	22.77c	12.95ab	2.68cd
Steamed 0 kg/cm^2	61.32cd	22.91c	12.65abc	2.68cd
Dry heat	61.65cd	22.35c	12.93ab	2.76d
Cooked	62.52d	24.43b	11.17c	2.56bc
Steamed 6.8 kg/cm^2	62.61de	21.99c	12.65abc	2.85d
Popped	64.45e	20.07d	12.40bc	3.21e
SE	0.25	0.14	0.21	0.02

a,b,c,d,e Treatment means in the same column bearing different letters are significantly different ($P < 0.01$)

barley are given in Table 13. The amount of data was insufficient to compare the two cultivars statistically, since only a single experimental run was carried out.

VFA patterns of waxy and normal barley were very similar among treatments. Generally, steam processing and reconstitution resulted in higher levels of acetate and lower levels of propionate for both varieties of barley compared to cooked, dry heated and dry ground barley. In addition, steaming, either at 0 or 6.8 kg/cm^2 tended to increase the level of butyrate. For both varieties the A/P ratios were significantly ($P < 0.01$) lower for cooked than for reconstituted whole, reconstituted ground barley or barley steamed at 0 kg/cm^2.

d. Oats

Although the two varieties of oats showed nearly identical response to treatment in dry matter digestibility trials, fewer similarities in VFA production were readily discernable (Table 14). Steam processing normal oats either at 0 or 6.8 kg/cm^2 and reconstituting ground normal oats increased acetate production and decreased propionate production significantly ($P < 0.01$) compared to dry ground normal oats. A similar response was obtained with the high-fat oats but it was non-significant ($P > 0.01$). However, steaming high-fat oats at 0 kg/cm^2 significantly ($P < 0.01$) increased acetate and decreased propionate compared to cooked and reconstituted whole high-fat oats. Again a similar response was noted with normal oats with the only significant ($P < 0.01$) difference occurring in propionate production.

Cooked high-fat oats produced the most favorable A/P ratio which

Table 13. Effect of processing barley on *in vitro* VFA production

Treatment	VFA						A/P ratio	
	Acetic		Propionic		Butyric			
	Waxy	Normal	Waxy	Normal	Waxy	Normal	Waxy	Normal
Cooked	56.95a	59.31ab	23.15a	23.59a	15.46a	13.23a	2.45a	2.51a
Dry ground	57.99ab	57.09a	22.13ab	21.27b	15.62ab	16.06bc	2.62ab	2.68ab
Dry heat	58.64ab	58.02a	22.30ab	20.84b	15.11a	16.56bc	2.63ab	2.78ab
Reconstituted whole	59.09ab	59.26ab	19.62c	20.43b	16.48abc	15.40abc	3.01bc	2.90b
Steamed 0 kg/cm^2	59.37ab	58.48ab	19.38c	19.98b	17.32c	17.70c	3.06c	2.92b
Steamed 6.8 kg/cm^2	59.59ab	58.94ab	19.78c	20.55b	17.20bc	17.20c	3.01bc	2.87ab
Reconstituted ground	60.55b	60.47b	20.08c	20.22b	15.28a	14.71ab	3.02c	2.99b
SE	0.46	0.29	0.20	0.29	0.22	0.32	0.05	0.05

a,b,c Treatment means in the same column with different letters differ significantly ($P < 0.01$).

Table 14. Effect of processing oats on *in vitro* VFA production

Treatment	VFA						A/P ratio	
	Acetic		Propionic		Butyric			
	High-fat	Normal	High-fat	Normal	High-fat	Normal	High-fat	Normal
Cooked	61.05a	60.85ab	22.02a	21.55ab	13.38	13.41	2.77a	2.82ab
Reconstituted whole	61.31a	61.14ab	21.93a	21.01abc	12.71	13.11	2.80a	2.91bc
Dry heat	61.54ab	62.25b	21.83a	20.31bc	12.84	13.35	2.82a	3.06bc
Dry ground	61.98ab	58.49a	19.78abc	22.67a	13.77	14.47	3.13ab	2.58a
Reconstituted ground	62.80ab	61.86b	20.58ab	20.94bc	12.70	12.62	3.05ab	2.95bc
Steamed 6.8 kg/cm^2	64.27ab	62.48b	18.04c	19.67cd	13.93	14.44	3.56b	3.18cd
Steamed 0 kg/cm^2	65.17b	62.93b	18.58bc	18.53d	12.76	14.14	3.51b	3.40d
SE	0.50	0.35	0.31	0.21	0.27	0.29	0.08	0.04

a,b,c,d Treatment means in the same column bearing different letters differ significantly ($P < 0.01$).

was significantly ($P < 0.01$) lower than that obtained from fermentation of steam processed high-fat oats. The lowest A/P ratio for normal oats was obtained with the dry ground treatment while steam processing produced the least favorable A/P ratio. All other A/P ratios were intermediate.

C. Starch Gelatinization and Chemical Analysis

A measurement of gelatinization of starch in heat treated grain was made to assess the extent of starch structural change. Gelatinization determinations for various treatments are given in Table 15.

The gelatinization data for corn is consistent with the expectation that increasing cooking intensity will increase the extent of gelatinization. For normal barley and normal oats the extent of gelatinization for dry heated and steam treated (0 kg/cm^2) grain was similar to dry ground grain. Dry heated and dry ground high-fat oats also showed the same degree of gelatinization. The mg maltose/g grain released from dry ground waxy barley was the same as that from waxy barley steamed at 0 kg/cm^2 but significantly ($P < 0.05$) greater than that from dry heated waxy barley. Rye, which was dry heated and steamed at 0 kg/cm^2 showed a significantly ($P < 0.05$) lower degree of gelatinization than dry ground and steam pressure cooked (6.8 kg/cm^2) rye. For all of the grains studied, cooking resulted in a significantly ($P < 0.05$) higher degree of gelatinization than any other heat processing method.

Chemical analysis for rye, oats, barley and corn are given in Table 16. High-fat oats and normal oats differed substantially in crude fat and crude fiber content whereas the barley varieties were similar.

Table 15. Degree of gelatinization of processed cereal grains

Treatment	Gelatinization (mg maltose/g grain)					
	Normal Barley	Waxy Barley	Rye	High-fat Oats	Normal Oats	Corn
Dry ground	115.4a	128.2a	145.3a	116.9a	104.6a	72.7a
Dry heat	113.8a	115.7b	97.7b	115.6a	103.1a	80.5a
Steamed 0 kg/cm^2	108.8a	123.2ab	100.7b	121.6b	113.3a	91.3b
Steamed 6.8 kg/cm^2	145.9b	152.7c	137.2a	200.5c	186.0b	106.0c
Cooked	454.3c	514.0d	392.0c	347.3d	371.0c	262.5e
Roasted	-	-	-	-	-	77.5a
Popped	-	-	-	-	-	137.6d
SE	2.00	1.74	1.57	0.68	2.20	1.67

a,b,c,d,e Treatment means in the same column bearing different letters are significantly different ($P < 0.05$)

Table 16. Chemical analyses of barley, oats, rye and corn

	Dry matter (%)		Protein (N × 6.25) (%)		Crude fat (%)		Crude fiber (%)		Starch (%)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Normal Barley	93.42	0.10	14.73	0.10	1.74	0.10	5.73	0.10	54.51	0.34
Waxy Barley	93.11	0.10	14.15	0.10	1.88	0.10	5.64	0.10	52.03	0.31
Normal Oats	93.77	0.10	12.52	0.10	4.02	0.10	13.08	0.10	41.80	0.34
High-fat Oats	93.70	0.10	13.50	0.10	7.01	0.10	9.25	0.10	43.87	0.00
Rye	93.14	0.10	11.59	0.10	1.56	0.10	2.18	0.10	67.70	0.45
Corn	92.57	0.10	9.50	0.10	3.94	0.10	1.98	0.10	72.04	0.67

DISCUSSION

A. Closed *In Vitro* Systems

a. Microbial Populations

In vitro systems designed for the quantitation of a few processes occurring in the microbial population are marked by simplicity of their design and procedure and also the ability to conduct large numbers of studies. This simplicity, however, has caused the system to be subject to criticism and question as to whether the microorganisms being propagated are typical of the rumen population in the intact animal. Johnson (60) believes that there is enough published evidence to demonstrate that bacteria propagated *in vitro* can be truly representative of those found in the intact rumen. Thus, the assumption can be made that the activities being measured are similar to those occurring *in vivo*.

Another major criticism of closed systems is the elimination of protozoa from the population. What effect this would have had on the present investigation of *in vitro* feed grain degradation by rumen microorganisms is unknown.

b. pH

Since the buffering capacity of the buffer medium is the only means by which pH can be controlled internally in an all-glass system, the pH in these systems often changes during the process of fermentation. The pH optimum for starch digestion has been found to be around 6.8 (73). Moore *et al* (73) found that pH did not drop markedly

until between 6 and 12 hours after inoculation. After this time the pH dropped rapidly necessitating hourly readjustment of the pH to 6.8 in order to maximize starch degradation activity.

The buffer media used by Moore et al (73) had a much lower buffering capacity than the media used in the present investigation. It was felt that any benefit to be had from adjusting pH of the fermentation mixture would be offset by loss of anaerobic conditions.

c. Inoculum Source

Source of inoculum has been found to have a profound effect on dry matter disappearance of various physical forms of corn (126). With inoculum from steers fed a high concentrate ration the IVDMD of raw corn was significantly greater than that of reconstituted corn (126). This was reversed when inoculum was taken from a steer fed an alfalfa-hay ration (126). It is likely that in vitro results more truly represent in vivo activities if the donor ration and the sample to be examined are similar in composition.

It has been reported elsewhere (126) and was noted in the present investigation that inoculum from a steer fed a high concentrate ration creates sample inoculation and filtering problems with resulting error. In addition, the inoculum which is very thick in consistency produces large blank weights which can be extremely variable within trials. Rumen fluid from steers fed a 65% grain ration or 2.7 kg of coarsely-ground corn was somewhat viscous but pipetting and filtering were carried out with a minimum of difficulty. Whole rumen fluid was desired since it contains nutrients and growth factors for the microorganisms.

d. Incubation Time Period

Tonroy and Perry (117) have emphasized a need for more than one incubation period to evaluate grain processing effects. Apparently, comparisons based on only one length of incubation period do not give a complete picture of changes in ease of utilization or total utilization that may result from processing. However, upon evaluating recent observations on in vitro dry matter digestibility of various processed grains over different incubation periods (3, 29, 39, 116), it appeared that a 24 hour fermentation period would be suitable for the present investigation. It was felt this length of incubation period would best represent changes in total utilization that may have resulted from processing. Due to the large number of grain processing treatments to be screened, particularly with barley and oats, it was not feasible to use more than one incubation period.

B. In Vitro Dry Matter Disappearance

a. Rye

Recent experimentation with concentrate rations containing 0, 40, 60 and 80% rye at Federal Research Stations (19, 20) has indicated that 60-80% ergot free rye can be included in growing and finishing rations without a reduction in intake or gain. To date there are no reports of rye digestibility either in the intact bovine or in vitro since rye is not considered an important feed grain due to its low palatability and tendency to produce digestive upsets.

The IVDMD value for dry ground rye (Table 7) was similar to that reported for the 24 hour incubation of wheat (24). The high starch

and low fiber content could be factors contributing to high IVDMD. It appears that digestibility of rye like wheat (3, 24) is not improved by processing, and may, as in the case of cooked rye, be reduced by intensive processing. Perhaps the nature of the starch granule or the protein matrix surrounding the starch is such that unprocessed rye starch is readily available to hydrolytic enzymes.

Aimone and Wagner (3) suggested that heat treatment during micronization lowered protein solubility of wheat in in vitro rumen cultures having wheat as the only source of nitrogen. Supposedly a lack of available protein resulted in lower IVDMD of micronized wheat compared to dry-rolled wheat. However, this cannot be the case with cooked rye or rye steamed at 6.8 kg/cm^2 since 38 mg of urea were added to each culture tube. A probable explanation for the decreased in vitro digestibility of rye treated in this manner is retrogradation of the starch.

Since the IVDMD of rye so closely approaches that for wheat, the feeding value of rye may also approach that of wheat, however, it has been suggested that rye may contain growth-limiting factors (25).

b. Corn

Reconstituting whole corn (Table 8) did not significantly ($P > 0.05$) affect IVDMD compared to dry grinding. Similar results were obtained by Tonroy and Perry (116) but White et al (126) , using a 48 hour incubation period and a buffer media without supplemental nitrogen, found reconstituted whole corn to be more digestible than untreated corn. Further experimentation (126) showed that addition of urea to the incubation media altered relative IVDMD values of

processed corn.

In the present investigation, corn which was ground before reconstitution (Table 8) had a significantly ($P < 0.05$) greater IVDMD than dry ground corn and whole reconstituted corn. These results are not in agreement with those of Newhaus and Totusek (85) who found that reconstituting sorghum grain in the ground form resulted in little improvement in in vitro digestibility, while digestibility of the grain reconstituted in the whole form was increased greatly. They (85) reasoned that beneficial effects of reconstitution result from partial germination within the intact grain rather than a simple fermentation process. From their microscopic examinations Sullins and Rooney (110) established that a high degree of disorganization of subcellular constituents occurs in the whole sorghum kernel during reconstitution. They believe this disorganization is probably caused by enzyme activity similar to that which takes place during malting. Sullins and Rooney (110) contend however, that some proteolytic and amylolytic activity took place in reconstituted ground sorghum. Furthermore, it was observed that water added to the ground sample penetrated the particles thoroughly, softening the protein matrix (110).

The nature of the seed coat of corn may be responsible for lack of a favorable response both in vitro (116) and in vivo (17, 71) when dry whole corn is reconstituted. It is very difficult to distribute moisture evenly in whole corn to raise the moisture level to 30% without the addition of heat. Grinding prior to reconstitution increases ease of moisture penetration and perhaps, as a result, the enzymatic hydrolysis of protein, starch and other carbohydrates begins earlier in the storage period. This could account for the improvement

in IVDMD of dry corn when it was subjected to grinding prior to reconstitution in the present investigation (Table 8).

Processing corn by roasting or pressure-flaking (117, 126) has been found to decrease IVDMD although in vitro starch digestion may be improved by roasting (117). IVDMD values in Table 8 indicate that heat treatment reduces dry matter digestibility by rumen organisms compared to unheated corn. Cooked corn had a significantly ($P < 0.05$) higher IVDMD than popped, roasted and pressure steamed corn which is surprising since the starch must certainly have been mostly, if not completely, retrograded by the moist cooking process. The gelatinization results (Table 15) show that changes in starch structure due to cooking were very extensive. Heating corn at low moisture levels was evidently more detrimental to IVDMD than overcooking in the presence of excess moisture.

It is possible that the nature of corn starch is such that some of the retrograded starch resolubilized in the fermentation media becoming susceptible to enzymatic degradation. Another possibility is that in the presence of 8-20% moisture the addition of heat resulted in some of the undesirable reactions described by Sieb (107), these being: (i) formation of unnatural glycosidic linkages between sugar units in the polymer chains of starch; (ii) depolymerization of starch followed by reaction of newly exposed aldehyde groups with the e-amino group of lysine; (iii) encapsulation of starch within a tough rubbery protein matrix and (iv) production of free fatty acids by hydrolysis of fats and subsequent formation of relatively stable complexes of amylose with the newly formed fatty acids and monoglycerides.

Lack of optimum processing conditions and equipment may account for the very low IVDMD observed for popped corn (Table 8).

c. Barley

Since it has been reported that amylopectin is more readily digested than amylose (103), it was considered desirable to introduce the waxy gene into a commercial feed barley variety. Although waxy barley and normal barley used in this study were not analyzed for amylopectin content, it is known that starches from common varieties of cereals contain 73-80% amylopectin and starches from waxy varieties of corn, sorghum, barley and rice contain 93-100% amylopectin (107). In the present investigation no significant ($P > 0.05$) differences in IVDMD were found between waxy and normal barley, however, waxy barley appeared to be slightly more digestible than normal barley. Perhaps further study of these two varieties is warranted to determine the relative value of waxy barley as a feed grain.

Barley starch has been found to be highly digested in the rumen (94% ± 2.4) and very little variation in digestibility occurs from using different processing methods (123). Nevertheless, it is possible that processing methods could enhance the digestibility of other components of barley. The results for IVDMD in Table 9 indicate that most processing methods did not significantly ($P > 0.05$) affect dry matter digestibility of barley. However, cooking of barley increased IVDMD of both varieties of barley compared to other treatments. This increase may have been due to the effect of cooking on the less digestible fiber portion of the grain.

Statistical analysis of the data revealed a significant ($P < 0.05$)

interaction between variety of barley and processing method. If *in vitro* digestibility techniques are sensitive enough to indicate such interactions, possibly these techniques may be useful in quality control of premixed feedstuffs and commercially processed feed grain.

d. Oats

Oats are not considered an important feed grain due to low energy content compared to other feed grains. Oats also have a high fiber content which makes them undesirable for swine or poultry rations. However, oats are a popular feed grain for horses, dry cows and young ruminants because of their palatability. In addition, oats are useful in diluting the energy content of grain mixtures while maintaining protein level.

Recently a high-fat oat variety has been bred which contains approximately 7% crude fat (Table 16). A total energy determination by bomb calorimetry showed that high-fat oats contain 100 cal/g more gross energy than normal oats. Crude fiber content of high-fat oats has been found to be substantially lower than that of normal oats.

Processing beyond a dry grinding treatment had little effect on IVDMD of oats (Table 10). Intensive cooking with 2 parts water to 1 part grain increased dry matter digestibility compared to other treatments, possibly by rendering the fiber component of oats more digestible. Since there were no significant ($P > 0.05$) variety or interaction effects, treatment means were averaged and IVDMD for cooked oats was found to be significantly ($P < 0.05$) greater than for any other treatment. The consistently higher IVDMD of high-fat oats compared to normal oats, though non-significant ($P > 0.05$) was probably

due to its lower crude fiber content and higher crude fat content rather than the effect of processing on starch. Waldo (123) suspects that intact oat starch is mostly if not completely digested in the rumen.

C. In Vitro Volatile Fatty Acid Production

A close relationship between VFA patterns and feedlot performance, particularly feed efficiency, would facilitate research with limited numbers of animals to circumvent costly, time-consuming feedlot trials and aid in interpretation of results. Unfortunately, to date there is little evidence that this relationship exists. The often accepted belief that a lower A/P ratio favors greater feeding efficiency due to a smaller heat increment for propionic acid may be conditional. It has been observed that feeding steam-flaked corn reduces the A/P ratio (39) and enhances feed conversion efficiency compared to raw rolled corn (53, 71). Reconstituting whole corn has been found to produce higher levels of acetate and lower levels of propionate than dry corn (116) but also enhances feed conversion efficiency (6). Most certainly every type of feed grain is not altered in the same way under the same processing conditions.

Although data in Tables 11, 12, 13, and 14 could not be compared statistically, there appears to be interactions between grain types and processing method. It is possible that day of inoculum collection and donor steer may be responsible, in part, for the different responses to treatment.

It is notable that cooked rye which had the lowest IVDMD in the rye trials (Table 7) also produced the lowest level of acetate and

highest level of propionate compared to other processing treatments (Table 11). As mentioned previously, the rye starch may have been retrograded during the cooking process thus reducing its susceptibility to enzymatic hydrolysis. Reconstituted rye which had a significantly ($P < 0.05$) greater IVDMD than cooked rye also had a significantly ($P < 0.01$) larger A/P ratio than cooked rye.

The VFA production data for processed corn (Table 12) shows a reversal of the situation noted for rye. The popped corn which had the lowest IVDMD (Table 8), produced the highest level of acetate and lowest level of propionate and reconstituted ground corn, which had the most favorable IVDMD also produced what is thought to be the most desirable A/P ratio. Therefore, it appears that a high molar proportion of propionate and a low A/P ratio are not always concomitant with maximum feed grain dry matter digestibility by a culture of rumen microorganisms. In addition, heat treatment does not necessarily result in a lower in vitro A/P ratio.

Processing of barley and oat cultivars (Tables 13 and 14) did not result in substantial alteration in the pattern of VFA production although cooking consistently produced a low A/P ratio for all grain varieties. It appears therefore, that the cooked grains, which contained the highly altered starch, tended to create conditions where propionate production was favored.

D. Gelatinization

It is difficult to explain the apparent reduction in degree of gelatinization in rye and waxy barley due to the dry heating process. Rye, in particular, presents an enigma in that only very severe

processing conditions appeared to gelatinize the starch. The mg maltose/g of rye and corn grain released upon incubation of the cooked sample with beta-amylase was somewhat lower than that for the barley varieties which may indicate that cooked rye and corn starch were less susceptible to beta-amylase. If this was the case, these results may not represent an absolute determination of gelatinization and are useful for comparing treatment effects only within each grain species. However, it is possible that corn and rye starch were not as extensively gelatinized by the cooking process as barley starch.

Pfost (94) has compared methods of measuring starch gelatinization in grains subjected to various cooking processes. He found that a microscopic method which determines the percentage of starch granules stained by Congo red dye indicates complete gelatinization before the enzymatic method which utilizes beta-amylase.

SUMMARY

Using an *in vitro* technique, the dry matter disappearance of processed rye, corn, normal and waxy barley, and normal and high-fat oats was studied. The effect of processing on *in vitro* volatile fatty acid production and gelatinization was also determined for each cereal.

There appears to be little advantage in terms of IVDMD in processing rye either by "hot" or "cold" processing methods. Analysis of *in vitro* VFA indicated that heat treated rye produced a lower A/P ratio than unheated rye. The cooked treatment, which had the greatest degree of starch gelatinization, had the lowest A/P ratio.

The IVDMD data with corn agree with other reports of a decrease in dry matter digestibility by treating corn with dry heat (117) or steam heat without flaking or rolling (60). Reconstituting ground corn resulted in the highest IVDMD value, followed by reconstituted whole and raw ground corn which had statistically similar IVDMD values. The A/P ratio was the lowest for reconstituted ground corn and raw ground corn while heat treatment appeared to elevate A/P ratio, with the exception of the cooked treatment which had a A/P ratio statistically similar to that of raw ground corn.

Processing treatments other than cooking did not affect IVDMD of waxy or normal barley. Possibly cooking for long periods with excess moisture increased the digestibility of non-starch components of the barley kernel, thus increasing IVDMD. Statistical analysis of the data indicated a variety-treatment interaction with the dry ground and steam pressure (6.8 kg/cm^2) treatments being responsible

for the interaction effect. All processing treatments tended to elevate A/P ratio compared to raw dry ground barley, with the exception of the cooked treatment. Similar trends in VFA production were noted for both waxy and normal barley.

IVDMD of normal and high-fat oats were not significantly ($P > 0.05$) affected by "hot" or "cold" processing. There were no apparent differences in IVDMD between the two oat varieties. Volatile fatty acid analysis for the two varieties was not similar but the cooked treatment tended to have a low A/P ratio for both varieties.

A search of the literature revealed that increasing ruminal digestion rather than postruminal digestion of cereal starch may enhance efficiency of feed conversion. If this supposition is correct, in vitro techniques utilizing rumen inocula could be useful in screening large numbers of processed grain samples to determine if various treatments are beneficial or if a newly developed cereal variety has value as a potential feedstuff.

BIBLIOGRAPHY

1. ADEEB, N. N., WILSON, G. F. and CAMPLING, R. C. 1971. Physical form of maize and its digestibility in cattle and sheep. J. Br. Soc. Anim. Prod. 13: 391 (abst.).

2. AIMONE, J. C. and WAGNER, D. G. 1977. Micronized wheat. I. Influence on feedlot performance, digestibility, VFA and lactate levels in cattle. J. Anim. Sci. 44: 1088-1095.

3. AIMONE, J. C. and WAGNER, D. G. 1977. Micronized wheat. II. influence on in vitro digestibility, in vitro gas production and gelatinization. J. Anim. Sci. 44: 1096-1099.

4. ANDERSON, D. C. and RALSTON, A. T. 1973. Chemical treatment of rye-grass straw: in vitro dry matter digestibility and compositional changes. J. Anim. Sci. 37: 148-152.

5. ANNISON, E. F. and ARMSTRONG, D. G. 1969. Volatile fatty acid metabolism and energy supply. Pages 422-437 in A. T. Phillipson, ed. Physiology of digestion and metabolism in the ruminant. Proc. 3rd Int. Symp., Cambridge, England. Oriel Press Ltd., London, England.

6. ARMBRUSTER, S. 1976. Processing feeds for growing and finishing cattle. Beef Cattle Science Handbook. Vol. 13. The International Stockmen's School, San Antonio, Texas. p. 537-541.

7. ARMSTRONG, D. G. 1972. Developments in cereal processing -- ruminants. Cereal processing and digestion. Tech. Publ. U. S. Feed Grains Counc., Washington, D. C. p. 9-37.

8. ARMSTRONG, D. G. and BEEVER, D. E. 1969. Post-abomasal digestion of carbohydrate in the adult ruminant. Nutr. Soc. Proc. 28: 121-131.

9. ARNETT, D. W. 1971. Effect of method of processing wheat for finishing steers. J. Anim. Sci. 26: 925 (abst.).

10. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1970. Official methods of analysis. 11th ed. Washington 4, D. C.

11. BANKS, W. and GREENWOOD, C. T. 1975. Starch and its components. John Wiley and Sons, Inc., Toronto, Ontario.

12. BAUMGARDT, B. R., TAYLOR, M. W. and CASON, J. L. 1962. Evaluation of forages in the laboratory. II. Simplified artificial rumen procedure for obtaining repeatable estimates of forage nutritive value. J. Dairy Sci. 45: 62-68.

13. BEESON, W. M. 1973. Effect of steam-flaking, roasting, popping, and extrusion of grains on their nutritional value for beef cattle. Pages 326-337 in Effect of processing on the nutritional value of feeds. Proceedings of a symposium. N. A. S., Washington, D. C.

14. BRETHOUR, J. R. and DUITSMAN, W. W. 1972. Roasted wheat in a beef finishing ration. Kansas Agr. Exp. Sta. Bull. 556. p. 19-22.

15. BRIS, E. J., DYER, I. A. and HOWES, D. 1966. Wheat as a grain for fattening cattle. J. Anim. Sci. 25: 594 (abst.).

16. BUCHANAN-SMITH, J. G., TOTUSEK, R. and TILLMAN, A. D. 1968. Effect of methods of processing on digestibility and utilization of grain sorghum by cattle and sheep. J. Anim. Sci. 27: 525-530.

17. BURKHARDT, J. D., EMBRY, L. B. and LUTHER, R. M. 1969. Corn grain processing methods for high concentrate beef cattle diets. J. Anim. Sci. 29: 153 (abst.).

18. BURT, A. W. A. 1973. Effect of processing on the nutritive value of cereals in animal feeds. Proc. Nutr. Soc. 32: 31-39.

19. CANADA DEPARTMENT OF AGRICULTURE. 1974. Research Branch Report, Charlottetown, P. E. I.

20. CANADA DEPARTMENT OF AGRICULTURE. 1976. Growing fall rye for grain in the Atlantic provinces. Publ. No. 1578. Ottawa, Ontario.

21. CHANDLER, C. H. and FULTON, D. A. 1968. Grain processing for feed. Feedstuffs 40: 26.

22. CHRISTENSEN, D. A., DUCK, B. A. and NICHOLSON, H. H. 1968. Effect of composition and processing of diets on performance of holstein steers. Can. J. Anim. Sci. 48: 263-267.

23. CHRISTIANSEN, R. R. and WAGNER, D. G. 1974. Reconstituted wheat. I. Influence on feedlot performance of cattle. J. Anim. Sci. 38: 456-462.

24. CHRISTIANSEN, R. R. and WAGNER, D. G. 1974. Reconstituted wheat. II. Influence of physical form on in vitro digestibility. J. Anim. Sci. 38: 463-466.

25. CHURCH, D. C. 1977. Feeds and feeding. O and B Books, Corvallis, Oregon. p. 90.

26. CLANTON, D. C. and WOODS, W. 1966. Performance of steers and rumen fermentation as influenced by physical form of ingredients and alfalfa:corn ratio. J. Anim. Sci. 25: 102-106.

27. COLE, N. A., JOHNSON, R. R. and OWENS, F. N. 1976. Influence of roughage level and corn processing method on the site and extent of digestion by beef steers. J. Anim. Sci. 43: 490-496.

28. CORNETT, C. D., SHERROD, L. B. and ALBIN, R. C. 1971. Effect of methods of processing upon digestibility of a new wheat by sheep and cattle. J. Anim. Sci. 32: 716-720.

29. CROKA, D. C. and WAGNER, D. G. 1975. Micronized sorghum grain. II. Influence on in vitro digestibility, in vitro gas production and gelatinization. J. Anim. Sci. 40: 931-935.

30. CROKA, D. C. and WAGNER, D. G. 1975. Micronized sorghum grain. III. Energetic efficiency for feedlot cattle. J. Anim. Sci. 40: 936-939.

31. DeBIE, W. H. and WOODS, W. 1964. Rumen fermentation and animal performance as influenced by gelatinized corn and enzyme supplementation. J. Anim. Sci. 23: 872 (abst.).

32. ELLIS, G. F., Jr. and CARPENTER, J. A. 1966. Popped milo in fattening rations for beef cattle. J. Anim. Sci. 25: 594 (abst).

33. ERWIN, E. S. 1966. Pressure cooking grain for cattle rations. Feedstuffs 38: 60.

34. ERWIN, E. S., MARCO, G. J. and EMERY, E. M. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44: 1768-1771.

35. FAICHNEY, G. J. 1969. Production of volatile fatty acids in the sheep caecum. Aust. J. Agric. Res. 20: 491-498.

36. FELSMAN, R. J., HARVEY, R. W., LINNERUD, A. C. and SMITH, F. H. 1976. Effect of roasting temperature on corn grain characteristics. J. Anim. Sci. 42: 476-480.

37. FRANKS, L. G., NEWSOM, J. R., RENBARGER, R. E. and TOTUSEK, R. 1972. Relationship of rumen volatile fatty acids to type of grain, sorghum grain processing method and feedlot performance. J. Anim. Sci. 35: 404-409.

38. FRENCH, D. 1973. Chemical and physical properties of starch. J. Anim. Sci. 37: 1048-1061.

39. GALYEAN, M. L., WAGNER, D. G. and JOHNSON, R. R. 1976. Site and extent of starch digestion in steers fed processed corn rations. J. Anim. Sci. 43: 1088-1094.

40. GARRETT, W. N., LOFGREEN, G. P. and HULL, J. L. 1968. Steam pressure processed sorghum grain for steers. J. Anim. Sci. 27: 1164 (abst).

41. GARRETT, W. N., LOFGREEN, G. P. and HULL, J. L. 1971. Influence of processing method on energy utilization of feed grains. Hilgardia 41: 123-156.

42. GERKEN, H. J., WISE, M. B., HARVEY, R. W. and BARRICK, E. R. 1971. Whole corn, liquid supplements and polyethylene for beef cattle. J. Anim. Sci. 32: 379 (abst).

43. GOERING, K. J., ESLICK, R. and DeHAAS, B. W. 1973. Barley starch. V. A comparison of the properties of waxy compana barley starch with the starches of its parents. Cereal Chem. 50: 322-328.

44. HALE, W. H. 1967. Effect of moist heat treatment of cereal grains on growth, feed utilization by cattle. Feedstuffs 39: 29.

45. HALE, W. H. 1971. Grain processing as it affects beef cattle. Feedstuffs 43: 19.

46. HALE, W. H. 1973. Influence of processing on the utilization of grains (starch) by ruminants. J. Anim. Sci. 37: 1075-1080.

47. HALE, W. H., CUITUN, L., SABA, W. J., TAYLOR, B. and THEURER, B. 1966. Effect of steam processing and flaking milo and barley on performance and digestion by steers. J. Anim. Sci. 25: 392-396.

48. HALE, W. H. and THEURER, C. B. 1972. Feed preparation and processing. Pages 49-67 in D. C. Church, ed. Digestive physiology and nutrition of ruminants. Vol. 3. Oregon State Univ., Corvallis, Oregon.

49. HARBERS, L. H. 1975. Starch granule structural changes and amylolytic patterns in processed sorghum grain. J. Anim. Sci. 41: 1496-1501.

50. HARBERS, L. H. and DAVIS, A. B. 1974. Digestion of sorghum grain endosperm in the rat and pig observed by scanning electron microscopy. J. Anim. Sci. 39: 1099-1105.

51. HATFIELD, E. E. and WILSON, W. M. D. 1973. Effect of drying temperature and heat treatment on the nutritive value of corn. Pages 151-170 in Effect of processing on the nutritional value of feeds. Proceedings of a symposium. N. A. S., Washington, D. C.

52. HELLMAN, N. N., BOESCH, T. F. and MELVIN, E. H. 1952. Starch granule swelling in water vapor sorption. J. Amer. Chem. Soc. 74: 348-351.

53. HENTGES, J. F., Jr., CABEZAS, M., PALMER, A. Z. and CARPENTER, J. W. 1961. Effect of physical form of corn on cattle response. J. Anim. Sci. 20: 935 (abst.).

54. HINDERS, R. and ENG, K. 1970. Effect of grain sorghum type on starch degradation due to pressure cooking and micronizing. Feedstuffs 42: 20.

55. HINMAN, D. D. and JOHNSON, R. R. 1974. Influence of processing methods on digestion of sorghum starch in high concentrate beef cattle rations. J. Anim. Sci. 39: 417-422.

56. HINMAN, D. D. and JOHNSON, R. R. 1974. Influence of degree of micronization on the site and extent of sorghum starch digestion in beef cattle fed high concentrate rations. J. Anim. Sci. 39: 958-963.

57. HOLMES, J. H. G., DRENNAN, M. J. and GARRETT, W. N. 1970. Digestion of steam-processed milo by ruminants. J. Anim. Sci. 31: 409-413.

58. HUSTED, W. T., MEHEN, S., HALE, W. H., LITTLE, M. and THEURER, B. 1968. Digestibility of milo processed by different methods. J. Anim. Sci. 27: 531-534.

59. INGALLS, J. R. 1964. Nutritive value of several forage species as measured by in vitro and in vivo methods. PhD Thesis. Michigan State Univ., East Lansing, Michigan.

60. JOHNSON, R. R. 1966. Techniques and procedures for in vitro and in vivo studies. J. Anim. Sci. 25: 855-875.

61. JOHNSON, R. R., DeHORITY, B. A. and BENTLEY, O. G. 1958. Studies on the in vitro rumen procedure: improved inoculum preparation and the effects of volatile fatty acids on cellulose digestion. J. Anim. Sci. 17: 841-849.

62. JOHNSON, D. E., MATSUSHIMA, J. K. and KNOX, K. L. 1968. Utilization of flaked vs cracked corn by steers with observations on starch modification. J. Anim. Sci. 27: 1431-1437.

63. KARR, M. R., LITTLE, C. O. and MITCHELL, G. E., Jr. 1966. Starch disappearance from different segments of the digestive tract of steers. J. Anim. Sci. 25: 652-654.

64. KARTCHNER, R. J., THEURER, B., BURT, J. G. and HALE, W. H. 1973. Starch intake level and sorghum grain processing on bovine starch digestion. J. Anim. Sci. 37: 347 (abst).

65. KEATING, E. K., SABA, W. J., HALE, W. H. and TAYLOR, B. 1965. Further observations on the digestion of milo and barley by steers and lambs. J. Anim. Sci. 24: 1080-1085.

66. KIRK, R. E. 1968. Experimental design: procedures for the behavior sciences. Brooks/Cole Publ. Co., Belmont, Calif. p. 91-99.

67. LIANG, Y. T., MORRILL, J. L., ANSTAETT, F. R., DAYTON, A. D. and PFOST, H. B. 1970. Effect of pressure, moisture, and cooking time on susceptibility of corn or sorghum grain starch to enzymatic attack. J. Dairy Sci. 53: 336-341.

68. LINDSAY, D. B. 1969. Carbohydrate metabolism in ruminants. Pages 438-451 in A. T. Phillipson, ed. Physiology of digestion and metabolism in the ruminant. Proc. 3rd Int. Symp., Cambridge, England. Oriel Press Ltd., London, England.

69. LITTLE, C. O., MITCHELL, G. E. and REITNOUR, C. M. 1968. Post-ruminal digestion of corn starch in steers. J. Anim. Sci. 27: 790-792.

70. LITTLE, T. M. and HILLS, F. J. 1972. Statistical methods in agricultural research. Univ. Calif., Davis, Calif. p. 67-74.

71. MATSUSHIMA, J. K. and STENQUIST, N. J. 1967. Reconstituted, ensiled and flaked corn for cattle. J. Anim. Sci. 26: 925 (abst).

72. MEHEN, S. M., HALE, W. H., THEURER, B., LITTLE, M. and TAYLOR, B. 1966. Effect of dry rolling, fine grinding, steam processing and pressure cooking on the digestion of milo ration by steers. J. Anim. Sci. 25: 594 (abst).

73. MOORE, J. E., JOHNSON, R. R. and DeHORITY, B. A. 1962. Adaptation of an in vitro system to the study of starch fermentation by rumen bacteria. J. Nutr. 76: 414-422.

74. MORRISON, F. B. 1956. Feeds and feeding. 22 ed. Morrison Publ. Co., Clinton, Iowa.

75. MUDD, C. A. and PERRY, T. W. 1969. Raw cracked vs expanded gelatinized corn for beef cattle. J. Anim. Sci. 28: 822-826.

76. MYERS, L. L., JACKSON, H. D. and PACKETT, L. V. 1967. Absorption of volatile fatty acids from the cecum of sheep. J. Anim. Sci. 26: 1450-1458.

77. McCULLOUGH, M. W. and MATSUSHIMA, J. K. 1973. Effect of corn processing on starch digestibility. J. Anim. Sci. 37: 350 (abst).

78. McGINTY, D. D., BREUER, L. H. and RIGGS, J. R. 1967. Digestibility of dry and reconstituted sorghum grain by cattle. J. Anim. Sci. 26: 223 (abst).

79. McGINTY, D. D. and RIGGS, J. K. 1967. Moist sorghum grains for finishing cattle. J. Anim. Sci. 26: 925 (abst).

80. McLAREN, R. J. and MATSUSHIMA, J. K. 1971. Gelatinized corn starch for beef finishing ration. J. Anim. Sci. 33: 292 (abst).

81. McNEILL, J. W., POTTER, G. D. and RIGGS, J. K. 1971. Ruminal and postruminal carbohydrate utilization in steers fed processed sorghum grain. J. Anim. Sci. 33: 1371-1374.

82. McNEILL, J. W., POTTER, G. D., RIGGS, J. K. and ROONEY, L. W. 1970. Chemical and physical properties of processed sorghum grain. J. Anim. Sci. 30: 314 (abst).

83. McNEILL, J. W., POTTER, G. D., RIGGS, J. K. and ROONEY, L. W. 1975. Chemical and physical properties of processed sorghum grain carbohydrates. J. Anim. Sci. 40: 335-341.

84. NATIONAL ACADEMY OF SCIENCES - NATIONAL RESEARCH COUNCIL. 1958. Composition of cereal grains and forages. N. R. C. Publ. 585. Washington, D. C.

85. NEUHAUS, V. and TOTUSEK, R. 1971. Factors affecting the in vitro digestibility of high moisture sorghum grain. J. Anim. Sci. 33: 1321-1326.

86. NEWLAND, H. W., MAGEE, W. T., BRANAMAN, G. A. and BLAKESLEE, L. H. 1962. Effect of heat-processing and pelleting corn for steers and lambs. J. Anim. Sci. 21: 711-715.

87. OSMAN, H. F., THEURER, B., HALE, W. H. and MEHEN, S. M. 1970. Influence of grain processing on in vitro enzymatic starch digestion of barley and sorghum grain. J. Nutr. 100: 1133-1140.

88. ØRSKOV, E. R. 1976. The effect of processing on digestion and utilization of cereals by ruminants. Proc. Nutr. Soc. 35: 245-252.

89. PAPASOLOMONTOS, S. A. and WILKINSON, J. I. D. 1976. Aspects of digestion of heat processed cereals by ruminants. Optimising the utilization of cereal energy by cattle and pigs. Tech. Publ. U. S. Feed Grains Counc., Washington, D. C. p. 31-61.

90. PARRETT, N. A. and RIGGS, J. K. 1967. Dry, reconstituted and early harvested sorghum grain for cattle. J. Anim. Sci. 26: 224 (abst).

91. PARROTT, J. C., MEHEN, S., HALE, W. H., LITTLE, M. and THEURER, B. 1969. Digestibility of dry rolled and steam processed flaked barley. J. Anim. Sci. 28: 425-428.

92. PERRY, T. W., PETERSON, R. C. and BEESON, W. M. 1974. Roasted corn for finishing beef cattle. J. Anim. Sci. 39: 248 (abst).

93. PERRY, T. W., TONROY, B. R., PETERSON, R. C. and BEESON, W. M. 1975. Comparative nutritive value of raw, roasted, ensiled high-moisture and pressure flaked corn for growing-finishing beef cattle. J. Anim. Sci. 40: 1214-1221

94. PFOST, H. B. 1971. Equipment and techniques in starch gelatinization. Feedstuffs 43: 24.

95. POTTER, G. D., McNEILL, J. W. and RIGGS, J. K. 1971. Utilization of processed sorghum grain proteins by steers. J. Anim. Sci. 32: 540-543.

96. PRASAD, D. A., MORRILL, J. L., MELTON, S. L., DAYTON, A. D., ARNETT, D. W. and PFOST, H. B. 1975. Evaluation of processed sorghum grain and wheat by cattle and by in vitro techniques. J. Anim. Sci. 41: 578-587.

97. PRESTON, T. R. 1963. II. Barley-beef production. Vet. Rec. 75:1399-1402.

98. RIGGS, J. K., SORENSON, J. W., Jr., ADAME, J. L. and SCHAKE, L. M. 1970. Popped sorghum grain for finishing beef cattle. J. Anim. Sci. 30: 634-638.

99. RILEY, P. W., CARDON, B. P., BROWN, W. H. and STULL, J. W. 1965. Effect on cattle performance of intense moist cooking of the rolled grain portion of fattening rations. J. Anim. Sci. 24: 599 (abst).

100. SALSBURY, R. L., HOEFER, J. A. and LUECKE, R. W. 1960. Effect of heating starch on its digestion by rumen microorganisms. J. Anim. Sci. 20: 569-572.

101. SALSBURY, R. L., SMITH, C. K. and HUFFMAN, C. F. 1956. The effect of high levels of cobalt on the in vitro digestion of cellulose by rumen microorganisms. J. Anim. Sci. 15: 863-868.

102. SALSBURY, R. L., VANDERKOLK, A. L., BALTZER, B. V. and LUECKE, R. W. 1958. The rates of digestion of the cellulose of some plant fractions by rumen microorganisms in vitro. J. Anim. Sci. 17: 293-297.

103. SANDSTEDT, R. M., HITES, B. D. and SCHROEDER, H. 1968. Genetic variations in maize; effects on the properties of starch. Cereal Sci. Today 13: 82-87.

104. SANDSTEDT, R. M., STRAHAN, D., UEDA, S. and ABBOT, R. C. 1962. The digestibility of high-amylose corn starches compared to that of other starches. The apparent effect of the ae gene on susceptibility to amylase action. Cereal Chem. 39: 123-131.

105. SCHAKE, L. M., RIGGS, J. K. and BUTLER, O. D. 1972. Commercial feedlot evaluation of four methods of sorghum grain processing. J. Anim. Sci. 34: 926-930.

106. SCHOCH, T. J. and MAYWALD, E. C. 1956. Microscopic examination of modified starches. Anal. Chem. 28: 382-387.

107. SEIB, P. 1971. Starch gelatinization: chemical and physical effects. Feedstuffs 43: 44.

108. SHAW, J. C., ENSOR, W. L., TELLECHEA, H. F. and LEE, S. D. 1960. Relation of diet to rumen volatile fatty acids, digestibility, efficiency of gain, and degree of unsaturation of body fat. J. Nutr. 71: 203-208.

109. STEEL, R. G. D. and TORRIE, J. H. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., Toronto, Ontario.

110. SULLINS, R. D. and ROONEY, L. W. 1971. Physical changes in the kernel during reconstitution of sorghum grain. Cereal Chem. 48: 567-575.

111. SUNG, A. 1969. Enzymatic evaluation of changes in processed grains. Feedstuffs 19: 22.

112. THEURER, B., TREI, J. and HALE, W. H. 1967. In vitro VFA production as influenced by steam processing and flaking milo and barley. J. Anim. Sci. 26: 930 (abst).

113. THOMAS, O. O. and MYERS, L. L. 1961. Steam or dry rolled barley in high concentrate rations for fattening beef steers. J. Anim. Sci. 20: 953 (abst).

114. THOMPSON, J. T., BRADLEY, N. W. and LITTLE, C. O. 1965. Ruminal volatile fatty acid concentrations and performance of steers fed different levels and forms of hay and grain. J. Anim. Sci. 24: 1179-1183.

115. TILLEY, J. M. A. and TERRY, R. A. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18: 104-111.

116. TONROY, B. R. and PERRY, T. W. 1974. Effect of corn preservation treatments on in vitro digestibility, ruminal pH and volatile fatty acid formation. J. Anim. Sci. 38: 676-680.

117. TONROY, B. R. and PERRY, T. W. 1974. In vitro digestion of raw, roasted, and pressure-flaked corn. J. Dairy Sci. 57: 1508-1511.

118. TREI, J. E., HALE, W. H. and THEURER, B. 1966. Influence of grain processing factors on in vitro fermentation rate. J. Anim. Sci. 25: 910 (abst).

119. TREI, J. E., HALE, W. H. and THEURER, B. 1970. Effects of grain processing on in vitro gas production. J. Anim. Sci. 30: 825-831.

120. UTLEY, P. R. and McCORMICK, W. C. 1975. Comparison of cattle finishing diets containing various physical forms of corn. J. Anim. Sci. 40: 952-956.

121. UTLEY, P. R. and McCORMICK, W. C. 1975. Dry or high-moisture corn for finishing steers in dry lot or on oat pasture. J. Anim. Sci. 41: 495-499.

122. WAGNER, D. G., SCHNEIDER, W. and RENBARGER, R. 1970. Influence of steaming time on the nutritive value of steam flaked milo. Okla. Agric. Exp. Sta. Misc. Publ. 84. p. 33.

123. WALDO, D. R. 1973. Extent and partition of cereal grain starch digestion in ruminants. J. Anim. Sci. 37: 1062-1074.

124. WALKER, H. G., Jr., LAI, B., ROCKWELL, W. C. and KOHLER, G. O. 1970. Preparation and evaluation of popped grains for feed use. Cereal Chem. 47: 513-521.

125. WHITE, T. W. and HEMBRY, F. G. 1971. Shelled and ground corn rations for steers. J. Anim. Sci. 33: 304 (abst).

126. WHITE, T. W., PERRY, T. W., TONROY, B. R. and LECHTENBERG, V. L. 1973. Influence of processing on *in vitro* and *in vivo* digestibility of corn. J. Anim. Sci. 37: 1414-1418.

127. WILLIAMSON, J. L. 1967. Increasing biological utilization of grain through processing. Feedstuffs 39: 34.

128. WOODS, W. and WILSON, B. B. 1967. Influence of level of particle size of gelatinized corn on animal performance and rumen fermentation. J. Anim. Sci. 26: 933 (abst).

APPENDIX A

Filtering Crucible Preparation

Fifty milliliter pyrex crucibles fitted with a porous sintered-glass filter were used to filter the fermentation mixture upon termination of the incubation period. A pad of Celite 545 filtering agent (approximately 5 mm thick) was prepared for each crucible by mixing a slurry of Celite and distilled water, pouring the mixture into the crucible and drawing off the excess moisture by light vacuum. These crucibles were then dried for 24 hours in an oven at 80 C, placed in the dessicator to cool then weighed to four decimal places. It is essential to have a pad of Celite to filter the rumen--buffer liquor. A precaution that must be taken is that all the rumen--buffer liquor must be filtered off before washing through with distilled water. If any liquor remains in the crucible when the water is added, frequently a collodial precipitate is formed which slows the filtering process.

After several weeks of use, the filtering process becomes slow due to the partial blocking of the sintered-glass filter by very small particles of Celite. This can be avoided by first immersing the Celite in a large volume of distilled water, allowing it to stand for a few minutes and decanting the suspended material. The Celite remaining is then dried in an oven before use.

APPENDIX B

Summary of Statistical Analysis of IVDMD Data for Processed Rye, Corn, Oats and Barley

Cereal	Source	df	MS	F
Rye	Total	27		
	Treatment	6	34.45	4.62*
	Block	3	113.01	15.50*
	Residual	18	7.46	
Corn	Total	35		
	Treatment	8	118.38	38.94*
	Block	3	75.73	24.91*
	Residual	24	3.04	
Waxy and Normal Barley	Total	55		
	Variety (V)	1	57.07	6.21
	Block	3	45.03	4.90
	Residual (a)	3	9.19	
	Treatment (T)	6	28.56	13.60
	T × V	6	9.14	4.35*
	DG	1	20.48	9.75*
	DH	1	1.94	0.92
	Cook	1	4.71	2.24
	RGr	1	2.83	1.35
	SA	1	6.23	2.97
	SP	1	37.21	17.72*
	Residual (b)	36	2.10	
High-fat and Normal Oats	Total	55		
	Variety (V)	1	318.73	9.88
	Block	3	54.74	1.70
	Residual (a)	3	32.26	
	Treatment (T)	6	41.19	13.91*
	T × V	6	2.19	0.74
	Residual (b)	36	2.96	

* $P < 0.05$

SECTION II

IN VITRO RUMEN DIGESTION OF FABA BEANS (*VICIA FABA*)

INTRODUCTION

Animal productivity from certain feedstuffs may be low because of the presence of plant constituents which reduce the quantity eaten and the availability of the nutrients. One important group of such compounds is the "tannins".

A number of investigations of the effect of tannins on digestibility of feedstuffs by livestock have been reported (for examples see 2, 9, 10, 14, 24, 27, 34). Presently, however, few investigations have involved digestion of tannin-containing substrates by ruminants, particularly bovine species. Section II of this thesis describes a preliminary investigation of *in vitro* digestibility of tannin-free and tannin-containing cultivars of *Vicia faba* L. var. major and *V. faba* L. var. minor using bovine inoculum. Attempts were also made to determine if extracts of pure condensed tannin from faba bean hulls had a direct effect on microbial enzyme activity.

LITERATURE REVIEW

Faba beans (*Vicia faba* L. var. minor) have recently been introduced into Canada as a potential protein supplement for animal feeds. Provided that reasonable yields are obtained, faba beans could offer an alternative to imported soybean protein and the less palatable rapeseed meal. However, the use of faba beans in livestock rations may be restricted due to the presence of a growth-limiting factor or factors. In a recent investigation with chicks, Marquardt *et al* (24) provided evidence that condensed tannin was a major growth-inhibiting substance in faba beans. Bond (4) maintains that the presence of tannin in faba bean hulls was associated with inhibition of *in vitro* dry matter digestibility of faba beans.

The importance of tannin in animal feedstuffs has been thoroughly reviewed by McLeod (26). In this thesis, recent literature regarding biological properties of tannins and their effect on digestibility of feedstuffs by animals will be reviewed. Of particular interest are the investigations involving faba beans.

A. Biological Properties of Tannins

The most widely accepted classification scheme in one in which tannins are grouped on the basis of structural type (26). On these two groups of tannins, hydrolysable and condensed, the latter is not hydrolyzed by either acids or enzymes (26). Only condensed tannins will be considered in the present review.

a. Enzyme Inhibition

In vitro studies (Milić *et al*, 25) on the effect of tannin isolated from lucerne upon activity of purified trypsin, L-amylase and lypase in incubation mixtures with casein, starch and refined olive oil substrate, respectively, showed the existence of interaction between enzymes and tannins. It is believed that hydrogen bonds were formed between the hydroxyl groups of polyphenol tannins and the carbonyl groups of peptide bonds of enzyme proteins (25). Uncertainty still exists regarding interaction of proteolytic enzymes, protein and tannins. Milić *et al* (25) suggest that tannins may inhibit a proteolytic enzyme either by forming complexes with the substrate protein or with the protein part of the enzyme. In addition, protein-tannin complex formation can occur in two ways, producing soluble and insoluble complexes depending upon the concentration of the reactants and their heterogenity (25).

Daiber (7) found that enzyme production and activity in malt produced from intact birdproof sorghum grains were unaffected by the endogenous polyphenols in the testa and nucellar layer of the grain. However, when the malt was milled and suspended in water for mashing or analysis, the tannins reacted with proteins and enzymes to form insoluble complexes (7). In an earlier study, Strumeyer and Malin (32) isolated a condensed tannin from the germ fraction of *Leoti* sorghum grain. Upon incubating the tannin with a variety of enzymes, varying degrees of enzyme inactivation were observed.

It appears, therefore, that tannins are non-specific in the binding of enzyme protein. However, the degree of inhibition probably depends on the affinity of the tannin to bind a specific enzyme.

b. Physiological Effects of Tannins on Animals

McLeod (26) has reviewed the physiological investigations of tannins. A review of the most recent literature is given in Table 1.

B. Effect of Tannins on the Quality of Feedstuffs

a. Voluntary Intake

The presence of tannin in a feedstuff has been assumed to affect voluntary intake (10, 14, 27) but the exact significance of tannin as a cause of low intake is not known. Factors other than tannin may have an influence on intake since it was noted by Jambunathan and Mertz (13) that average intake of rats on high-tannin sorghum was greater than those on low-tannin sorghum.

b. Digestibility

(i). In Vitro

In vitro dry matter disappearance results obtained by Bond (4) indicate that tannin was associated with low digestibility of the hull fraction of faba beans. Those faba bean varieties which were determined to be tannin-free had, on the average, 4.7% higher whole bean dry matter digestibility. Bond (4) suggests that factors other than tannins may have affected in vitro digestibility of faba beans.

Cummins (6) found a strong linear relationship between tannin content and in vitro dry matter disappearance of whole plant sorghum prior to ensiling. Ensiling increased in vitro dry matter disappearance of high-tannin sorghum apparently through loss of tannins from the

Table 1. Effects of tannins from different sources on animals

Source	Concentration	Effects on animal	Reference
		Chickens	
Faba beans	7.6 (tannin in % crude protein)	No effect on egg production.	16
Faba beans	1.7% of the diet.	Decreased weight gain. Decreased pancreas weight.	24
Faba beans	N.A.† Dietary.	Depressed growth rate. Reduced metabolizable energy.	34
Sorghum grain	N.A. Dietary.	Reduced growth rate and feed efficiency. Addition of methionine did not improve chick performance.	2
Sorghum grain + 1% tannic acid	N.A. Dietary.		
		Rats	
Sorghum grain	N.A. Dietary.	Depressed weight gain and feed efficiency.	30
Sorghum grain	2.69% (catechin equivalent)	Depressed rate of gain.	13
		Sheep	
Acacia aneura (Mulga)	6.1% (tannic acid equivalent)	Depressed intake, rate of gain and wool production.	10

Table 1. (cont.)

Source	Concentration	Effects on animal	Reference
Spent coffee grounds	N.A. Dietary.	Depressed dry matter intake and protein digestion.	27
Oak Kernel	3.52% of the diet	Decreased protein and fiber digestibility. Depressed intake.	14
Faba bean hulls *V. faba* L. var. minor (62% of ration)	N.A. Dietary.	No effect	9

N.A.† The concentration of tannin is not available.

grain portion (6).

An investigation of *in vitro* dry matter digestibilities of high- and low-tannin sorghum grain by Schaffer *et al* (29) showed that there was a negative linear relationship between tannin content and *in vitro* dry matter disappearance. Upon addition of urea to the buffer media, the percent *in vitro* dry matter disappearance of the high-tannin varieties was more than doubled. Schaffert *et al* (29) suggested that tannin does not inhibit digestion of sorghum grain directly but rather indirectly by reducing the amount of nitrogen available for bacterial growth and rapid substrate digestion. They (29) believe that if tannin inhibited bacterial growth directly, then little or no increase in *in vitro* digestibility would be expected by adding a small amount of urea.

Lyford *et al* (17) have provided evidence that tannins may inhibit *in vitro* enzymatic hydrolysis of cellulose directly. Addition of casein to the incubation media afforded considerable protection to the cellulose degrading system but at high levels of extracted tannins, casein had no effect.

(ii). *In Vivo*

Schaffert *et al* (30) observed that supplementation of a high-tannin sorghum diet with 5 and 10% soybean meal did not increase rate of gain of rats. A supplementation of 15% soybean meal allowed rats to gain at the same rate with the same efficiency as those fed low-tannin sorghum. Their results indicate therefore, that the influence of tannins is not a toxic effect but rather affects the availability of dietary protein (30). Results of other investigations with chicks

(28) and sheep (27) support the possibility that tannins affect performance by binding dietary protein. Gartner and Hurwood (10) deduced from their analysis of mulga leaves, that supplemental dietary sulfur improves sheep and cattle performance by overcoming a sulfur deficiency created by tannins binding to sulfur-containing amino acids.

Katiyar et al (14) found a decrease in protein and fiber digestibility in sheep fed oak-kernel. They (14) assume that cellulose assimilation was decreased due to inactivation of cellulolytic enzymes by tannins in oak-kernel. Low crude fiber retention has been observed in chicks fed faba bean hulls (33) and condensed tannin extracts from faba bean hulls (24); in some cases there was a negative retention of crude fiber. This was attributed to an enhanced excretion of lignin-like condensed tannin-protein complex which was included in the analysis of crude fiber (24, 33).

The effect of tannins may be more pronounced in one species of livestock than in another. This species specificity is illustrated in the work of Ward et al (33) and Edwards et al (9). It has been observed that feeding 40% raw faba bean hulls in chick rations will reduce rate of weight gain and feed efficiency compared to chicks fed autoclaved faba bean hulls or water-extracted hulls (33). Edwards et al (9) found no evidence of toxicity in sheep consuming diets containing 62% faba bean hulls for 28 days. They (9) concluded that mechanically prepared faba bean hulls might be well utilized by sheep, comparing favorably with other fibrous feedstuffs.

C. Summary

Thus far most of the work investigating tannins has been done with

animals other than ruminants. It is quite possible that ruminants can ingest larger amounts of tannin per unit of body weight with little adverse effect due to their unique physiological characteristics. Whether tannins reduce enzyme activity in the rumen is not known, however, it is probable that tannins which bind dietary protein will decrease efficiency of utilization of the ration.

MATERIALS AND METHODS

A. Animals

One rumen fistulated steer, maintained on an alfalfa-hay diet, was used for this investigation. Mineralized salt and fresh water were available at all times.

Collection of rumen fluid was carried out prior to consumption of the daily ration.

B. *In Vitro* Dry Matter Disappearance Technique

Preparation of rumen fluid, buffer media, sample inoculation, incubation and dry matter disappearance measurement procedures were the same as in Section I except urea was omitted from the buffer media. In every experiment except where specified, sample weights were approximately 0.3000 g and incubation of the samples took place over a 48 h time period.

a. Experiment 1

The purpose of this experiment was to determine *in vitro* dry matter disappearance (IVDMD) of several varieties of whole faba beans known to contain variable levels of condensed tannin (Table 2).

Triplicate samples of each faba bean variety were incubated with rumen inoculum in two digestion trials. The data were statistically analyzed using a randomized complete block design (31) and treatment means were subjected to Student-Newman-Keul's Multiple Range test (15). Regression analysis was used to determine if a correlation existed between IVDMD and tannin content and/or IVDMD and lignin content of

the faba bean varieties examined.

Table 2. Faba bean varieties, seed coat color and location of supply

Variety	Seed Color	Location of supply
Vicia faba major		
Fidrim	white	Holland
Triple White	white	Holland
Kodrim	white	Holland
Small Pod	brown	Manitoba
Broad Windsor White	white	Manitoba
Exhibition Long Pod	tan	Manitoba
Vicia faba minor		
Diana	dark brown	Manitoba
Hertz-Freya	brown	Manitoba

b. Experiment 2

Two faba bean varieties containing the least amount of condensed tannin (Kodrim and Triple White) and two containing the greatest concentration of tannin (Hertz-Freya and Diana) were subjected to comparative IVDMD analysis. The effect of the addition of urea to the fermentation media on IVDMD of the four varieties of faba beans was examined to determine if availability of nitrogen was a limiting factor in the utilization of high-tannin faba beans by rumen microorganisms. Two digestion trials with triplicate observations of each treatment were carried out. Data was analyzed by using a two-factor model and

following the appropriate F tests, treatment means were subjected to Student-Newman-Keul's Multiple Range test.

c. Experiment 3

IVDMD of hulls, cotyledons and whole faba beans of Broad White Windsor, Triple White and Diana varieties were determined in a 48 h incubation period. The faba bean fractions of Diana were obtained as described by Ward et al (33). Fractions from Broad White Windsor and Triple White were obtained by splitting the beans by hand. The purpose of this experiment was to determine which fraction was responsible for the low in vitro digestibilities observed with tannin-containing faba beans. Two digestion trials with triplicate observations were performed and the data were subjected to analysis of variance using a randomized complete block design and treatment means tested for significant differences using Student-Newman-Keul's Multiple Range test.

d. Experiment 4

IVDMD of protein concentrate (cultivar, Diana; 56% crude protein) was determined for different incubation periods with and without urea in the buffer media. A single digestion trial with triplicate observations of each treatment; 0 and 30 mg urea, was carried out for each incubation period.

e. Experiment 5

The effect of heat treatment on IVDMD of various fractions of the faba bean variety Diana, was determined. Cotyledon and hull fractions were obtained as in Experiment 3. Starch and protein

concentrate were obtained by an air-classification process in a commercial plant. The faba bean fractions were divided into two parts and one part of each fraction was heat treated while the other was left untreated. Heat treatment was carried out as described by Marquardt et al (22). Two digestion trials with triplicate observations in each trial were performed. One-way analysis of variance was used to assess significance of heat treatment effect.

f. Experiment 6

In this experiment, faba bean protein concentrate (cultivar, Diana) was incubated with a water soluble extract and an acetone soluble extract from faba bean hulls (cultivar, Diana) prepared and described by Marquardt et al (24). The water and acetone extracts contained approximately 50 and 75% pure condensed tannin, respectively. IVDMD and in vitro protein disappearance (IVPD) were determined following a 24 h incubation period.

The dried tannin extracts were dissolved in distilled water and made up to concentrations of 0.0325 g/ml. One milliliter of the tannin extract solution was added to the samples of protein concentrate in the fermentation vessel prior to the addition of buffer or rumen fluid. The experimental treatments for both IVDMD and IVPD determinations were: (i) protein concentrate, (ii) protein concentrate plus water extract and (iii) protein concentrate plus acetone extract. The remaining tannin extract solutions were sealed in a flask and refrigerated for further use.

IVPD analysis was carried out in 50 ml centrifuge tubes fitted with bunsen valves. Four blanks were also included in the analysis.

Buffer media, tube inoculation procedure and incubation were the same as for IVDMD determinations. Following a 24 h incubation period, the tubes were centrifuged at 1930 g for 10 min, the supernatant decanted and the residue dried at 60 C in a forced air oven for 48 h. The residue was then weighed, ground in a mortar, mixed to secure a uniform sample and nitrogen content determined on 0.05 g duplicate samples by microkjeldahl procedure (3). The amount of protein degraded was calculated from the following formula:

$$\text{IVPD} = \frac{\text{g protein in substrate} - \left(\text{residual protein} - \text{g protein in 20 ml of inoculum}\right)}{\text{g protein in substrate}} \times 100\%$$

One digestion trial with triplicate observations for IVDMD determinations and quadruplicate observations for IVPD was performed.

g. Experiment 7

Corn starch (feed grade) was mixed with distilled water to make up a solution of approximately 0.05 g/ml. This mixture was heated to 60 C on a hot plate and held at that temperature for 3 min in order to slightly gelatinize the starch, then cooled gradually to 22 C. It was found earlier that treating the starch in this manner reduced the variability between triplicate in vitro determinations. The gelatinized starch stayed in suspension in the incubation media rather than settling to the bottom of the tube, thus making mixing easier. In addition, starch samples could be pipetted rather than weighed into the fermentation vessels and this considerably shortened preparation time.

Aliquots of the gelatinized starch (10 ml) were pipetted into

fermentation vessels while stirring the starch solution continually with a magnetic stirrer. At four intervals during sampling, 10 ml of starch solution were pipetted into four dried, preweighed beakers in order to determine exact sample weight. The beakers were dried for 24 h at 80 C in a forced air oven, cooled for at least 2 h in a dessicator and reweighed. The average weight of the starch sample in the four beakers was used as the sample weight for all fermentation vessels. The greatest variation between starch sample weights using this method did not exceed 0.001 g.

To determine the effect of the condensed tannins on starch digestion, 1 ml aliquots of water and acetone faba bean hull extracts, as described in Experiment 6, were added to the starch samples. The treatments were: (i) starch control, (ii) starch plus water extract and (iii) starch plus acetone extract.

Two digestion trials were performed with six observations for each treatment in the first run and three observations for each treatment in the second. Urea was added to the buffer media to ensure that available nitrogen was not a limiting factor in starch digestion. Four blanks were included to determine residual dry matter of the inoculum. The data were tested for significant differences using a completely randomized design with variability due to day of inoculum collection considered as error.

h. Experiment 8

This experiment was carried out in the same manner as Experiment 7. It was felt that there may be such an excess of microorganisms, therefore alfa-amylase activity, in the rumen inoculum that inhibi-

tion of alfa-amylase by tannins could not be detected. The three treatments described in Experiment 7 were incubated with 20 ml of buffer and 20 ml of rumen fluid diluted to various concentrations with buffer. The dilutions were: (i) whole rumen fluid, (ii) 66:33 rumen fluid/buffer and (iii) 33:66 rumen fluid/buffer. As in Experiment 7, urea was added to the buffer. One digestion trial with triplicate observations for each treatment within each inoculum dilution was performed.

C. Analyses

Dry matter, crude protein (N × 6.25), fat (ether extract), crude fiber, acid detergent fiber, lignin and ash were determined by methods according to the Association of Official Agricultural Chemists (3). Starch analysis was carried out as described in Section I. Assays for condensed tannins were carried out according to the vanillin-hydrochloric acid method of Burns (5).

RESULTS

In Vitro Dry Matter Disappearance

a. Experiment 1

Ranking the cultivars in ascending order of mean percentage IVDMD indicated a distinct difference between tannin-containing and tannin-free faba beans (Table 3). There was also some indication of greater digestibility in broad beans (V. faba major) than field beans (V. faba minor). A high negative correlation ($Y_i = 79.28 - 1.45X$; $r = -0.88$) existed between tannin content and IVDMD. Those varieties which were tannin-free had consistently higher IVDMD than the other cultivars. In addition, there was also a high negative correlation ($Y_i = 86.42 - 2.36X$; $r = -0.85$) between percent lignin in the hull and IVDMD. Percent hull for each cultivar was quite similar. Chemical composition of the eight cultivars is given in Table 1 of the Appendix.

b. Experiment 2

The addition of urea to fermentation media had no significant ($P > 0.05$) effect on IVDMD values for high-tannin and tannin-free cultivars of V. faba (Table 4). The difference in IVDMD between cultivar Diana and the tannin-free faba beans was non-significant ($P > 0.05$) when urea was added to the buffer.

c. Experiment 3

Bond (4) attributed differences in IVDMD of whole faba bean

Table 3. IVDMD, % hull, hull lignin content and tannin content of eight cultivars of Vicia faba

Variety	IVDMD (%)	% lignin in hull†	Hull tannin content (%)	% hull
V. faba minor				
Hertz-Freya	71.09a	5.52	6.0	14
Diana	72.52a	6.34	4.4	13
V. faba major				
Exhibition	73.41a	4.86	2.3	15
Small Pod	75.03ab	4.82	3.8	15
White Windsor	75.69b	5.28	2.4	14
Kodrim	79.04c	3.15	0.0	13
Fidrim	79.45c	2.87	0.0	13
Triple White	80.61c	2.98	0.0	13
SE	1.06			

a,b,c Treatment means in the first column bearing different letters are significantly different ($P < 0.05$).

†Dry matter basis.

Table 4. IVDMD of high-tannin and tannin-free faba bean cultivars

Cultivar	IVDMD (%) Without urea	IVDMD (%) With urea	Hull tannin content (%)
Hertz-Freya	68.15a	67.40a	6.0
Diana	68.90a	72.54ab	4.4
Triple White	75.37b	76.55b	0.0
Kodrim	77.41b	78.94b	0.0

a,b Treatment means in the first two columns with different letters are significantly different ($P < 0.05$).
SE = 1.96

cultivars to the presence of a factor or factors contained in the hull portion of the seeds. In the present study there were significant differences ($P < 0.05$) in IVDMD between tannin-containing and tannin-free hulls in the three cultivars evaluated (Table 5). In addition, there was a significant ($P < 0.05$) difference in IVDMD between V. faba minor and V. faba major cotyledons. Whole bean IVDMD of the three cultivars examined were all significantly ($P < 0.05$) different from one another, the trend in digestibility being in agreement with results of Experiment 1 although values were somewhat higher. Chemical composition of cotyledons and hulls is given in Table 2 of the Appendix.

Table 5. IVDMD of whole beans, cotyledons and hulls of three cultivars of V. faba

Cultivar	IVDMD (%)		
	Whole bean	Cotyledon	Hull
Diana	73.04a	79.46a	44.54a
White Windsor	77.91b	83.76b	41.26a
Triple White	86.11c	85.04b	80.47b
SE	1.35	1.24	1.74

a,b,c Treatment means in the same column bearing different letters differ significantly ($P < 0.05$).

d. Experiment 4

This experiment was conducted to determine a suitable incubation time for protein concentrate. IVDMD of protein concentrate of V. faba cultivar, Diana, for different incubation periods, is shown in Figure 1

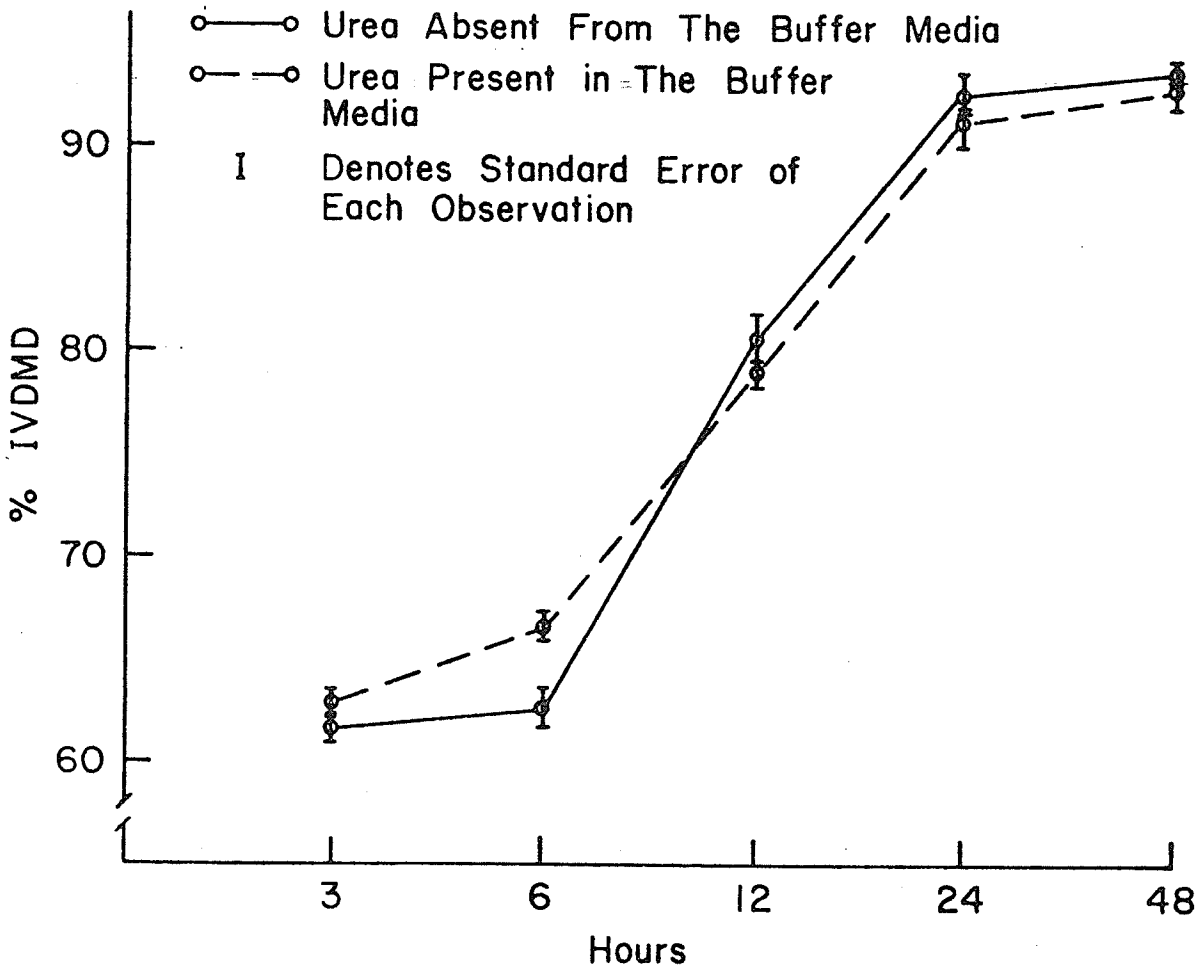

Figure 1. IVDMD of faba bean protein concentrate (cultivar, Diana) at 3, 6, 12, 24 and 48 hours after the initiation of fermentation.

together with the standard errors. It appears that the protein concentrate is almost completely digested 24 h after the beginning of the incubation period.

e. Experiment 5

Autoclaving had no significant ($P > 0.05$) effect on IVDMD of faba bean starch or hulls (Table 6). However, autoclaving significantly ($P < 0.05$) decreased the IVDMD of the whole bean, cotyledons and protein concentrate compared to raw (unheated) faba bean fractions. Chemical composition of the various faba bean fractions is given in Table 3 of the Appendix.

Table 6. The effect of autoclaving on IVDMD of various fractions of V. faba, cultivar Diana

	IVDMD (%)				
Treatment	Hulls	Whole bean	Protein concentrate	Cotyledons	Starch
raw	39.21	72.12a	84.00a	76.73a	80.35
autoclaved	41.47	65.40b	78.69b	70.55b	81.61
SE	1.49	2.21	1.51	0.98	1.79

a,b Treatment means in the same column bearing different letters differ significantly ($P < 0.05$).

f. Experiment 6

Condensed tannin added to the incubation media reduced IVDMD and IVPD (Table 7) of the faba bean protein concentrate (PC). Since only one laboratory run was performed the amount of data was

insufficient for statistical analysis but standard errors for each value are given. The water and acetone extract depressed digestibility to the same magnitude. In the protein concentrate control, nearly all of the protein was digested in vito in 24 h.

Table 7. The effect of added condensed tannin on IVDMD and IVPD of faba bean protein concentrate (PC)

Treatment	IVDMD (%)	IVPD (%)
PC control	88.64 ± 1.08	92.04 ± 0.55
PC + water extract	79.16 ± 0.44	67.69 ± 0.31
PC + acetone extract	78.21 ± 0.96	66.08 ± 1.33

g. Experiment 7

Condensed tannin added to the incubation media had no significant ($P > 0.05$) effect on in vitro starch digestion (Table 8). This

Table 8. Effect of condensed tannin on IVDMD of corn starch

Treatment	IVDMD (%)
Starch control	65.46
Starch + water extract	64.43
Starch + acetone extract	64.24

SE = 0.92

may indicate that there was no inhibition of microbial alfa-amylase by condensed tannins or that an excess of microbial alfa-amylase was present.

h. Experiment 8

The effect of condensed tannins on *in vitro* corn starch digestion, using a number of different rumen inoculum dilutions, is unclear (Table 9). Again, only one laboratory run was performed providing only a small amount of data. For some treatment means the standard errors were unusually large. Clearly a number of digestion trials would be necessary before a statment can be made whether or not tannins affect starch digestion *in vitro*.

Table 9. Effect of condensed tannins on IVDMD of corn starch

Dilution (rumen fluid/buffer)	IVDMD (%) of Starch		
	Starch control	Starch + water extract	Starch + acetone extract
1:0	66.65 ± 0.67	62.37 ± 0.39	62.49 ± 2.45
66:33	74.06 ± 2.55	65.42 ± 0.87	61.26 ± 1.23
33:66	69.80 ± 1.85	68.80 ± 2.78	72.91 ± 3.98

DISCUSSION

The higher digestibility of the tannin-free faba beans would make them more desirable as a livestock feed (Table 3). While there was a significant ($P < 0.05$) linear relationship between tannin content and IVDMD, it was suspected that another factor affected digestibility. The present study shows that digestibility of the whole faba bean in the rumen may be affected to the same degree by the amount of lignin in the hull, as the amount of condensed tannin in the hull. Lignin may be the unknown factor affecting *in vitro* digestibility which Bond (4) felt must be present.

Unlike the findings of Schaffert *et al* (29) with sorghum, the addition of urea to the incubation media did not increase IVDMD of high-tannin faba beans significantly ($P > 0.05$) (Table 4). This could be due to the fact that faba beans have large amounts of available protein compared to sorghum grain, and the small amount of protein bound by the tannins would not easily be detected *in vitro*. According to Schaffert *et al* (29) lack of an increase in IVDMD upon addition of urea would indicate direct inhibition of bacterial growth, however, this may not be true of a high-protein substrate.

The data contained in Table 5 indicate that the tannin-free faba bean hulls had a high IVDMD compared to the hulls of those varieties containing tannin. This is in agreement with the *in vitro* trials of Bond (4) who found that the testa accounted for differences in digestibility values of various faba bean cultivars. Unlike Bond (4) it was found that not all cotyledons from various cultivars had the same digestibilities. Cotyledons of the high-tannin variety,

Diana, had a significantly ($P < 0.05$) lower digestibility than low-tannin or tannin-free cultivars. This may be an indication of the presence of an inhibitor substance in the cotyledon, but, it might also reflect differences in chemical and physical properties of the constituents of cotyledons from different cultivars.

A number of studies have demonstrated that faba beans (Vicia faba L. var. minor) contain a thermolabile growth depressing factor (18, 19, 20, 21, 22, 23, 33, 34). In a recent investigation, Marquardt et al (24) identified this factor as a condensed tannin. The results in Table 6 indicate that autoclaving did not improve in vitro digestibility of various components of faba beans, in fact, autoclaving appeared to be detrimental to in vitro protein digestion, possibly due to denaturation of the protein. Digestibility of faba bean hulls was slightly though non-significantly ($P > 0.05$) increased by autoclaving. Since nitrogen is low in the hulls (4.4% crude protein), additional nitrogen in the form of urea could have increased the utilization of faba bean hulls but these results indicate that heat processing of faba bean hulls for ruminants may not be warranted.

The data presented in Table 7, though limited, confirms other observations (11, 14, 24, 27, 29, 30) on the effect of condensed tannin on protein utilization or retention. Whether the decreased protein digestibility was due to binding of proteolytic enzymes or binding of dietary protein is not known.

In an in vitro rumen system where microorganisms are intact, difficulties are encountered in determining the effect of inhibitors on enzyme activity. Cellular disruption increases ease of enzyme assay but destroys the normal system. Also, little is known about the

association between rumen organisms and substrates. Protozoa can engulf food particles or other microorganisms and digest them rather than releasing free enzymes to degrade the substrate (12). Electron microscopy techniques have been used by Akin (1) to study the manner of attachment of different morphological types of bacteria to plant cell walls during degradation. In the rumen ecosystem, bacteria appeared to adhere closely to plant substrates during degradation by capsule-like material and by small amounts of extracellular material, as well as by other means not observable by electron microscopy (1).

There is a possibility, therefore, that tannins may not have access to microbial enzymes because of the nature of the close association between microorganisms and substrates. This hypothesis is supported by data shown in Table 8 which shows starch IVDMD to be slightly but non-significantly ($P > 0.05$) reduced by adding condensed tannin to the sample. Possibily alfa-amylase was in great excess compared to the amount of condensed tannin added, although this is thought to be unlikely since added tannin extract was 7.4% of the weight of starch substrate. Although the results contained in Table 9 are largely inconclusive, it may be useful to carry out a number of such trials in attempt to determine the effect of condensed tannin on microbial systems. A dilution trial such as this would be more meaningful than disruption of microorganisms in order that enzyme assays can be performed.

SUMMARY

This preliminary investigation was undertaken in order to determine the *in vitro* digestibility of tannin-containing and tannin-free faba bean cultivars by rumen microorganisms. The effect of condensed tannins, previously extracted from faba bean hulls, on IVDMD of faba bean protein concentrate and corn starch was also studied.

The results indicate that tannin-free cultivars are more digestible *in vitro* than tannin-containing faba beans probably due to the much higher digestibility of the hull portion of the tannin-free beans. It appears that both tannin content and lignin content of the hull are equally responsible for the lower IVDMD of tannin-containing faba beans. Autoclaving various faba bean fractions to destroy the thermolabile growth depressing factor had no effect on IVDMD of hulls and starch but reduced IVDMD of the protein concentrate, cotyledons and whole bean fractions. Condensed tannin added to the incubation media decreased IVDMD and IVPD of protein concentrate but did not affect IVDMD of corn starch.

Further investigations are necessary to determine if the tannins affect proteolytic and amylolytic activity of intact rumen microbial systems or if the major effect of tannin is mediated through the binding of available dietary protein.

BIBLIOGRAPHY

1. AKIN, D. E. 1976. Ultrastructure of rumen bacterial attachment to forage cell walls. Appl. Environ. Microbiol. 31: 562-568.

2. ARMSTRONG, W. D., FEATHERSTON, W. R. and ROGLER, J. C. 1974. Effects of bird resistant sorghum grain and various commercial tannins on chick performance. Poult. Sci. 53: 2137-2142.

3. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1970. Official methods of analysis. 11th ed. Washinton 4, D. C.

4. BOND, D. A. 1976. In vitro digestibility of the testa in tannin-free field beans (Vicia faba L.). J. Agric. Sci., Camb. 86: 561-566.

5. BURNS, R. E. 1971. Method for estimation of tannin in grain sorghum. Agron. J. 63: 511-512.

6. CUMMINS, D. G. 1971. Relationships between tannin content and forage digestibility in sorghum. Agron. J. 63: 500-502.

7. DAIBER, K. H. 1975. Enzyme inhibition by polyphenols of sorghum grain and malt. J. Sci. Food Agric. 26: 1399-1411.

8. DUNN, O. J. and CLARK, V. A. 1974. Applied statistics: analysis of variance and regression. John Wiley and Sons, Inc., New York, N. Y.

9. EDWARDS, D. G., DUTHIE, I. F. and ROGERS, B. M. 1973. A note on the digestibility by sheep of hulls from the field bean (Vicia faba L.). Anim. Prod. 17: 329-332.

10. GARTNER, R. J. W. and HURWOOD, I. S. 1976. The tannin and oxalic acid content of Acacia aneura (mulga) and their possible effects on sulphur and calcium availability. Aust. Vet. J. 52: 94-95.

11. GLICK, Z. and JOSLYN, M. A. 1970. Effect of tannic acid and related compounds on the absorption and utilization of proteins in the rat. J. Nutr. 100: 516-520.

12. HUNGATE, R. E. 1966. The rumen and its microbes. Academic Press, New York, N. Y.

13. JAMBUNATHAN, R. and MERTZ, E. T. 1973. Relationship between tannin levels, rat growth, and distribution of proteins in sorghum. J. Agric. Food Chem. 21: 692-696.

14. KATIYAR, R. C., PRAKASH, V. R. and RANJHAN, S. K. 1973. Utilization of oak-kernel as energy source for sheep. Indian J. Anim. Sci. 43: 515-518.

15. KIRK, R. E. 1968. Experimental design: procedures for the behavior sciences. Brooks/Cole Publ. Co., Belmont, Calif. p. 91-99.

16. LINDGREN, E. 1975. The nutritive value of peas and field beans for hens. Swed. J. Agric. Res. 5: 159-161.

17. LYFORD, S. J., Jr., SMART, W. W. G., Jr. and BELL, T. A. 1967. Inhibition of rumen cellulose digestion by extracts of Sericea lespedeza. J. Anim. Sci. 26: 632-637.

18. MARQUARDT, R. R. and CAMPBELL, L. D. 1973. Raw and autoclaved faba beans in chick diets. Can. J. Anim. Sci. 53: 741-746.

19. MARQUARDT, R. R. and CAMPBELL, L. D. 1974. Deficiency of methionine in raw and autoclaved faba beans in chick diets. Can. J. Anim. Sci. 54: 437-442.

20. MARQUARDT, R. R. and CAMPBELL, L. D. 1975. Performance of chicks fed faba bean (Vicia faba) diets supplemented with methionine, sulfate and cystine. Can. J. Anim. Sci. 55: 213-218.

21. MARQUARDT, R. R., CAMPBELL, L. D., STOTHERS, S. C. and McKIRDY, J. A. 1974. Growth responses of chicks and rats fed diets containing four cultivars of raw or autoclaved faba beans. Can. J. Anim. Sci. 54: 177-182.

22. MARQUARDT, R. R., CAMPBELL, L. D. and WARD, T. 1976. Studies with chicks on the growth depressing factor(s) in faba beans (Vicia faba L. var. minor). J. Nutr. 106: 275-284.

23. MARQUARDT, R. R., McKIRDY, J. A., WARD, T. and CAMPBELL, L. D. 1975. Amino acid, hemagglutinin and trypsin inhibitor levels, and proximate analyses of faba beans (Vicia faba) and faba bean fractions. Can. J. Anim. Sci. 55: 421-429.

24. MARQUARDT, R. R., WARD, A. T., CAMPBELL, L. D. and CANSFIELD, P. E. 1977. Purification, identification and characterization of a growth inhibitor in faba beans (Vicia faba L. var. minor). J. Nutr. 107: 1313-1324.

25. MILIĆ, B. L., STOJANOVIĆ, S. and VUČUREVIĆ, N. 1972. Lucerne tannins. II. Isolation of tannins from lucerne, their nature and influence on the digestive enzymes in vitro. J. Sci. Food Agric. 23: 1157-1162.

26. McLEOD, M. N. 1974. Plant tannins - their role in forage quality. Nutr. Abst. Rev. 44: 804-815.

27. McNIVEN, M., FLIPOT, P., SUMMERS, J. D. and LEESON, S. 1977. The feeding value of spent coffee grounds for ruminants. Nutr. Rep. Int. 15: 99-103.

28. NELSON, T. S., STEPHENSON, E. L., BURGOS, A., FLOYD, J. and YORK, J. O. 1975. Effect of tannin content and dry matter digestion on energy utilization and average amino acid availability of hybrid sorghum grains. Poult. Sci. 54: 1620-1623.

29. SCHAFFERT, R. E., LECHTENBERG, V. L., OSWALT, D. L., AXTELL, J. D., PICKETT, R. C. and RHYKERD, C. L. 1974. Effect of tannin on in vitro dry matter and protein disappearance in sorghum grain. Crop Sci. 14: 640-643.

30. SCHAFFERT, R. E., OSWALT, D. L. and AXTELL, J. D. 1974. Effect of supplemental protein on the nutritive value of high and low tannin Sorghum bicolor (L.) moench grain for the growing rat. J. Anim. Sci. 39: 500-505.

31. STEEL, R. G. D. and TORRIE, J. H. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., Toronto, Ontario.

32. STRUMEYER, D. H. and MALIN, M. J. 1969. Identification of the amylase inhibitor from seeds of Leoti sorghum. Biochim. Biophys. Acta 184: 643-645.

33. WARD, A. T., MARQUARDT, R. R. and CAMPBELL, L. D. 1977. Further studies on the isolation of the thermolabile growth inhibitor from the faba bean (Vicia faba L. var. minor). J. Nutr. 107: 1325-1334.

34. WILSON, F. J. and McNAB, J. M. 1972. The effect of autoclaving and methionine supplementation on the growth of chicks given diets containing field beans (Vicia faba L.). Br. Poult. Sci. 13: 67-73.

APPENDIX

Table 1. Chemical analysis of eight faba bean cultivars

Cultivar	DM	Fat	Fiber	Protein (N×6.25)	Starch
White Windsor	90.79	1.24	7.54	27.3	41.17
Kodrim	90.29	1.51	8.58	25.5	40.17
Fidrim	90.67	1.16	8.50	27.3	39.90
Diana	89.39	1.46	6.99	26.1	43.21
Triple White	90.08	1.00	8.87	27.8	40.35
Small Pod	91.63	1.15	8.66	26.5	41.89
Hertz-Freya	88.32	0.95	7.44	26.6	41.13
Exhibition	90.56	1.37	7.91	28.9	42.57

Table 2. Chemical analysis of faba bean cotyledons and hulls

	Diana		Triple White		White Windsor	
	Cotyledons	Hulls	Cotyledons	Hulls	Cotyledons	Hulls
DM	89.03	91.30	90.49	90.85	92.82	92.82
Nitrogen %	4.40	0.70	5.22	0.49	5.27	0.48
Protein (N × 6.25)	27.47	4.37	32.63	3.08	32.91	3.01
Fat (ether extract)	1.17	0.14	1.22	0.25	1.31	0.34
Fiber (crude)	1.12	47.87	1.56	54.68	1.35	48.61
Ash	2.75	2.43	4.03	3.19	3.35	3.56
Neutral Detergent Fiber		60.76		69.82		-
Acid Detergent Fiber		63.71		68.27		64.24
Lignin		6.95		3.28		6.26
Cellulose		56.41		64.72		58.08

Table 3. Analysis of faba bean fractions (cultivar, Diana)

Fraction	DM	Nitrogen	Protein	Fat	Fiber	Ash
Whole						
-raw	88.24	4.244	26.53	1.42	7.65	3.14
-autoclaved	87.74	4.187	26.17	1.56	7.43	3.11
Cotyledons						
-raw	88.41	4.711	29.44	1.37	0.97	2.78
-autoclaved	92.51	4.995	31.22	1.78	1.18	2.96
Hulls						
-raw	93.61	0.752	4.70	0.24	46.68	2.48
-autoclaved	92.37	0.786	4.91	0.23	46.60	2.53
Protein Concentrate						
-raw	93.77	9.516	59.48	3.00	2.03	6.13
-autoclaved	93.26	9.772	61.08	3.91	2.43	6.25
Starch						
-raw	88.58	0.730	4.56	0.38	1.31	0.94
-autoclaved	88.39	0.731	4.57	0.43	1.37	0.89