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THE UNIVERSITY OF ALBERTA

INFLUENCE OF SULFUR DIOXIDE ON THE PRESERVATION AND NUTRITIVE VALUE OF HIGH MOISTURE BARLEY FOR CATTLE AND SWINE

By

DAVID MARTIN GIBSON

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

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled INFLUENCE OF SULFUR DIOXIDE ON THE PRESERVATION AND NUTRITIVE VALUE OF HIGH MOISTURE BARLEY FOR CATTLE AND SWINE submitted by DAVID MARTIN GIBSON in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

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Supervisor

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Date November 26 1986

Dedication

To my wife April

and in loving memory

of my brother Stuart.

ABSTRACT

Three studies were designed to investigate the usefulness of liquid sulfur dioxide (SO_2) as a grain preservative. In experiment one, a 1% (wt/wt) SO_2 application rate did not effectively preserve high moisture barley (HMB, 30 to 32% moisture) for large scale (69 t), near anaerobic, storage. Heating and mould growth resulted in 30% loss of grain through spoilage. The treated grain was preserved successfully for 9 mo with small scale (700 kg) aerobic storage. Moisture migration and resultant loss of molecular SO_2 via binding with protein, sugar and lignin components of the grain were probably the major cause for failure in large scale storage. A second experiment was conducted to investigate the feeding value of SO_2 treated HMB for cattle. Feeding SO_2 treated HMB to lactating dairy cows for 6 wk at 35% of the diet (DM basis) was not detrimental to performance, milk taste or odour ($P>0.05$). Feedlot steers fed a thiamin supplemented (5.5 mg kg^{-1} DM) diet, containing an 85% DM inclusion level of SO_2 treated HMB, had 10.1% lower DM intake, 31% lower average daily gain (ADG), and required 21% more DM per kg of gain than those fed thiamin supplemented dry or ensiled HMB diets ($P<0.05$). The results of the feedlot trial were confounded by the feeding of spoiled SO_2 treated HMB at approximately 40% of the diet (DM basis) between day 29 and 57 of the 96 d trial. Four steers fed SO_2 treated HMB developed polioencephalomalacia in this period. The feeding value of SO_2 treated HMB for pigs was examined in experiment three. Feeding of SO_2 treated HMB to weanling pigs, at 47% of the diet for 28 d, was not detrimental to performance ($P>0.05$). SO_2 effectively preserved a HMB based diet for 18 d at 16°C, but 61% of the dietary thiamin was destroyed by 7 d after mixing the rations. Growing-finishing pigs fed SO_2 treated HMB to 85 kg had a 9.8% lower feed intake and 9.0% lower ADG than pigs fed the HMB control diet. The reduced performance of growing-finishing pigs fed SO_2 treated HMB was due primarily to thiamin depletion. Reduced ration palatability and toxicological effects on the digestive tract due to 75% SO_2 treated grain in the diet (DM basis) may have also contributed to the reduced performance of growing-finishing pigs. It was concluded that the use of SO_2 as a grain preservative cannot be recommended.

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I. INTRODUCTION

In many parts of the world, a short growing season and/or adverse weather conditions at harvest make it necessary to harvest grain before it has been field-dried. Unless artificially dried, such high moisture grain (HMG) is traditionally ensiled or preserved chemically with a grain preservative (GP) in order to prevent spoilage.

Fungi (moulds) are the principal cause of spoilage in grain (Alberta Agriculture 1984). Changes that occur in grain as a result of fungal invasion include: (1) loss of germination, (2) heat damage and mustiness, (3) possible production of mycotoxins, and (4) loss of nutritive value (Lynch 1972). Consumption of mould contaminated feed by livestock is thus undesirable as it will often cause reduced feed intake and animal performance, as well as possible liver damage (Jones et al. 1974). Furthermore, prolonged intake of mould contaminated feed by livestock can result in mycotoxin residue in meat and milk (Jones et al. 1974; Mertens 1978).

Grain preservatives were developed as an alternative to artificial drying of HMG, as their antifungal activity enables HMG to be stored aerobically without spoilage (Jones et al. 1974). Their use first began in the late 1960's in Europe and in the early 1970's spread to Canada and the United States (Christensen and Sauer 1982).

The most widely used GP have been propionic and acetic acids and their mixtures (Campbell 1972; Jones et al. 1974). These organic acids have been shown to be effective in inhibiting mould growth and do not adversely effect the feeding value of the grain (Livingstone et al. 1971; Ingalls et al. 1974; Jones et al. 1974). Nevertheless, their use as GP has not been extensive due to corrosion of metal equipment, safety precautions required to prevent inhalation of acid fumes, and acid contact to the hands and eyes (Jones et al. 1974). Additionally, a practical application system which ensures even application of preservative at the required application rate is lacking (Campbell 1972; Jones et al. 1974; Harrison 1985). Presently, organic acids are considered too expensive for routine use (Mathison et al. 1985).

Alkali treatment with ammonia (NH_3) (Bothast et al. 1973; Laksesvela 1981) or sodium hydroxide (NaOH) (Bothast et al. 1975; Berger et al. 1981) has been shown to be effective in controlling microbial proliferation in moist grain. Alkali treatment has an additional benefit of increasing fibre digestibility in ruminants (Orskov et al. 1980; Low and Kellaway 1983). However, both NH_3 (Peplinski et al. 1978) and NaOH (Orskov 1979; Pringle et al. 1983) treatments have resulted in grain handling problems due to

bridging and clumping. Pringle et al. (1983) did not recommend NaOH preservation since it significantly reduced protein digestibility of high moisture barley (HMB) for pigs and rats. Further concern pertaining to the use of NH_3 as a GP is that it inhibits mould growth but not bacterial growth (Jones et al. 1974).

The need for economical and effective GP remains, not only because weather conditions may dictate emergency harvesting, but also because:

1. HMB may have some potential yield advantages which have ranged from 6.7 to 19.7% on a dry matter basis (Krall 1972; Marx 1981; Kennelly et al. 1984).
2. Grain drying is expensive, particularly at moisture levels in excess of 25% (Sauer 1972), and it takes little time for spoilage to begin when on-farm drying capacity is exceeded.
3. Oxygen limiting silos used for ensiling HMG require a high capital investment.
4. Chemically treated HMG should be rolled or ground before storage in pit or bunker silos in order to minimize spoilage losses (Merrill 1971; Krall 1972). Such processing can create problems at harvest, however, since the rate of rolling or grinding is often slower than the rate of combining (Windels 1972).
5. Diets based on HMG need to be mixed on a daily basis and fed in amounts which can be consumed within 24 to 48 hr, depending on environmental temperature, to prevent heating and substantial mould growth (Livingstone and Livingston 1970; Jones et al. 1974; Brooker et al. 1981).

Sulfur dioxide (SO_2) is a chemical that warrants consideration as a GP. Its antimicrobial activity is well known due to its long history as a preservative in the food and beverage industry (Schroeter 1966). In animal feedstuffs, the first use of SO_2 as a silage preservative was in 1885 (Watson and Nash 1960). In the early 1950's SO_2 was shown to be an an effective silage preservative, cutting fermentation losses in half (Kennedy and Allred 1953); and, when SO_2 treated silage was fed to lactating dairy cows, it had no adverse effect on performance (Knodt et al. 1952; Dufour et al. 1954). However, due to the obnoxious properties of the gas, SO_2 was never used other than under experimental conditions (Woolford 1984). The salt of SO_2, sodium metabisulfite ($Na_2S_2O_5$), was promoted as a silage preservative in the later 1950's as it was easier to apply, safer to handle, and more economical than SO_2 (Bratzler et al. 1956), but it was not consistently effective and was believed to reduce palatability (Woolford 1984). More

recently, Mathison et al. (1979; 1984) re-examined the usefulness of SO_2 as a silage preservative because: (1) past research showed favourable results with SO_2, (2) the liquified gas is now more easily applied, and (3) a large and inexpensive supply of SO_2 exists in central and western Canada. They determined SO_2 to be a good silage preservative for legume and legume/grass silages when applied at rates of 0.36-0.66% (wt/wt basis), and that the feeding of such treated silage had no adverse influence on steer performance.

As a silage preservative, SO_2 is valued for: (1) reducing fermentation losses as measured by organic acid and NH_3 nitrogen levels (Knodt et al. 1952; Skaggs and Knodt 1962; Petrosyan 1967; Mathison et al. 1979) and (2) enhancing conservation of water-soluble carbohydrates (Knodt et al. 1952; Petrosyan 1967; Mathison et al. 1984).

While it has been reported that SO_2 is an effective microbial growth inhibitor in low temperature grain drying systems (Eckoff et al. 1979; 1983), only one study has examined its usefulness as a GP. Mathison et al. (1985) showed that SO_2 has potential as an effective and economical GP and that the feeding value of SO_2 treated HMB for feedlot steers compares favourably with that of dry barley.

The studies described herein were designed to investigate the efficacy of SO_2 as a GP and, to examine the influence of feeding SO_2 treated HMB on the performance of pigs, lactating dairy cows and feedlot cattle.

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II. EFFICACY OF SULFUR DIOXIDE AS A GRAIN PRESERVATIVE

A. INTRODUCTION

Chemical preservation with grain preservatives (GP) is an alternative to ensiling or drying high moisture grain (HMG). The most widely used GP have been propionic and acetic acids and their mixtures (Campbell 1972; Jones et al. 1974). These have proven to be effective but cost and convenience militate against their routine use.

The antimicrobial activity of sulfur dioxide (SO_2) and its salt, sodium metabisulfite, is well known due to its long history as a preservative in the food and beverage industry (Schroeter 1966). As a silage additive, SO_2 enhances conservation of water-soluble carbohydrates (Knodt et al. 1952; Petrosyan 1967; Mathison et al. 1984) and reduces fermentation as measured by organic acid and ammonia levels (Knodt et al. 1952; Skaggs and Knodt 1952; Petrosyan 1967; Mathison et al. 1979). SO_2 is also valued as a microbial growth inhibitor in low temperature grain drying systems (Eckoff et al. 1979; 1983).

A readily available and inexpensive supply of SO_2 in central and western Canada warrants its investigation as a GP. The objective of this experiment was to determine the efficacy of SO_2 as a GP, on the basis of chemical and microbiological characteristics of SO_2 treated high moisture barley (HMB).

B. MATERIALS AND METHODS

Barley treatment and storage

HMB (30–32% moisture) was ensiled in a Harvestore® silo (control), or treated with 1% (wt/wt) SO_2 as it was augered into storage. Liquid SO_2 was applied from a pressurized cylinder via a plastic tube (1.27 cm i.d.) through a hole made 1 m from the auger boot. SO_2 flow rate was regulated by the cylinder needle valve, on the basis of weight change of the SO_2 cylinder as monitored by a digital readout platform scale. An SO_2 flow rate of 5 kg min^{-1} was maintained with a 500 kg min^{-1} flow of barley through the auger. Treated barley was stored in a 69 t capacity upright bag (3.7 m height, 5.6 m diameter) woven of polyester (12 mil) with 4 mil of polyethylene coating (Nilex Geotechnical Products Inc., Edmonton) and resembling a conventional truck tarp. The

bag was supported in a steel grid cage (Aktieselskabet SORENCO, Rodovre, Denmark). The storage system was 'near anaerobic' since the material used for the upright bag had an oxygen permeability (at 25°C) of 42.5 L m^{-2} day^{-1}, approximately five times more permeable to oxygen than the 8.5 mil polyethylene used in the manufacture of some silage bags. Prior to filling, three thermocouples were put in the centre of each storage structure using plastic conduit to fix them in place at three different depths: top, middle and bottom. Temperatures of control and treated HMB were monitored daily for 30 d using a portable thermocouple temperature indicator (Bailey Instrument, Model Bat 8, Saddlebrook, NJ).

Chemical analyses

Barley samples were selected at harvest, bin opening (74 d post filling) and at intervals throughout a total storage time of 9 mo. Samples were frozen immediately and stored at -22°C until analyzed.

Dry matter (DM) was determined both by oven drying at 110°C for 24 h (AOAC 1980) and by gas chromatography (GC) (Fenton et al. 1981) using an Aerograph 660 gas chromatograph. Methanol (MeOH) rather than ethanol was used for sample water extraction since this is the preferred alcohol for extracting water from HMG (Fenton et al. 1981). To ensure complete extraction of water, barley was rolled before adding MeOH, and samples were shaken continuously for 48 h.

Volatile fatty acids (VFA), ethanol (EtOH) and furfural were determined by GC. Between 9 and 10 g of rolled HMB were put into a 50 mL test tube. To this was added: 20 mL of deionized water, 2 mL of 0.2% (w/v) n-caproic acid as an internal standard, and 0.5 mL of 25% phosphoric acid. Tubes were fitted with screw caps and shaken continuously for 12 h at room temperature. Two mL of this extract were centrifuged for 5 min (17,000 x g) and the supernatent was used for GC analysis. A standard containing EtOH, acetate, propionate, n-butyrate and furfural with n-caproic acid as an internal standard was prepared in 25% phosphoric acid and made up to volume with deionized water. A Varian 3700 gas chromatograph equipped with a flame ionization detector and an OV 351 30m (0.25 mm i.d.) fused silica capillary column was used for chromatographic analysis. Conditions were as follows: oven, programmed temperature rise, from an initial 80°C for 2 min, at 15°C min^{-1} increasing to 180°C and held for 2

min; injector, 170°C; detector, 190°C; carrier gas, N_2, with a flow rate of 1 mL min^{-1}; detector gases, air and hydrogen at 300 and 30 mL min^{-1} flow rates, respectively.

Lactic acid was also determined by GC. To 5 g of frozen rolled HMB, placed in a 50 mL test tube, was added 20 mL of MeOH and 3 mg of n-caproic acid as an internal standard. Tubes were sealed and shaken continuously for a 48 h extraction and equilibration. One hundred uL of 3N NaOH in MeOH was added to 0.5 mL of extract with the sodium lactate salt recovered by evaporating to dryness under N_2 at 70°C. Esterification to methyl lactate was achieved by adding 0.5 mL of 3N HCl in MeOH to reduce the pH to 4–5 and then by heating the capped tube for 20 min at 100°C. Duplicate 1 uL injections were made after the salt had settled. GC conditions were similar to that used for VFA determinations with the exception that the oven temperature was programmed from 80°C and held for 2 min, then increased at 20°C min^{-1} to a temperature of 190°C which was held for 2 min. The standard solution consisted of 2 mg mL^{-1} DL-lactic acid, in MeOH, with 0.2% (w/v) n-caproic acid as the internal standard.

Ammonia nitrogen (NH_3-N) and pH were determined in an aqueous extract derived from 1–3 g rolled barley samples. Twenty mL of deionized water were used for the 12 h extraction which was done at 5°C with continual shaking. NH_3-N was measured by the method of Fawcett and Scott (1960) using a dipping probe colorimeter (Brinkman PC 800) fitted with a 600 nm filter.

All barley samples were freeze-dried and ground to a homogeneous powder through a 0.5 mm mesh prior to sulfur (S), acid detergent fibre (ADF), and acid detergent insoluble nitrogen (ADIN) determinations. Sulfur was determined using a LECO Sulfur Determinator SC132 (St. Joseph, MI). Duplicate samples of 0.2 to 0.3 g were incinerated at 1370°C in an oxygen atmosphere. Vanadium pentoxide was used as a combustion catalyst. Moisture and dust were removed using a magnesium perchlorate drying tube; evolved SO_2 gas was measured by a solid state infrared detector. Citrus leaf (National Bureau of Standards #1572, Washington, D.C.) and coal calibration standards (LECO #501-001, St. Joseph, MI) were used as standards. ADF and ADIN were analyzed according to Goering and Van Soest (1970).

Microbiological

Microbiological analysis of samples taken at bin opening (74 d post filling) and 1 mo later (day 102) was conducted. Fungi (yeasts and moulds) and bacterial populations of fresh samples were estimated by pour plate technique, similar to that used for the bacteriologic examination of milk (Richardson 1985). Fresh weight, 1 g samples were diluted with 9 mL of phosphate buffer pH 7.2 (Bacto-FA, Difco Laboratories) and agitated. One mL aliquots of four dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were plated using Bacto Plate Count Agar (Difco Laboratories), pH 7.0, and then incubated at 35°C for 48 h. Anaerobic counts were obtained by incubation in an anaerobic jar with a CO_2-H_2 gas generating kit (Oxoid, Basingstoke, England). Fungal population was estimated by colony morphology.

Statistical analyses

Barley temperature and chemical data were subjected to one way analysis of variance (Steel and Torrie 1980). Relationships between selected chemical characteristics, especially fermentation products, were determined by Pearson correlation coefficients while linear regression between time and chemical characteristics were calculated for SO_2 treated HMB (Steel and Torrie 1980).

C. RESULTS AND DISCUSSION

Barley temperature during storage

SO_2 treatment decreased ($P<0.01$) mean maximum barley temperature by 1.1°C (Figure II-1). Mathison et al. (1984) reported mean maximum silage temperatures decreased by 2.5 and 4.6°C compared with untreated controls for alfalfa/bromegrass and barley silages treated with 0.33 and 0.40% SO_2 (wt/wt), respectively. Mean maximum temperatures were 39.0 and 37.9°C for the control and SO_2 treated HMB respectively, approximately 10.5°C higher than barley temperatures measured on the day of filling. Treatment did not influence the number of days at which temperatures stayed within 1°C of the maximum but did influence the number of days after filling at which maximum temperature was reached. Mean maximum temperatures occurred 6 to 11 and 14 to 19 d after filling for control and SO_2 treatments, respectively (Figure II-1).

Observed lower maximum temperature and delay in fermentation for SO_2 treated HMB are consistent with the results obtained previously with SO_2 treated silages (Knodt et al. 1952; Petrosyan, 1967; Mathison et al. 1979; 1984).

Chemical characteristics of barley

Chemical characteristics of SO_2 treated HMB after 74 d of storage (bin opening) reflect its action as a fermentation inhibitor (Table II-1). EtOH concentration was significantly reduced ($P<0.001$) by SO_2 treatment; from 20.6 to 0.8 mg g^{-1} DM in the ensiled and SO_2 treated barleys, respectively. The EtOH content of the HMB control was within the range of 4 to 22 mg g^{-1} DM reported by others (Windels 1972; Goodrich et al. 1975). SO_2 treatment also reduced acetate ($P<0.01$) and lactate ($P<0.05$) levels in HMB. Propionate could not be detected in either barley sample. This is consistent with other reports where either no or trace amounts of propionate were measured in HMB (Sogn 1973; Flipot and Pelletier 1980).

Despite reduced fermentation in the SO_2 treated HMB, chemical treatment reduced ($P<0.001$) pH from 5.7 in the control to 3.3 in the treated HMB. The solubility of SO_2 in the free water of the barley results in the formation of sulfurous acid (H_2SO_3) and the dissociation of this acid to hydrogen ions and either bisulfite (HSO_3^-) or sulfite (SO_3^-).

Ammonia-N was lower ($P<0.05$) in the SO_2 treated than in the ensiled HMB; measuring 78.1 and 96.1 ug g^{-1} DM, respectively. Adjusting for NH_3-N produced in the freshly harvested barley via plant enzyme respiration and proteolysis prior to treatment (14.7 ug g^{-1} DM), 84.7 and 81.2% of the NH_3-N measured could be attributed to microbial activity in the control and SO_2 treatments, respectively. Lowered NH_3-N concentration indicated reduced proteolysis and is consistent with observations made with SO_2 treated silages (Knodt et al. 1952; Skaggs and Knodt 1952; Petrosyan 1967; Mathison et al. 1979).

The S content of HMB treated with SO_2 was higher ($P<0.001$) than that of ensiled HMB. Fifty percent of the applied S was retained between application and bin opening (74 d post filling). Mathison et al. (1979) reported S retention, between time of application and feeding, of 38 and 59% for SO_2 treated silages of 55.9 and 65.1% moisture, respectively. More recently, Mathison et al. (1984) reported S retention of 17

to 40% in SO_2 treated silages ranging from 62 to 72% moisture. Their work showed a trend for higher retention of added S in higher moisture forages, explained presumably by the solubility of SO_2 in water. Considering that a retention of 50% was achieved in this experiment for HMB of 30% moisture, our retention of applied S was considerably greater than that reported by Mathison et al. (1984) with silage.

Sulfur dioxide treatment reduced ADF ($P<0.01$) from 9.3 to 6.8% of the barley DM. This may be explained by the delignifying capability of SO_2 and/or its ability to inhibit non-enzymatic browning (Green 1976), commonly referred to as the Maillard reaction. Ben-Ghedalia and Miron (1981, 1984) have demonstrated the delignifying capability of SO_2 at application levels of approximately 5%, and at temperatures of 70°C for 72 h. However, Mathison et al. (1979, 1984) observed no consistent effect of SO_2 on either NDF, ADF or hemicellulose contents of silage material treated with SO_2 at 0.14 to 0.72% of fresh weight. It would appear that prolonged exposure of HMB to high concentrations of SO_2, at elevated temperatures, are necessary to elicit delignification. The results reported by these researchers, coupled with the lowered ($P<0.01$) ADIN of SO_2 treated HMB observed in this experiment imply that inhibition of non-enzymatic browning contributed more than did delignification in reducing ADF, as the bisulfite and sulfite ions of SO_2 are valued as Maillard reaction inhibitors (Schroeter 1966; Van Soest 1982). Further support of this viewpoint is provided by the fact that the ADF of SO_2 treated HMB was reduced by 2.5%, although the lignin content of barley is negligible. The ADIN levels of 6.1 and 2.7% of total N in ensiled and SO_2 treated HMB, respectively, suggest no heat damage as 10% of total N as ADIN (DM basis) is considered normal in forages which are not heat damaged (Bath et al. 1985).

SO_2 treatment of HMB had no effect ($P>0.05$) on moisture, crude protein or furfural levels, the latter being an indication of the extent of carbohydrate thermal degradation under acidic conditions (Van Soest 1982).

Microbiological characteristics of barley

On the basis of bacterial and fungal (yeasts and moulds) counts taken after 74 d of storage, SO_2 appears to be effective as a bacteriocide but ineffective as a fungicide (Figure II-2). A fungi population of 10^3 colonies g^{-1} was observed in both control and treated HMB at bin opening. In his work with GP, Bernier (1973) concluded that fungi

counts at or in excess of 10^4 colonies g^{-1} indicated ineffective preservation. He observed that at such counts, slight mould growth was usually visible.

The microbiological populations observed for HMB in this experiment are consistent with the values reported in a limited number of experiments involving ensiled or chemically treated HMB. Livingstone et al. (1971) observed no values in excess of 10^2 and 10^3 colonies g^{-1}, for fungi and bacteria, respectively, when 1.3% (wt/wt) propionic acid treated HMB (29% moisture) was stored in hessian sacks, at room temperature, for a 4 mo period. Bacterial and fungal populations in their control HMB which was stored in sealed polyethylene sacks, averaged 10^6 and 10^7 colonies g^{-1}, respectively.

Two explanations seem likely as to the questionable efficacy of SO_2 as a fungicide observed in this experiment. First- as with other preservatives, the efficacy of SO_2 varies with different micro-organisms (Sofos and Busta 1982). For example, some yeast species can resist up to 500 ppm SO_2 (Reed and Peppler 1973). However it is likely that moulds, not yeasts were the predominant fungi observed at bin opening. This conclusion arises from the fact that EtOH levels in SO_2 treated HMB were very low and that the more usual fermentation involving yeasts is the ethanolic one (Woolford 1984). At 25°C and 77% RH, the bag material used for the upright bag was five times more permeable to oxygen transmission than 8.5 mil polyethylene used in the manufacture of some silage bags. Nevertheless, the oxygen:fresh HMB ratio (mL g^{-1}) of 0.29 calculated for the upright bag, on the basis of the permeability measurement, would classify the system as anaerobic (McDonald 1981). A continual air infusion into the grain estimated at .03 L sec^{-1} m^{-3} grain, because of the bag's oxygen permeability, would more accurately describe the storage system as being 'near anaerobic'. This distinction is significant, because continual air infusion into the grain during storage would have encouraged the persistence of aerobic micro-organisms (McDonald 1981), especially moulds since they are usually aerobic and prefer acidic conditions (Jackson and Wolfe 1978). A second possible explanation for the lack of effectiveness of SO_2 as a fungicide is that the concentration of molecular SO_2, the effective antimicrobial form of the preservative was inadequate (Schroeter 1966; Green 1976; Hammond and Carr 1976).

SO_2 reduced lactate concentration in HMB by only 34.3% whereas EtOH and acetate levels were reduced by 96.1 and 73.2%, respectively. These relative levels of fermentation products in treated HMB would suggest the presence of a significant

population of active lactic acid bacteria. This is in agreement with Carr and Davies (1971) who reported viable lactobacilli present in ciders which contained appreciable amounts of "free" SO_2 (cited by Hammond and Carr 1976).

Changes in SO_2 treated HMB

The chemical and microbiological characteristics of SO_2 treated HMB noted at 74 d of storage (bin opening) did not persist with increasing storage time. Barley at the bin perimeter remained well preserved but heating and mould growth within the bin core resulted in about 30% (21 t) loss through spoilage. Spoilage was first observed 28 d after bin opening. In the time period between bin opening and spoilage, barley was removed daily for feeding purposes, otherwise the bin was closed. Approximately 56 d after bin opening (28 d after spoilage was observed), the well preserved treated HMB was removed from the upright bag and stored in uncovered boxes of 700 kg capacity. This grain showed no visible signs of spoilage throughout a total storage period of 9 mo.

Spoiled SO_2 treated HMB was characterized by temperatures of 40 to 60°C. Similar temperatures have been reported by Wallace (1973) who stated that poorly preserved grain will heat to temperatures of up to 56°C or slightly above. Spoiled SO_2 treated HMB had higher ($P<0.05$) pH and increased ($P<0.001$) concentrations of acetate, NH_3-N, furfural and ADIN ($P<0.01$) (Table II-2). Thermal degradation of carbohydrates to produce furfural and its subsequent condensation with amino acids to form ADIN, through the Maillard reaction, likely explain the high levels of furfural and ADIN in spoiled SO_2 treated HMB. Grain temperatures in excess of 50°C are conducive to this process (Van Soest 1965). Furfural formation is favoured under acidic conditions (Van Soest 1982) and in this study, pentosans for furfural formation would have come primarily from the hemicellulose portion of the barley. Van Soest (1982) identified hemicellulose and soluble carbohydrates as the most reactive carbohydrates in the Maillard reaction. Since more water soluble carbohydrates are preserved in SO_2 treated silages (Knodt et al. 1952; Petrosyan 1967; Mathison et al. 1984) we would expect the same in SO_2 treated HMG. The characteristic dark brown colour and peculiar odour of the spoiled grain were consistent with both the elevated furfural and ADIN levels measured. Furfural has a characteristic odour resembling benzaldehyde and on exposure to air or light turns brown (Merck 1976) while ADIN is also a brown coloured polymer.

The bisulfite and sulfite ions of SO_2 do not limit thermal carbohydrate breakdown but are powerful Maillard reaction inhibitors. This would suggest that high furfural levels would be expected in spoiled SO_2 treated HMB, but that elevated ADIN levels would not be anticipated. An ADIN level of 25% of total N in the spoiled SO_2 treated HMB suggests extensive heat damage, because 10% of total N as ADIN (DM basis) in forages, which are not heat damaged, is the acceptable limit (Bath et al. 1985). It also indicates reduced nutritive value because protein is rendered relatively indigestible as ADIN (Van Soest 1982). To explain why SO_2 did not inhibit heat damage more effectively is difficult. The pH of the treated barley would not have reduced the efficacy of SO_2 in inhibiting heat damage, as the pH (3.6 to 3.9) favoured the ionization of H_2SO_3 to its bisulfite and sulfite ions (Green 1976). However, any reduction in molecular SO_2 concentration via binding, oxidation or volatilization would have reduced its ability to inhibit non-enzymatic browning and such losses would have been accelerated at the 40 to 60°C temperatures observed.

In spoiled SO_2 treated HMB, EtOH, acetate and lactate concentrations diminished significantly ($P<0.05$) over time ($r=-0.66$, -0.62, -0.88, respectively; $n=10$). Conversely, NH_3-N, moisture, and pH tended ($P<0.10$) to increase with time. These trends are consistent with chemical changes that occur in the aerobic deterioration of silage: (1) loss of primary fermentation acids, proteins and residual water soluble carbohydrates, and (2) production of CO_2, ammonia, water and heat (McDonald 1981; Woolford 1984).

Spoiled SO_2 treated HMB was also characterized by 10- and 100-fold increases in aerobic bacteria and fungi populations, respectively, over those measured for preserved SO_2 treated HMB at the same time. The grain temperatures of 40 to 60°C would require the presence of thermotolerant microflora; several Aspergillus fungi, as well as Penicillium arenarium are thermotolerant (Wallace 1973). Traces of black mould were also observed on the surface of the spoiled grain. The increased NH_3-N levels observed with time would suggest proteolytic clostridial activity as these are thermotolerant anaerobic bacteria (Wallace 1973). However, the absence of butyric acid, normally associated with silage that has undergone a clostridial fermentation (Vetter et al. 1978), implies that the role of these bacteria in the spoilage process was secondary to that of fungi. Proliferation of thermophilic sulfate or sulfite reducing anaerobic bacteria may have also contributed to spoilage losses. While similar anaerobic bacteria numbers in

preserved and spoiled SO_2 treated HMB would not support this conclusion, the culture media used in our microbiological procedures would not have facilitated the growth of such microbes due to insufficient sulfur.

The SO_2 treated HMB which remained well preserved was characterized by increasing ($P<0.01$) lactate and acetate levels over time ($r = 0.66$, 0.64 respectively; $n=20$). No significant ($P>0.05$) loss of S or moisture was measured in the barley between bin opening and 7 mo thereafter. There was a tendency ($P>0.10$) for NH_3-N and pH to increase with storage time ($r = 0.29$, 0.15 respectively; $n=20$) while EtOH levels diminished ($P<0.05$) with time ($r = -0.21$, $n=20$). No apparent change in numbers of bacteria or fungi was observed for this barley between the time of bin opening and 28 d later.

Spoilage rationale

Spoilage was likely initiated by moisture migration within the bin. This phenomenon occurs in an unaerated grain bulk of uniform moisture when temperature differentials exist within the bin causing heat and moisture to move, via convection, from warmer to colder areas of the bin (Sauer 1972; Muir 1973). A number of observations support this rationale.

The observation of spoilage within the bin core, while good preservation of HMB was observed at the bin perimeter, is consistent with moisture migration patterns commonly seen when ambient temperature is lower than grain temperature (Figure II-3). Furthermore in review, Muir (1973) suggests that increased barley temperature and moisture, as well as bin size were all conducive to a rapid rate of moisture migration.

It is generally recommended that for the safe storage of dry barley, a temperature differential between grain and average ambient temperature of 5°C necessitates cooling or warming of the grain (depending on the season) by aeration to prevent spoilage by moisture migration (Friesen et al. 1986). HMB was 28°C at harvest, 7°C warmer than mean ambient temperature. During the first 30 d of storage the temperature differential between SO_2 treated HMB and ambient averaged 19.3°C.

Moisture migration is more of a problem with large bins than small bins because higher temperature gradients within the grain bulk cause a faster rate of moisture migration. For this reason, it is recommended that bins holding more than 30 t be

equipped with an aeration system to prevent moisture migration (Agriculture Canada 1983). Friesen et al. (1986) recommend grain aeration for large scale bins, 60 t or larger. The upright bag used in this study contained 69 t of treated HMB, and it is likely that the large temperature differential, and 30% grain moisture level, provided conditions suitable for moisture migration. The results of this study appear to validate Campbell's (1972) concern for aeration of chemically preserved HMG in large scale storage. It is important to note that moisture migration was halted once the grain was transferred to the unsealed boxes and stored in 700 kg portions. This observation further supports the role of moisture migration in causing spoilage while grain was stored in the large upright bag. Mathison et al. (1985) also observed no spoilage of HMB treated with 0.75% to 1.1% (wt/wt) SO_2 when stored aerobically in 300 kg to 26 t portions.

Data collected on the day spoilage was first observed reflect the occurrence of moisture migration. Spoiled grain samples taken from the bin core at 91, 31 and 5 cm depths from the upper surface had corresponding grain moistures of 28.3, 31.5 and 43.2%. Respective S levels (DM basis) were 0.41, 0.44 and 0.53% with the correlation between grain moisture and S being highly ($P<0.001$) significant ($r=0.99$, $n=6$). Conversely, preserved grain sampled the same day from the bin perimeter had equivalent moisture and S levels at the same three sampling depths. These observations are direct evidence of an upward flux of moisture and S through the bin core.

Loss of molecular SO_2 may have also contributed to spoilage. The concentration of molecular SO_2 is influenced by: (1) pH of the preserved material, (2) level of free or unbound SO_2, (3) extent of oxidation to sulfate, and (4) extent of volatilization (Hammond and Carr 1976; Green 1976).

The low pH of the treated barley favoured the ionization of molecular SO_2 to its bisulfite and sulfite ions. It was estimated (Green 1976) that only 4 to 5% of the total SO_2, retained by the HMB, would be in the molecular form at the pH of 3.3 measured at bin opening.

Free SO_2 refers to that which has not been bound or combined with feedstuff components. Components that could have provided opportunity for binding of free SO_2 include disulphide bonds in proteins, sugars, aldehydes (e.g., furfural) and lignin (Eckoff and Okos 1983). The extent of these binding reactions is important because bound SO_2 gives little or no preservative action (Green, 1976). In work done with $^{35}SO_2$, Lozeman

et al. (unpublished) reported a 63% increase in bound SO_2 when SO_2 (1.2% wt/wt) treated HMB (22% moisture) was stored for 63 d at room temperature. Ingram and Vas (1950) reported a similar trend with SO_2 treated orange juice concentrate (350 ppm SO_2; pH 3.4). Four to 5 d storage at 10, 20 and 30°C resulted in binding of 56, 67 and 80%, of the total SO_2, respectively. Work with sodium metabisulfite as a silage preservative also suggests that bound SO_2 has no benefit as a preservative, since SO_2 addition to silage did not affect ensiling temperatures when mean silage temperature exceeded 30°C during the first 20 d of ensiling (Lanigan 1961). In this study, we measured a mean temperature of 35°C for SO_2 treated HMB at the bin centre during the first 30 d of storage. Thus, barley storage temperature measured in this study together with data from previous research suggest that a significant loss of free SO_2 via binding reactions was highly probable.

Concentration of molecular SO_2 may have been further reduced via oxidation to sulfate. Lozeman et al. (unpublished) measured 26% of the total radioactivity as sulfate in $^{35}SO_2$ treated HMB after 63 d of "sealed storage conditions" at room temperature. Based on this, oxidation of SO_2 to sulfate would likely be more extensive in this study due to presumably more aerobic storage conditions.

A significant loss of molecular SO_2 via volatilization could have occurred depending on the SO_2 permeability of the bag. While the permeability of the upright bag to oxygen was determined, its permeability to SO_2 was not. However, an insignificant ($P>0.05$) loss of total S in both barley which spoiled, and that which remained preserved, between bin opening and 7 mo thereafter does not support this speculation. The successful preservation achieved by Mathison et al. (1985) with 0.75 to 1.1% (wt/wt) SO_2 treated HMB under aerobic storage conditions also implies that the loss of molecular SO_2 via volatilization should not be a concern. Nevertheless, Lozeman et al. (unpublished) measured a 17% volatile loss of S from $^{35}SO_2$ treated HMB over 63 d of "sealed storage", even though the grain remained well preserved. Similarly, food preservation research has shown that SO_2 is easily lost from products which are not stored in "airtight containers", the rate of this loss being influenced by storage temperature and moisture content of the product (Sawyer and Crosby 1980).

The data suggests that a 1% (wt/wt) SO_2 application level was adequate for 30% moisture barley since this grain, once removed from the upright bin, remained well preserved for the entire 9 mo of storage.

McDonald (1981) states that carbohydrate rich silages, in which fermentation has been restricted by an additive, are particularly prone to aerobic deterioration. It seems logical that SO_2 treatment of carbohydrate rich HMB would equally predispose the grain to aerobic deterioration if treatment efficacy began to fail during near anaerobic storage. Eckoff et al. (1983) suggested that SO_2 treatment initially suppressed the indigenous microflora, but that the most resilient and opportunistic microorganism dominated when treatment efficacy began to fail. Furthermore, they state that such single micro-organism cultures appear to proliferate more quickly than the mixed culture that would have otherwise been present due to a lack of competition. Thus, it appears that when SO_2 treatment began to fail in this study, high temperatures, oxygen supply, and low pH favoured the proliferation of moulds. A further concern is the caution of Green (1976) that: "moulds and yeasts can develop an immunity to SO_2, or they may spore and remain dormant even at ever-increasing high levels of preservative."

In conclusion, this study demonstrated that as a grain preservative, SO_2 preserves by inhibiting fermentation. Under these storage conditions, SO_2 treatment appeared to be efficacious as a bacteriocide but ineffective as a fungicide. A 1% (wt/wt) SO_2 application rate did not effectively preserve HMB for large scale (69 t) near anaerobic storage. Successful preservation was achieved with small scale (700 kg) aerobic storage. Our data leads us to conclude that moisture migration and resultant loss of molecular SO_2 via binding reactions at high grain temperatures were the major cause for failure with large scale storage.

Table II-1 Chemical characteristics of ensiled and well preserved SO_2 treated high moisture barley (HMB) at bin opening†

Item	Treatment		
	Ensiled HMB	SO_2 treated HMB	Significance
Ethanol (mg g^{-1})	20.57 (2.61)‡	0.80 (0.24)	***
Acetate (mg g^{-1})	1.38 (0.04)	0.37 (0.02)	**
Lactate (mg g^{-1})	8.13 (0.37)	5.34 (0.73)	*
pH	5.7 (0.03)	3.3 (0.10)	**
Furfural (mg g^{-1})	0.17 (0.03)	0.16 (0.01)	NS
Crude protein (%)	11.3 (0.20)	11.5 (0.22)	NS
Ammonia-N (ug g^{-1})	96.05 (1.65)	78.08 (3.36)	*
Sulfur (%)	0.15 (0.003)	0.50 (0.011)	***
ADF (%)§	9.28 (0.574)	6.79 (0.204)	**
ADIN (% of total N)¶	6.08 (0.44)	2.72 (0.16)	**
Moisture (%)	29.7 (0.18)	30.0 (0.17)	NS

†Means expressed on DM basis; four and six observations for HMB and SO_2 treatment respectively.

‡Values in parentheses represent standard error of mean.

§Acid detergent fibre.

¶Acid detergent insoluble nitrogen.

*P<0.05, ** P<0.01, *** P<0.001, NS P>0.05

Table II-2 Chemical characteristics of preserved and spoiled SO_2 treated high moisture barley (HMB) between 28 and 56 days after bin opening†

Item	Classification		Significance
	Preserved	Spoiled	
Ethanol (mg g^{-1})	0.44 (0.11)	0.33 (0.12)	NS
Acetate (mg g^{-1})	0.48 (0.09)	1.19 (0.25)	***
Lactate (mg g^{-1})	6.53 (0.37)	6.60 (0.35)	NS
pH	3.6 (0.12)	3.9 (0.15)	*
Furfural (mg g^{-1})	0.16 (0.01)	0.40 (0.07)	***
Ammonia N (ug g^{-1})	112.54 (24.94)	578.54 (66.98)	***
Sulfur (%)	0.48 (0.01)	0.49 (0.02)	NS
ADIN (% of total N)§	2.74 (0.16)	25.0 (4.2)	**
Moisture (%)	29.6 (0.27)	30.2 (1.15)	NS

†Means expressed on DM basis; 18 and 24 observations for preserved and spoiled SO_2 barley respectively.

‡Values in parentheses represent standard error of mean.

§Acid detergent insoluble nitrogen.

*P<0.05, ** P<0.01, *** P<0.001, NS P>0.05.

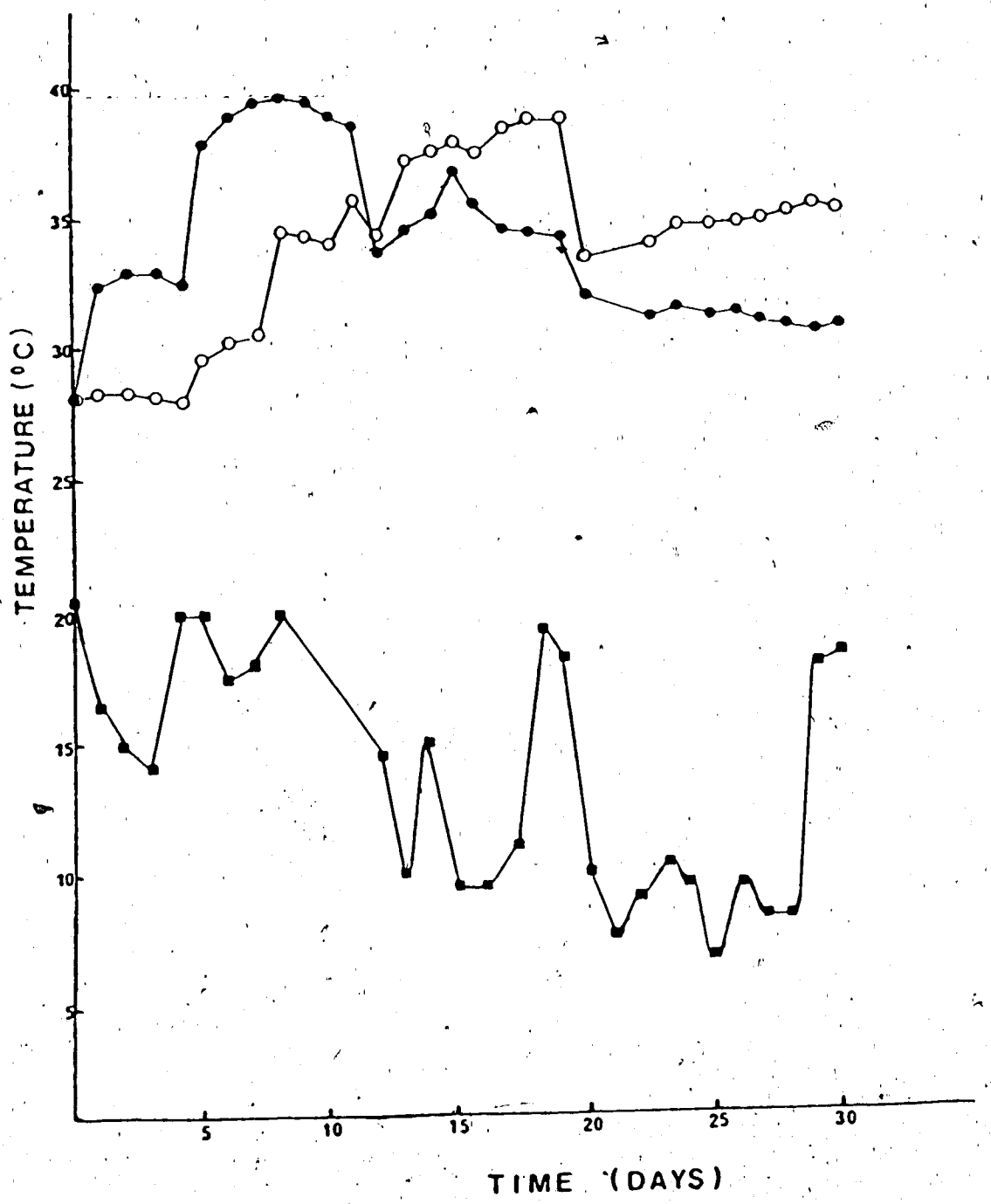

Figure II-1 Mean temperatures of ensiled (●) and SO_2 treated (O) high moisture barley during the first 30 d of anaerobic and near anaerobic storage respectively (■ = ambient).

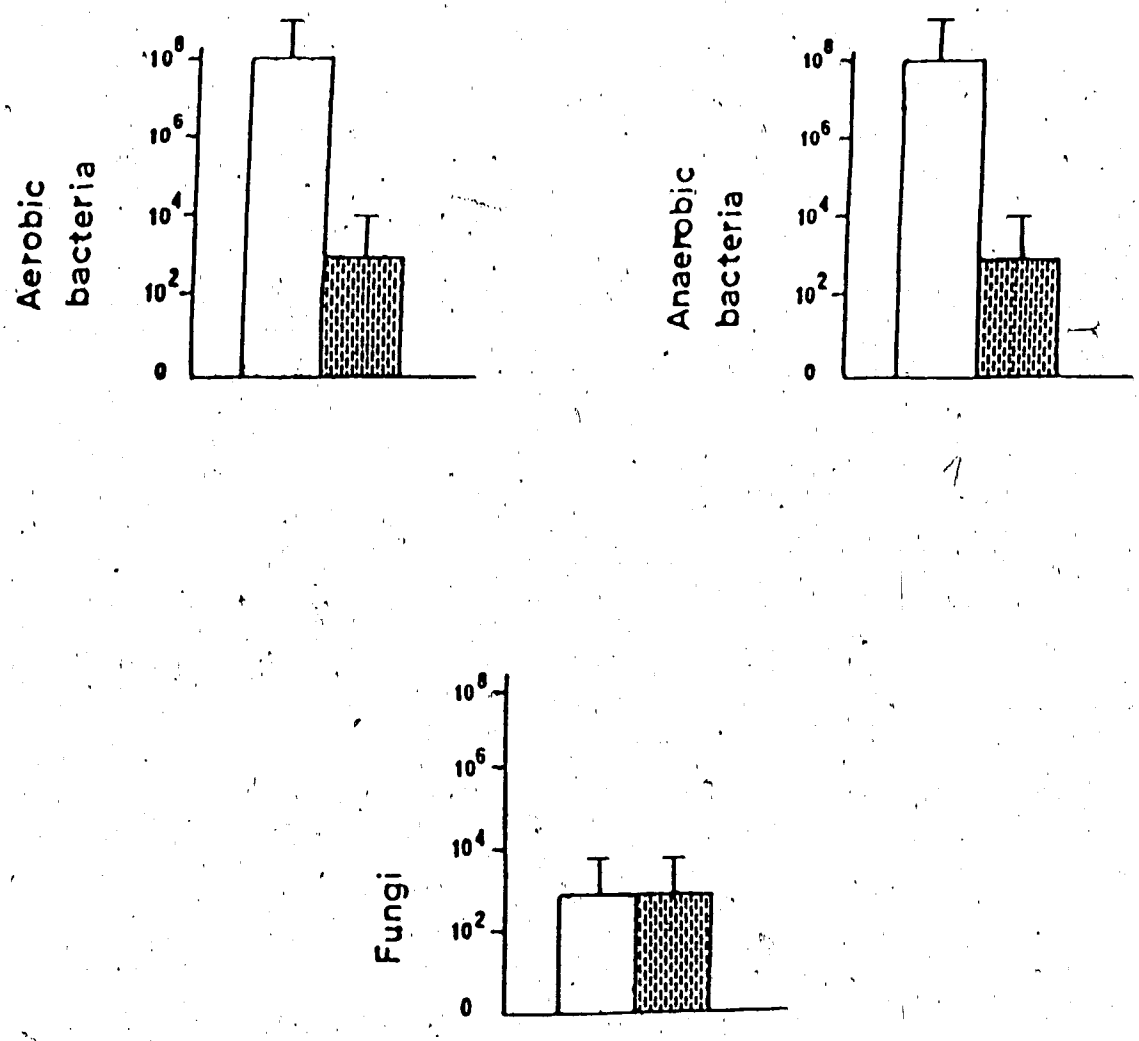

Figure II-2 Mean (4 replicates) bacterial and fungal numbers (colonies/fresh g) on ensiled (□) and SO_2 treated (▤) HMB at bin opening. Vertical bars are standard errors.

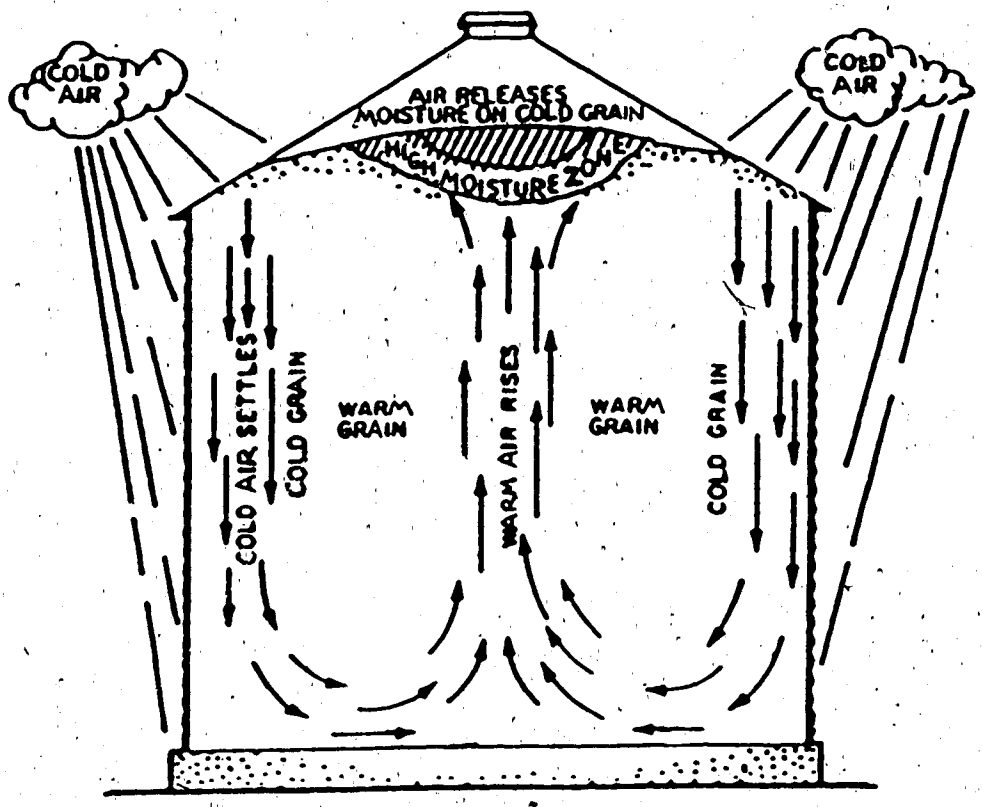

Figure II-3 Moisture migration when ambient temperature is lower than grain temperature (Adapted from Friesen et al. 1986).

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III. THE PERFORMANCE OF DAIRY AND FEEDLOT CATTLE FED SULFUR DIOXIDE PRESERVED HIGH MOISTURE BARLEY

A. INTRODUCTION

The feeding value of either ensiled or organic acid treated high moisture barley (HMB) is similar to dry barley for both lactating dairy cows and feedlot cattle when compared on a dry matter (DM) basis (BP Chemicals 1969; Merrill 1971; Wilton 1971; Marx 1973; Ingalls et al. 1974; Kung et al. 1983; Kennelly 1983; 1984; Kennelly et al. 1984). Farmers who feed HMB by preference or because of adverse weather conditions at harvest, have traditionally ensiled the material or preserved it chemically with organic acids. Presently, cost and convenience militate against the routine use of organic acids as grain preservatives (GP).

The antimicrobial activity of sulfur dioxide (SO_2) and its salt, sodium metabisulfite ($Na_2S_2O_5$), is well known due to its long history as a preservative in the food and beverage industry (Schroeter 1966). Recent work by Mathison et al. (1985) and Gibson (Chapter II) indicates that SO_2 may have potential as a GP. To date, there is no information on the feeding of SO_2 treated HMB to lactating dairy cows. The one previous trial conducted with SO_2 treated HMB for feedlot steers has shown its feeding value to compare favourably with that of dry barley (Mathison et al. 1985).

The objective of this experiment was to determine the influence of feeding SO_2 treated HMB on the performance of lactating dairy cows and feedlot cattle.

B. MATERIALS AND METHODS

Barley harvesting, treating and storage

All barley used in this experiment was of the same variety (Klondike) and grown under the same conditions. HMB was combined at 30 to 32% moisture immediately after swathing. Dry barley was harvested at 12 to 14% moisture after four additional days of drying in the swath. Two fields of 28.2 and 20.2 ha were divided into eight and four rectangular strips, respectively. Odd numbered strips were harvested as HMB, even numbered strips were harvested as dry barley. Plot areas harvested for HMB were about twice the size of those harvested for dry barley as double the amount of HMB was

required in subsequent feeding trials.

HMB was ensiled in a Harvestore® silo or treated with 1% (wt/wt) SO_2 as previously described (Chapter II). Dry barley was stored at 12 to 14% moisture. Treated HMB was stored for 130 d in a 69 t capacity upright bag (polyethylene lined polyester) which constituted a near anaerobic system (Chapter II). Subsequently, well preserved SO_2 treated HMB was removed from the upright bag and stored in unsealed boxes of 700 kg capacity. Feeding trials commenced 74 d after SO_2 application.

Experiment 1: SO_2 treated HMB for lactating dairy cows

Thirty-six Holstein cows were assigned to three groups of 12 with groups balanced on the basis of average milk production per cow during a 2 wk pre-trial period, days in milk and lactation number. Three dietary treatments (Table III-1) were randomly assigned to the three groups for the 6 wk trial. Diets consisted of 35% rolled barley (DM basis) and differed only in the type of barley fed: dry barley, ensiled HMB or SO_2 treated HMB. Diets were fed twice daily with forage and concentrate portions offered simultaneously, but not as a total mixed ration.

Milk yield, feed intake and feed refusals were recorded daily. Milk yield persistency was calculated according to procedures of Kung et al. (1983). Feed samples were taken weekly, frozen immediately and stored at -22°C until composited for chemical analyses. Cows were weighed once weekly. Both initial and final weights were the mean of measurements on two consecutive days.

Milk fat, protein and lactose were determined by infrared milk analysis (Alberta Agriculture, Central Milk Testing Laboratory, Edmonton, Alberta) on composite AM and PM samples, for each cow, collected once weekly. Additional milk samples were taken pre-trial and at weeks two and four of the trial, from each cow, and composited according to treatment group for sensory evaluation of both raw and pasteurized milk. Milk was batch pasteurized at 63°C for 30 min (Lampert 1975). Sensory evaluation of milk was conducted on these three occasions, using nine untrained consumer panelists in a triangle test, to identify the odd sample (Agriculture Canada 1977). These composite milk samples were also scored by a trained expert according to procedures developed by the American Dairy Science Association (Nelson and Trout 1964).

Experiment 2: SO_2 treated HMB for feedlot steers

Upon arrival at the feedlot, 64 Hereford-type steers were fed 2 kg head^{-1} of dehydrated alfalfa pellets and grass hay ad libitum. Trace mineral salt and a 1:1 mixture of salt and calcium phosphate were provided free-choice. Between feedlot arrival and commencement of the trial steers were eartagged; vaccinated for blackleg and malignant edema; and given an intramuscular injection of 1,000,000, 150,000 and 100 IU of vitamin A, D_3 and E, respectively. Steers were also implanted with zeranol (Ralgro®, International Minerals Co. Ltd., Terre Haute, Ind.).

Steers were divided equally between a heavy and light group with an average initial weight of 410 and 372 kg, respectively. Within each group, steers were ranked by weight, then randomly allocated within weight groups to eight identical pens. Pens were 4 x 8 m, bedded with wooden shavings and partially covered by an open-front shed. The four dietary treatments (Table III-2) consisted of dry barley, ensiled HMB, SO_2 treated HMB, and SO_2 treated HMB plus sodium bicarbonate ($NaHCO_3$). Sodium bicarbonate was included as a treatment to determine the influence of feeding a readily excretable cation to animals fed diets with a high sulfur (S) content. A secondary objective was to determine the effect of a buffer on the palatability of SO_2 treated HMB. Supplemental thiamin was included in the diets to supply 5.5 mg kg^{-1} DM, because SO_2 treatment destroys thiamin (Gray 1980).

Over a 14 d period, steers were brought onto a dietary regime consisting of: 85% rolled barley, 5% supplement and 10% chopped hay (DM basis). The feeding trial commenced with steers fed concentrate at 2.0 kg DM day^{-1}. Concentrate was increased at about 0.64 kg DM head^{-1} day^{-1} while hay was decreased by approximately 0.83 kg DM head^{-1} day^{-1}.

Feed intakes were recorded daily on a pen basis, weighbacks were measured every 2 wk. Initial weights were the mean of measurements on three consecutive days; thereafter, steers were weighed once every 2 wk during the 96 d trial. Final weights were the mean of measurements on two consecutive days. All weights were taken after water had been withheld for 16 h.

Barley samples were taken every 2 wk during the trial, frozen immediately and stored at -22°C until chemical analyses. Hay and supplement samples were taken on four occasions during the trial and stored in the same manner until composited for analyses.

Steers were slaughtered 2 d after termination of the feeding trial. At slaughter, livers were inspected and the incidence of abscessed livers recorded. Additionally, heads of two steers from each of the two SO_2 treatments and from the dry barley control were subjected to postmortem examination (Alberta Agriculture, Pathology Branch, Animal Health Division, Edmonton, Alberta). Animals were checked for possible brain lesions indicative of acute thiamin deficiency, otherwise known as polioencephalomalacia (PEM). After overnight chilling, carcasses were graded by Agriculture Canada Food Production and Inspection Branch personnel according to beef carcass grading procedures (Agriculture Canada 1972). Dressing percentages were calculated from warm carcass weights and final feedlot weights.

Analytical

Feed dry matters were determined by oven drying at 110°C for 24 h as well as by gas chromatography (Fenton et al. 1981) using an Aerograph 660 gas chromatograph. In the latter method, methanol was used for sample water extraction and samples were shaken for 48 h continuously for complete extraction. Crude protein and thiamin were determined according to the methods of the Association of the Official Analytical Chemists (AOAC 1980). Feed samples were freeze-dried and ground to a homogenous powder (0.5 mm) prior to thiamin, acid detergent fibre (ADF) and S measurements. Sulfur was determined using a LECO Sulfur Determinator SC 132 (St. Joseph, MI) as previously described (Chapter II). Acid detergent fibre was determined according to Goering and Van Soest (1970). Calcium and phosphorus were measured by the meta-vanadate method of the AOAC (1980) (Alberta Agriculture, Soil and Animal Nutrition Laboratory, Edmonton, Alberta).

Statistical Analyses

Yield differences between high moisture and dry barley were subjected to one way analysis of variance (ANOVA) (Steel and Torrie 1980). One way ANOVA was applied to feed intake, performance and milk composition data of dairy cows to assess differences between treatments. Dairy cow performance data were also subjected to two way ANOVA with treatment and time as the sources of variation. Triangle test data on milk were analyzed using tables of Roessler et al. (1948). A two way ANOVA was used to

analyze feedlot data with treatment and animal weight groups as sources of variation. Treatment differences in feedlot performance were determined for complete data as well as data collected for each of the three time periods; days 1–28, 29–57 and 58–96. Multiple comparison of means was by Student–Neuman–Keuls' test (Steel and Torrie 1980).

C. RESULTS

Barley harvest and preservation

Fresh weight yields of HMB and dry barley were 5.44 and 4.31 t ha^{-1}, respectively. On a DM basis, these yields corresponded to 3.81 and 3.75 t ha^{-1}, respectively. The 1.6% higher DM yield for HMB over dry barley was nonsignificant ($P>0.05$).

The ensiled HMB, as well as the dry stored barley showed no visible signs of spoilage throughout the feeding trials. SO_2 treated HMB remained well preserved in the 69 t near anaerobic upright bag until 130 d after treatment; 28 d after the bin was opened for feeding purposes. Barley at the bin perimeter remained well preserved, but heating (40 to 60°C) and mould growth within the bin core resulted in about 30% loss through spoilage. This spoiled grain was discoloured and had elevated levels of furfural, ammonia–nitrogen (NH_3–N) and acid detergent insoluble nitrogen (ADIN) (Chapter II), all indicative of reduced nutritive value. Approximately 56 d into the feeding trials (28 d after spoilage was observed), the well preserved SO_2 treated HMB was removed from the upright bag and stored in uncovered boxes of 700 kg capacity. This grain showed no visible signs of spoilage for the duration of the feeding trials.

Experiment 1

Despite preservation problems with SO_2 treated HMB, cows were fed well preserved SO_2 treated HMB for the entire 6 wk. This was possible because the small amount required daily could be removed from the bin perimeter without contamination of spoiled barley from the bin core.

One cow was removed from the trial because her appetite for SO_2 treated HMB and supplement was erratic, diminishing markedly in the last week of the trial. This cow

had no apparent health problems, but had a history of being a finicky eater.

No differences ($P>0.05$) were observed in DM intake (DMI), milk yield or persistency, milk composition, or liveweight change for cows fed the three diets (Table III-3). Nevertheless, cows fed diets based on dry barley produced 6.8 and 9.7% more daily fat corrected milk (FCM) than cows fed diets containing HMB and SO_2 treated HMB, respectively. There was a trend, which approached significance ($P<0.13$), for cows fed diets containing dry barley to convert DMI more efficiently to FCM (FCM:DMI). In wk 1, cows fed diets based on HMB ate 2.3 kg more ($P<0.05$) total DM than cows fed diets containing either dry or SO_2 treated HMB, but this did not result in any production differences ($P>0.05$) between treatment groups in wk 1. As expected, feed intake, milk production and FCM:DMI diminished ($P<0.05$) between wk 1 and 6 while cow body weight increased ($P<0.05$). No treatment x week interaction was observed ($P>0.05$) for any of the parameters measured.

Sensory evaluation of both raw and pasteurized milk detected no differences ($P>0.05$) in milk flavour or odour between treatments (data not shown).

Experiment 2

Due to a limited quantity of well preserved SO_2 treated HMB, steers were fed spoiled SO_2 treated HMB at an estimated 40% DM inclusion level, primarily during period two (29 to 57 d) of the finishing period. While traces of black mould were observed in spoiled SO_2 treated HMB, barley fed to steers had no visible evidence of mould growth.

Four steers fed the SO_2 treatments (two from each treatment) developed PEM as determined by post-mortem brain examination. Two of these steers died before the trial ended. Prior to death both steers exhibited clinical signs of PEM, namely prodromal anorexia, impaired vision and head-pressing (Merck 1979).

Feedlot performance and carcass characteristics of steers are presented in Tables III-4 and III-5, respectively. Steers fed SO_2 treated HMB had a 10.1% lower DMI, 31% lower average daily gain (ADG) and required 21% more feed per kg of gain (DMI:gain) than those fed either dry or ensiled HMB ($P<0.05$). Steers from the heavy group consumed more ($P<0.01$) feed and tended ($P<0.13$) to gain faster than those from the light group. The addition of $NaHCO_3$ had no apparent effect on palatability of SO_2

treated HMB and feedlot performance was not influenced ($P>0.05$) by its addition. The lower DMI of steers fed the SO_2 treatments tended to become significant ($P<0.10$) in the last two periods of the trial, day 29 to 57 and 58 to 96, respectively. While feeding of SO_2 treated HMB adversely affected ADG and DMI:gain, by individual periods this effect was significant ($P<0.05$) only in period one. Neither feedlot performance or carcass characteristics differed between steers fed HMB or dry barley diets ($P>0.05$). While the difference was nonsignificant ($P>0.10$), steers fed the dry barley diet required 13.3% less feed per kg of gain (DMI:gain) than those fed the HMB diet.

The negative influence of feeding SO_2 treated HMB to steers resulted in lower ($P<0.10$) final feedlot and carcass weights, but no other carcass characteristics were influenced ($P>0.05$) by treatment. Weight group affected ($P<0.05$) carcass weight, dressing percent and ribeye measurement; the heavy group being higher than the light group by 10.0, 1.4 and 7.5%, respectively. Steers fed SO_2 treated HMB tended to have reduced ($P>0.05$) carcass fat depth; however, the addition of $NaHCO_3$ to the diet appeared to counteract this effect. The only significant ($P<0.05$) treatment x weight group interaction effect observed was in carcass fat depth. There was no apparent difference between treatments in the incidence of abscessed livers.

D. DISCUSSION (EXPERIMENTS 1 AND 2)

Barley harvest, preservation and nutritive value

Reported DM yield advantages of HMB over dry barley range from 6.7 to 19.7% (Krall 1972; Windels 1972; Marx 1981; Kennelly et al. 1984) with 7–10% being more common (Alberta Agriculture 1982a). The yield benefit of HMB over dry barley is primarily due to the threshing characteristics of a high moisture product which results in less shattering and more wild oats (Windels 1972), as well as more thin (small light weight) kernels (Krall 1972; Marx 1981; Alberta Agriculture 1982a).

Three factors may help to explain the lack of yield advantage in favour of HMB observed in this study. First, HMB was harvested conventionally and not by direct combining. Direct combining, according to Alberta Agriculture (1982b), accounts for 2 to 3% of the DM yield advantage of HMB by reducing handling losses. Second, the contribution that thin kernels normally make to the yield advantage of HMB ranges from

2.1 (Krall 1972) to 3% (Alberta Agriculture 1982b). However, for this contribution to be realized, a slower combine ground speed is required because moist kernels do not sift through the straw as easily as dry barley (Krall 1972; Lauder 1979). Ground speed and grain losses for combining dry and HMB were not measured in this study. Third, field losses of dry barley would have been minimal as drying conditions were optimal during the 4 d between swathing and combining.

The causes and extent of the grain spoilage observed with SO_2 treated HMB have been discussed previously (Chapter II).

The spoiled SO_2 treated HMB fed to steers contained elevated furfural, NH_3-N and ADIN levels, indicating a loss of energy (carbohydrate degradation) and digestible protein, and presumably, palatability. An ADIN level of 25% of total N was measured for the spoiled SO_2 treated HMB, those measured for ensiled and well preserved SO_2 treated HMB were 6.1 and 2.7%, respectively (Chapter II). Bath et al. (1985) state that 10% of the total N as ADIN is normal in forages which are not heat damaged. Thus, the ADIN level of spoiled SO_2 treated HMB would have rendered about 15% of the total N or 1.7% of the barley crude protein (CP) as unavailable or indigestible (Van Soest 1982). As a result, the available CP of the diets containing spoiled SO_2 treated HMB would have been reduced to 9.5%, almost 2% below the CP level of the dry barley and ensiled HMB diets.

Animal performance

The comparable performance and normal health status of cows fed ensiled or SO_2 treated HMB versus dry barley in experiment 1 is in general agreement with the results of other trials conducted with lactating cows in which diets included either ensiled or organic acid treated HMB (Marx 1973; 1974; Ingalls et al. 1974; Kennelly et al. 1984). Feeding silage treated with 0.0 to 0.28% (wt/wt) SO_2 to dairy cows has also resulted in similar feed intake (Dufour et al. 1954), milk production and body weight change (Knodt et al. 1952; Dufour et al. 1954) when not confounded by excessive (Stallcup 1955 cited by Mathison et al. 1979) or uneven SO_2 application (Bratzler et al. 1955). More recently, Weigand et al. (1972) fed 18 to 20 g of S cow^{-1} day^{-1} for 110 d via beet pulp with added $Na_2S_2O_5$ with no adverse effect on milk yield or butterfat test, rumen motility, hematology, or animal behavior. Cows fed SO_2 treated HMB in experiment 1

consumed, on average, 24 g of S from SO_2 $head^{-1}$ day^{-1}.

The primary factor responsible for both the reduced performance and a 12.5% incidence of PEM in steers fed SO_2 treated HMB in experiment 2 was probably the feeding of spoiled grain. This conclusion is based on the fact that the distinguishing feature of this trial from those of Mathison et al. (1985), in which feeding SO_2 treated HMB to feedlot cattle had no adverse effects, was the feeding of spoiled SO_2 treated HMB. This conclusion was strengthened by the fact that eight comparisons with steers wintered on legume, legume/grass or cereal silages treated with 0.14 to 0.66% (wt/wt) SO_2 have shown either no or even a beneficial influence on steer performance as measured by ADG and DMI:gain (Mathison et al. 1979; 1984). Examination of steer performance by period (Table III-4) reflected the reduced nutritive value of the spoiled SO_2 treated HMB fed during period two (29 to 57 d) of the finishing period. Dry matter intake by steers fed SO_2 treated HMB was reduced ($P<0.10$) during period two, while ADG and DMI:gain improved substantially in period three when steers were again consuming well preserved SO_2 treated grain. The lack of change in DMI for steers fed SO_2 treated HMB between period two and three, coupled with the improvement in ADG and DMI:gain suggests that spoilage had a more marked influence on grain nutritive value than on its palatability.

The feeding of SO_2 treated HMB to dairy and beef cattle in these experiments, when not confounded by spoilage, had no readily apparent physiological or toxicological effect. This was in agreement with observations made in two other feedlot trials in which SO_2 treated HMB has been fed (Mathison et al. 1985). The feeding of SO_2 treated HMB to cattle in experiments 1 and 2 and in the trials by Mathison et al. (1985) have involved intakes of S from SO_2 ranging from 20 to 25 g $head^{-1}$ day^{-1}, equivalent to 40 to 50 mg of S from SO_2 per kg of body weight (bw). Luedke et al. (1959) reported that a cow receiving 26.5 g of S from $Na_2S_2O_5$ (approximately 50 mg of S from $Na_2S_2O_5$ kg of bw^{-1}) for 180 d via rumen fistula was also uneffected by treatment. At the end of the treatment period the cow calved normally, producing a healthy calf. Similarly, they reported that 600 cattle were fed $Na_2S_2O_5$ treated silage for a period of 5 yr without adverse effect. Luedke et al. (1959) concluded that a daily 40 to 80 g dose of S from SO_2 or $Na_2S_2O_5$ would produce anorexia and because of this, an acute S toxicity would be unlikely.

Implications of elevated dietary sulfur due to SO_2 treatment of HMB

In experiment 1, the concentration of dietary S (DM basis) was 0.20, 0.20 and 0.32% for the dry barley, ensiled and SO_2 treated HMB diets, respectively (Table III-1). At 0.20% S, cows fed dry barley or ensiled HMB received National Academy of Sciences - National Research Council (NAS-NRC 1978) recommendations for S. The diet based on SO_2 treated HMB, at 0.32% with 0.12% S from SO_2, was 160% of NAS-NRC (1978) recommendations. Steers in experiment 2 consumed diets of 0.15, 0.17, 0.46 and 0.46% S for the dry barley, ensiled HMB, SO_2 treated HMB and SO_2 treated HMB plus $NaHCO_3$ diets, respectively (Table III-2). Steers fed the dry barley or ensiled HMB received adequate S according to NAS-NRC (1984) recommendations. The NAS-NRC (1984) recommends a dietary level of 0.10% S for beef cattle diets, but emphasizes that requirements are not well defined. The two diets based on SO_2 treated HMB, with 0.30% of the 0.46% S from SO_2, were 460% of NAS-NRC (1984) S recommendations.

The maximum tolerable level of S is known to be influenced by its chemical form (NAS-NRC 1980). Thus, NAS-NRC (1978) suggests a dietary maximum of 0.35% S with no more than 0.20% from added sulfate. NAS-NRC (1980) states that 0.40% is the maximum tolerable S level as sulfate while Kandylis (1984) concluded that 0.3 to 0.4% S as sulfate or elemental S in ruminant diets may cause toxic effects. Presumably, the concern about dietary sulfate level is its rapid reduction to sulfide in the rumen (Goodrich 1975), since if not incorporated into microbial protein, sulfide is potentially lethal because of hydrogen sulfide production in the rumen and the formation of sulfhemoglobin in the blood (Kandylis 1984). Work by Lozeman et al. (unpublished) and by this author (Chapter II), indicates that S from SO_2 treated HMB would be ingested primarily as bound SO_2 (e.g. hydroxysulfonates), to a lesser extent as bisulfite, and to the least extent as sulfate. Even if all the 0.12% added S from SO_2 was oxidized to sulfate, cows fed SO_2 treated HMB in experiment 1 were 70% below the NAS-NRC (1980) maximum tolerable S level as sulfate. Nevertheless, since excessive amounts of S reduce feed intake (NAS-NRC 1978), it is not surprising that one cow in experiment 1, previously determined to be a finicky eater, did not relish the SO_2 treated grain. If all the 0.30% added S from SO_2 was oxidized to sulfate, steers fed the SO_2 treated HMB in experiment 2 were only 25% below the NAS-NRC (1980) maximum tolerable S level as sulfate. The fact that DMI by steers fed the SO_2 treatments was consistently lower throughout the trial than that by steers fed either dry barley or ensiled HMB treatments,

suggests that the dietary S level in the SO_2 treatments was high enough to negatively influence diet palatability. If S level in the SO_2 treatments did depress DMI by steers in experiment 2, this effect would have been confounded by the feeding of spoiled grain between day 29 to 57 of the trial. The work of Mathison et al. (1984; 1985) suggests, however, that the maximum tolerable level may not have been reached since dietary S levels of 0.46 and 0.69%, with 0.25% and 0.40% S from SO_2 respectively, did not adversely effect steer performance or health with either cereal silage or high concentrate diets. Similarly, Rumsey (1978) reported that the feeding of all-concentrate diets in which 0.42% sublimed S was added did not significantly reduce ADG or DMI:gain of finishing cattle. The addition of 0.72% sulfate to the diet however, has been reported to contribute to a high incidence of PEM in cattle fed 70% concentrate (DM basis) diets (Sadler et al. 1983).

The high S level associated with feeding SO_2 treated HMB to cattle in these experiments could have interfered with the metabolism of copper (Cu), molybdenum (Mo), and selenium (Se) (NAS-NRC 1978). Data by Mathison et al. (1984) indicate that the feeding of SO_2 treated silage to steers for up to 66 d does not influence animal Se status as measured by blood Se levels.

Sulfur:Nitrogen ratio

The dietary nitrogen (N) to S ratio for cows fed SO_2 treated HMB was 8:1, and for cows fed dry barley or ensiled HMB it was 13:1. Dietary N:S ratios in experiment 2 were 4:1 for the SO_2 treatments and 11:1 for the dry barley or ensiled HMB treatments. The most frequently recommended N:S ratio is 10:1 (Elam 1975; NAS-NRC 1978; Bull 1984), while Bouchard and Conrad (1973a) have shown a 12:1 N:S ratio is adequate in maintaining maximum DMI by lactating dairy cows. Since no diet in either experiment contained minimal S, and because the performance response to dietary S supplementation diminishes when the N:S ratio becomes 10:1 or less (Moir 1979 cited by Bull 1984), N:S ratio was likely of no consequence on animal performance in either trial.

Effect of sodium bicarbonate addition to SO_2 treated HMB diet

It is possible that the elevated dietary S levels caused by feeding SO_2 treated HMB could have overloaded the urinary excretion system (Keener et al. 1953; NAS-NRC

1978). Excessive S over and above metabolic needs is excreted chiefly as inorganic sulfate complexed with Na^+, K^+, NH_4^+ or H^+ cations in the urine. According to Denis and Hobson (1923) however, the excretion of inorganic sulfate is difficult relative to other inorganic ions usually present in the blood (cited by Keener et al. 1953). Presumably in experiment 2, the provision of Na^+ and H^+ cations through dietary $NaHCO_3$ addition helped in the excretion of excess sulfate generated by feeding SO_2 treated HMB. Urinary sulfate levels were not measured in either experiment. Keener et al. (1953) have stated that there is little chance that the excretion of large amounts of sulfate from feeding SO_2 treated grain would cause renal damage. However, they suggest that more work is needed in this area.

Influence of SO_2 on dietary thiamin

While diets containing SO_2 treated HMB in both experiments were lower in thiamin and higher in S relative to either dry barley or HMB diets (Tables III-1 and III-2), conditions which are predisposing to thiamin deficiency, it is not surprising that animal performance and thiamin status (on the basis of PEM) were adversely affected only in experiment 2. This is partially because diets in experiment 1, due to lower concentrate levels, were not as conducive to causing lactic acidosis as those fed in experiment 2. Lactic acidosis appears to establish rumen conditions conducive for PEM development (Brent 1976). More importantly though, steers in experiment 2 could have consumed spoiled SO_2 treated HMB which appeared acceptable by sight and smell in terms of mould contamination, but which was still mould contaminated. Evidence linking PEM to feeding of the spoiled grain is threefold. First, the four steers which developed PEM (two of these died), did so during the period in which spoiled grain was being fed (29 to 57 d). Second, no PEM was observed by Mathison et al. (1985) in two feedlot trials involving SO_2 treated HMB. Their trials were of similar duration and had similar dietary S and concentrate levels, and either 0.0 or 6.2 mg kg^{-1} DM of supplemental thiamin. Third, although evidence is contradictory and not conclusive, it has been determined that certain species of fungi associated with mouldy feed are thiaminase producers (Loew et al. 1972 cited by Blood et al. 1983). This is significant because destruction of thiamin and production of an anti-thiamin analog by ruminal thiaminase enzymes (thiaminase I, EC 2.5 1.2; thiaminase II, EC 3.5 99.2) have been shown to be

the most likely cause for PEM (Edwin et al. 1968; Edwin and Jackman 1982). Despite the strong case for acute thiamin deficiency in experiment 2, the SO_2 diet fed to cows in experiment 1 would also not have supplied the 21 to 47 mg day^{-1} thiamin requirement assumed by Zintzen (1973) for lactating dairy cows (cited by Breves et al. 1981). Animal thiamin status data were not determined in these experiments.

Influence of SO_2 on palatability of HMB

HMB has been considered by many to be more palatable than dry barley (Merrill 1971; Krall 1972; Windels 1972; Marx 1981). This is perhaps due to the organic acid end products of anaerobic fermentation (Weltzien 1986). Additional factors reported to contribute to its enhanced palatability include: (1) reduced dust (Marx 1974; 1981) and (2) less scouring and foundering in feedlot cattle due to more uniform daily feed consumption as they are brought onto a high concentrate diet (Krall 1972; Windels 1972; Marx 1974; 1981). The higher ($P<0.05$) DMI by cows fed diets containing HMB vs. those fed diets containing SO_2 treated HMB or dry barley during wk 1 of experiment 1 was a continued trend ($P<0.19$) throughout the 6 wk trial. This trend was consistent with the lower organic acid levels measured in both SO_2 treated HMB (Chapter II) and silages (Knodt et al. 1952; Mathison et al. 1979). The fact that steers in the study of Flipot and Pelletier (1980) tended ($P>0.05$) to consume more total DM for a diet based on organic acid treated HMB over another based on ensiled HMB also supports this, since total organic acid levels in the treated HMB were significantly higher. In their study, barley comprised approximately 85% of the diet (DM basis). However, work by Ingalls et al. (1974) in which dairy cows consumed slightly less ($P>0.05$) total DM, for diets containing 45% (DM basis) ensiled or organic acid treated HMB than on similar diets based on dry or organic acid treated HMB, does not support the hypothesis of improved palatability due to the organic acid end products of anaerobic fermentation.

According to Krall (1972), the superior palatability of HMB results in earlier gains and better final grades in feedlot finishing of cattle. Our results indicate no benefit to HMB, over dry barley, in terms of ADG in the first part of the finishing period nor in better final carcass grades. These results are consistent with that reported by others (Dinusson et al. 1964; Flipot and Pelletier 1980; Kennelly and Mathison 1982; Kennelly 1983; 1984). In interpreting the feedlot trial results, it is important to note that in

feedlot finishing of cattle, grain moisture level and processing, amount and/or type of roughage influence the feeding value of HM grains, particularly with HM corn and sorghum (Merrill 1971; Jones et al. 1974; Mathison et al. 1981). In a recent trial with feedlot calves, however, the feeding value of HMB was not influenced by the amount and/or source of roughage (silage vs. hay) as measured by performance (Kennelly 1984).

Despite the slightly higher DMI (1.1 kg) of cows fed SO_2 treated or ensiled HMB treatments in experiment 1, there was no trend for higher milk yield over cows fed the dry barley treatments. In fact, cows fed the dry barley diet tended ($P<0.13$) to convert DMI more efficiently to milk and FCM. Similarly, steers fed the dry barley diet in experiment 2 were 13.3% more ($P>0.05$) efficient in converting DMI to gain than those fed the ensiled HMB diet. Previous researchers have more often observed slightly better ($P>0.05$) feed conversion to meat or milk with HMB than for dry barley based diets (Merrill 1971; Ingalls et al. 1974; Marx 1981; Kennelly et al. 1984). However, some of these results reported may have been confounded by underestimation of DMI and feed efficiency due to oven DM determination of the fermented product (Mathison et al. 1981). Thus, the feeding value of either ensiled or organic acid treated HMB is similar to dry barley for both lactating dairy cows and feedlot cattle when compared on a DM basis (BP Chemicals 1969; Merrill 1971; Wilton 1971; Marx 1973; Ingalls et al. 1974; Schmidt and Van Vleck 1974; Kung et al. 1983; Kennelly 1983; 1984; Kennelly et al. 1984).

Influence of SO_2 on carcass and milk characteristics

The adverse effect of feeding SO_2 treated HMB on final feedlot weight and carcass weight in experiment 2 was logical considering the reduced performance of steers fed the treated grain. The influence ($P>0.05$) of $NaHCO_3$ addition on increasing carcass fat depth might be partially explained by the slight tendency ($P>0.05$) for steers fed SO_2 treated HMB with $NaHCO_3$ to consume more DM and gain better than steers fed SO_2 treated HMB without $NaHCO_3$. Mathison et al. (1985) also reported that $NaHCO_3$ supplementation of SO_2 treated HMB for feedlot steers improved carcass fat depth, but there was no tendency for improved DMI or ADG over steers fed the same treated grain without $NaHCO_3$. The lack of performance effect to date by $NaHCO_3$ supplementation of SO_2 treated HMB for feedlot steers suggests that the mechanism by which $NaHCO_3$ may counteract the nonsignificant tendency for SO_2 treated HMB to depress carcass fat

is metabolic or biochemical in nature. The response may be ruminal since S supplementation of ruminant diets can change microbial and protozoal populations and thereby influence fermentation end product concentrations (Bull 1984). For example, Kennedy (1974) reported that supplemental sulfate reduced rumen proportions of propionate and isovalerate in cattle fed grass hay. Enhanced propionate levels, through the feeding of high concentrate diets, are associated with an increase in adipose tissue deposition (Van Soest 1982). Thus, if the feeding of SO_2 treated HMB were to have a similar rumen effect to that reported by Kennedy (1974), then tissue lipogenesis would be reduced resulting in a lower carcass fat measurement. Furthermore, if $NaHCO_3$ enhances the disposal of high urinary sulfate levels, then the elevated rumen S level from feeding SO_2 treated HMB might be reduced due to less sulfate recycled via saliva. Assuming such an effect from $NaHCO_3$ addition would facilitate a higher proportion of propionate in the rumen, which would lead to high carcass fat through enhanced tissue lipogenesis.

The treatment x weight group interaction for carcass fat depth is difficult to explain. Steers from the heavy group fed either dry barley or ensiled HMB had 20.1% lower ($P>0.05$) carcass fat depth than those from the light group. This was not surprising, since the higher ribeye measurement associated with faster gaining cattle, as observed for the heavy group of cattle in experiment 2, is known to be negatively correlated with carcass fat depth (Lasley 1978). While $NaHCO_3$ supplementation tended to counteract the effect of reduced ($P>0.05$) carcass fat depth associated with feeding SO_2 treated HMB, its influence differed between the light and heavy group, increasing carcass fat depth by 4.0 and 11.1%, respectively. Furthermore, heavy cattle from both SO_2 treatments had a 14.8% greater ($P<0.05$) carcass fat depth than light cattle fed the same diet. This treatment x weight group interaction for carcass fat depth cannot be compared with the results of Mathison et al. (1985) since treatment x weight group was not included in their experimental design.

Milk sulfide levels from cows fed the three diets in experiment 1 were not measured. However, sensory evaluation of milk suggests that sulfide levels in milk from cows fed SO_2 treated HMB were not markedly elevated. Other studies conducted with lactating cows fed varying levels of S via $^{35}SO_2$, sulfate or $Na_2S_2O_5$ have not shown significant increases in milk of total S (Bouchard and Conrad 1973b; Grieve et al. 1973), sulfate (Keener et al. 1953) or sulfide (Weigand et al. 1972).

Conclusion

In conclusion, no DM yield advantage was measured between high moisture and dry barley harvesting of swathed grain. Feeding of SO_2 treated HMB to lactating dairy cows for 6 wk at 35% of the diet (DM basis) was not detrimental to performance, health, milk taste or odour. Despite the high dietary S level associated with feeding SO_2 treated HMB at 85% of the diet (DM basis), it was believed that the reduced performance of feedlot steers in a 96 d trial was due primarily to spoilage of the treated grain and acute thiamin deficiency.

Table III-1 Formulation and chemical composition of diets for lactating dairy cows in experiment 1

Treatment	Dry barley	Ensiled HMB†	SO_2 treated HMB
Ingredients (% dry matter)			
Cereal silage	37.0	37.0	37.0
Chopped alfalfa hay	13.0	13.0	13.0
Rolled barley	35.0	35.0	35.0
Canola meal	11.8	11.8	11.8
Molasses	1.35	1.35	1.35
Dicalcium phosphate	0.75	0.75	0.75
Calcium carbonate	0.53	0.53	0.53
Trace mineralized salt‡	0.53	0.53	0.53
Vitamin premix§	0.04	0.04	0.04
Chemical analysis (Dry matter basis)			
Dry matter (%)	54.7	51.8	51.4
Crude protein (%)	16.0	15.9	15.9
Acid detergent fibre (%)	20.0	20.8	19.9
Calcium (%)	0.98	0.98	0.95
Phosphorus (%)	0.46	0.46	0.44
Sulfur (%)	0.20	0.20	0.32
Thiamin (mg kg^{-1})	1.67	1.67	0.83

†High moisture barley.

‡Provided per kg of dry matter diet: 0.53 mg iodine, 40 mg zinc, 0.21 mg cobalt, 13 mg copper, 19 mg manganese, 0.13 mg selenium.

§Provided per kg of dry matter diet: 4500 IU vitamin A, 1600 IU vitamin D, 14 IU vitamin E.

Table III-2 Formulation and chemical composition of diets for feedlot steers in experiment 2

Treatment	Dry barley	Ensiled HMB†	SO_2 treated HMB	SO_2 treated HMB + buffer
Ingredients (% dry matter)				
Chopped grass hay	10.0	10.0	10.0	10.0
Rolled barley	85.0	85.0	85.0	84.25
Supplement‡	4.75	4.75	4.75	4.75
Vitamin-rumensin premix§	0.25	0.25	0.25	0.25
Sodium bicarbonate	—	—	—	0.75
Chemical analysis (Dry matter basis)				
Dry matter (%)	85.7	72.0	71.5	71.5
Crude protein (%)	11.5	11.0	11.3	11.3
Calcium (%)	0.51	0.51	0.49	0.49
Phosphorus (%)	0.30	0.31	0.27	0.27
Sulfur (%)	0.15	0.17	0.46	0.46
Thiamin (mg kg^{-1})	6.17	6.51	4.17	4.17

†High moisture barley.

‡Containing: 66.3% ground barley, 25.0% calcium carbonate, 5.7% trace mineralized salt, 3.0% wet molasses. TM salt provided per kg of dry matter diet: 0.27 mg iodine, 20 mg zinc, 0.10 mg cobalt, 7 mg copper, 10 mg manganese, 0.10 mg selenium.

§Provided per kg of dry matter diet: 5.5 mg thiamin as thiamin mononitrate, 5500 IU vitamin A, 900 IU vitamin D, 6 IU vitamin E, monensin activity of 11 and 33 mg for day 1-28 and day 29 to the end of the trial respectively.

Table III–3 Effect of treatment on feed intake, performance and milk composition of cows (exp. 1)

Parameter	Dry barley	Ensiled HMB†	SO_2 treated HMB	SEM‡	Sig
No. of cows	12	12	11		
DM intake (kg d^{-1})	19.6	21.2	20.2	0.63	NS
Pre–experimental milk yield (kg d^{-1})	25.8	24.4	24.0	0.86	NS
Milk yield (kg d^{-1})	24.6	22.6	21.7	1.43	NS
4% FCM (kg d^{-1})	23.7	22.1	21.4	1.43	NS
Milk fat (%)	3.81	3.91	3.93	0.15	NS
Milk protein (%)	3.31	3.32	3.43	0.09	NS
Milk lactose (%)	4.93	4.74	4.75	0.08	NS
Milk persistency (%)§	94.4	92.6	90.9	2.39	NS
FCM/DM intake	1.21	1.04	1.06	0.06	NS
Liveweight change (kg wk^{-1})	1.65	1.93	1.68	0.69	NS

†High moisture barley.

‡Standard error of the mean.

§Milk persistency = 100 x (treatment milk yield/pre–experimental milk yield).

NS $P>0.05$.

Table III-4 Effect of treatment on performance of feedlot steers (exp. 2)

Item	Dry barley	Ensiled HMB†	SO_2 treated HMB	SO_2 treated HMB + buffer	SEM‡	Sig
No. of steers	16	16	14	16		
Days on trial	96	96	96	96		
Initial wt (kg)	391	389	387	393	5.43	NS
Final wt (kg)	515^{c}	498^{c}	467^{d}	473^{d}	8.90	*
Daily gain (kg)						
Day 1-28	1.25^{a}	0.88^{ab}	0.59^{b}	0.53^{b}	0.05	**
Day 29-57	1.39	1.40	0.65	1.05	0.09	NS
Day 58-96	1.23	1.13	1.13	0.90	0.08	NS
Day 1-96	1.28^{a}	1.14^{a}	0.83^{b}	0.84^{b}	0.07	**
Daily DM intake (kg)						
Day 1-28	7.98	7.61	7.27	7.32	0.22	NS
Day 29-57	9.17^{c}	9.03^{c}	8.00^{d}	8.03^{d}	0.24	*
Day 58-96	9.04^{c}	8.96^{c}	7.96^{d}	8.01^{d}	0.27	*
Day 1-96	8.77^{a}	8.59^{a}	7.78^{b}	7.82^{b}	0.25	**
DM intake/gain						
Day 1-28	6.25^{a}	8.33^{a}	12.50^{b}	14.29^{b}	0.32	**
Day 29-57	6.67	6.67	12.50	7.69	0.42	NS
Day 58-96	7.14	7.69	7.14	9.09	0.40	NS
Day 1-96	6.67^{a}	7.69^{a}	9.09^{b}	9.09^{b}	0.38	**

†High moisture barley.

‡Standard error of the mean.

* $P<0.10$, ** $P<0.05$, NS $P>0.10$

$^{a-b}$Means within the same row with different letters differ significantly ($P<0.05$).

$^{c-d}$Means within the same row with different letters differ significantly ($P<0.10$).

Table III-5 Effect of treatment on carcass characteristics of finishing steers (exp. 2)

Item	Dry barley	Ensiled HMB†	SO_2 treated HMB	SO_2 treated HMB + buffer	SEM‡	Sig
No. of steers	16	16	14	16		
Warm carcass wt (kg)	288[a]	283[a]	264[b]	271[b]	4.67	*
Dressing (%)	55.9	56.8	56.4	57.2	0.36	NS
Rib eye area (cm^2)	74.9	73.7	68.5	73.0	0.59	NS
Fat depth (cm)	1.13	1.02	0.99	1.08	0.07	NS
Grade (no. of carcasses)						
A1	9	12	11	10		
A2	7	3	3	3		
A3						
B1		1		3		
Abscessed livers (%)	19	19	14	25		

†High moisture barley.

‡Standard error of the mean.

* $P<0.10$, NS $P>0.10$

[a-b]Means within the same row with different letters differ significantly ($P<0.10$).

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IV. THE PERFORMANCE AND THIAMIN STATUS OF PIGS FED SULFUR DIOXIDE PRESERVED HIGH MOISTURE BARLEY

A. INTRODUCTION

The feeding value of ensiled or organic acid treated high moisture barley (HMB) is similar to dry barley for pigs when compared on a dry matter (DM) basis (Cole et al. 1970; Perez-Aleman et al. 1971; Bowland and Corbett 1973; English et al. 1973; Cole et al. 1975; Cole et al. 1980; Weltzien 1986), providing HMB is stored in a manner which prevents spoilage (Livingstone and Livingston 1970; Livingstone et al. 1971). One disadvantage associated with HMB arises from the need to mix diets on a daily basis to prevent mould growth and spoilage (Jones et al. 1974). While the use of organic acids help in this regard (Livingstone et al. 1971; Jones et al. 1974), they are presently too expensive for routine use. Therefore, the need still exists for effective and economical grain preservatives (GP) as alternatives to drying grain.

The antimicrobial activity of sulfur dioxide (SO_2) and its salt, sodium metabisulfite ($Na_2S_2O_5$), is known and the chemical has a long history as a preservative in the food and beverage industry (Schroeter 1966). SO_2 has proven useful as a silage preservative (Knodt et al. 1952; Petrosyan 1967; Mathison et al. 1979; 1984) and as a microbial growth inhibitor in low temperature grain drying (Eckoff et al. 1979; 1983). Recently, the potential of SO_2 as a GP for HMB fed to cattle has been reported by Mathison et al. (1985), but information as to the feeding of SO_2 preserved high moisture grain to swine is not available.

This experiment was designed to: (1) determine the feeding value of SO_2 treated HMB for pigs; (2) determine the effect of feeding SO_2 treated HMB on the thiamin status of pigs; and (3) evaluate the effectiveness of SO_2 in preventing spoilage of a HMB based diet fed over several weeks.

B. MATERIALS AND METHODS

Barley

HMB (30 to 32% moisture) was ensiled in a Harvestore® silo or treated with 1% (wt/wt) SO_2 as previously described (Chapter II). Dry barley was stored at 12 to 14%

moisture. Treated HMB was initially stored in a large scale (69 t) near anaerobic bin and later in small scale (700 kg) aerobic boxes (Chapter II). The feeding trial commenced 167 d after SO_2 application.

Growth trial - Phase I

Forty-five weanling pigs, of an average weight of 10.6 kg and average age of 35 d, were allocated on the basis of sex and weight to five diets for a 28 d trial (Table IV-1). Barley was hammermilled through a 3.2 mm screen and included in all diets at a 47% inclusion level (DM basis). Treatments one and two were the control diets based on dry and HMB, respectively. Diets three, four and five were based on SO_2 treated HMB. Batches (500 kg) of diets one and three were mixed at the start of the experiment while diets two, four and five were mixed on a daily basis. Diet three was mixed once and stored in unsealed 25 kg bags at 16°C for the 28 d test period. Diets one, two and three were observed daily for any visible signs of spoilage. Fermentation end products of diet three were measured on a weekly basis. Feed from individual bags that either heated or looked or smelled mouldy, was not fed. Diets four and five were mixed daily; diet five differed from diet four by the addition of 2.0 mg thiamin kg^{-1} diet.

Feed and water were available ad libitum. Pigs were individually housed in raised weaner decks and in slatted floor pens. Barn temperature was maintained at 22 to 23°C. Pig weights were recorded weekly.

Three pigs were randomly chosen from each treatment, with the exception of treatment one due to limited time for analytical work, and blood samples were taken from the anterior vena cava from each pig on day one, 14 and 28 for thiamin determination.

Growth trial - Phase II

To assess the long term effect of feeding SO_2 treated HMB on pig performance and thiamin status, pigs fed daily mixed diets based on the HMB control and SO_2 treated HMB, treatments two and four respectively, were continued on test to 85 kg liveweight. Diets continued to be mixed daily, but were reformulated (Table IV-2) to contain 75% barley (DM basis). Barley was hammermilled through a 4.7 mm screen as higher ambient temperature made grinding through a 3.2 mm screen more difficult.

Feed and water were available to the pigs at all times. Pigs in each treatment group were initially housed as one group of nine per pen and later in two pens of five and four pigs per group. Barn temperature was maintained at 18 to 20°C throughout the trial. Individual pig weights and feed intake per pen were recorded weekly.

Pigs were slaughtered after 75 d on test at approximately 85 kg. The day before slaughter all pigs were blood sampled from the anterior vena cava for thiamin determination. At slaughter, six pigs per treatment group were selected (three heaviest, three lightest) and subjected to gross postmortem examination for possible abnormalities caused by high dietary sulfur (S) level and/or dietary thiamin deficiency. Heart, liver and small intestine were visually assessed for incidence of cardiac hypertrophy, colour and size; inflammation and hemorrhages. Pork chop samples were taken 20 min after slaughter from the longissimus dorsi muscle. This meat was frozen immediately and stored at -22°C until analyzed for total thiamin.

Analytical Procedures

Feed samples were taken weekly, frozen immediately and stored at -22°C until composited for chemical analyses. Diets were analyzed for DM, crude protein and thiamin according to the methods of the Association of Official Analytical Chemists (AOAC 1980). Feed samples were freeze-dried and ground to a homogeneous powder (0.5 mm) prior to thiamin and S determination. Sulfur was determined using a LECO Sulfur Determinator SC132 (St. Joseph, MI), as described in Chapter II. On a weekly basis, 5 to 6 g samples of diet three were analyzed for volatile fatty acids (VFA), ethanol (EtOH), lactic acid, furfural, ammonia-nitrogen (NH_3-N), pH, and moisture according to methods previously described (Chapter II). Acid detergent insoluble nitrogen (ADIN) was determined according to Goering and Van Soest (1970).

Thiamin status of the pigs was assessed by erythrocyte transketolase (EC 2.2.1.1) activity (ug of pentose utilized mL of hemolyzate^{-1} h^{-1}) and its in vitro stimulation by the addition of thiamin pyrophosphate (% TPP effect) (Brin 1970). This method is recognized as being a sensitive, specific and reliable measure of thiamin nutriture in humans and rats (Tanphaichitr and Wood 1984) as well as in pigs (Peng and Heitman 1973).

Meat samples (2 g) were analyzed for total thiamin by high performance liquid chromatography (HPLC) as per Fellman et al. (1982) after homogenization and deproteinization with 10% trichloracetic acid (Kazuhiko et al. 1979) (Alberta Agriculture, Food Laboratory Services Branch, Edmonton, Alberta). The procedure differed from that of Fellman et al. (1982) in that: (1) 2 mL of 12N HCl were added to each deproteinized sample followed by autoclaving at 121°C/103 kPa for 30 min, and (2) acid phosphatase (EC.3.1.3.2) from human prostrate (CalBiochem, San Diego, CA) was used to dephosphorylate thiamin-phosphate esters. A Lab Data Control Constametric III HPLC equipped with a fluorometer (Perkin-Elmer 650 10S) was used to separate thiamin and analyze for the thiochrome produced in the procedure.

Statistical Analyses

For diet three in Phase I of the growth trial, the relationship between storage time and fermentation products was determined by Pearson correlation coefficients (Steel and Torrie 1980). A t-test (Steel and Torrie 1980) was applied to chemical data collected for preserved and spoiled samples of this diet to assess differences. Thiamin changes in diet three, due to SO_2 exposure over the 28 d storage period, were examined by one way analysis of variance (ANOVA) (Steel and Torrie 1980) with time as the source of variation.

In Phase I of the growth trial, pig performance, % TPP effect, as well as mean dietary thiamin levels were subjected to one way ANOVA to examine differences between treatments. Using actual dietary thiamin levels and % TPP effects as distinguishing criteria, the performance of pigs from any treatment group assessed to be thiamin inadequate was compared, by orthogonal contrasts (Steel and Torrie 1980), to that of pigs from thiamin adequate treatment groups. The relationship between time on trial and % TPP effect was assessed by linear regression for all dietary treatments.

In Phase II of the growth trial, a t-test was applied to dietary thiamin, % TPP effect and pork chop thiamin data to examine differences between treatments. Average daily feed intake, average daily gain and feed:gain ratio data were not statistically analyzed for this portion of the experiment because there was only one pen per treatment.

C. RESULTS

Diet preservation during Phase I of growth trial

The SO_2 treated HMB that was stored throughout the 28 d trial (Diet 3) remained well preserved until day 18 of the trial when pockets of warm and mouldy feed were discovered in some of the feed bags. However, less than 5% of the 500 kg prepared on day one of the trial was lost due to spoilage. Spoiled feed containing SO_2 treated HMB was characterized by a higher ($P<0.05$) EtOH concentration and tended ($P>0.05$) to have higher concentrations of acetate and furfural than feed which remained preserved (Table IV-3). While higher ($P<0.01$) levels of ADIN and moisture were measured in spoiled feed, no difference ($P>0.05$) in NH_3-N concentration or pH was measured between spoiled and preserved portions of diet three.

During the 28 d trial, diet three was marked by diminishing ($P<0.05$) EtOH and acetate levels over time ($r=-0.66$ and -0.67 respectively; $n=14$). Ration moisture loss from the unsealed bags amounted to 5.77% during week one of storage, thereafter there was no significant ($P>0.05$) loss of moisture. Lactate and NH_3-N levels increased ($P<0.05$ and $P<0.001$, respectively) with time while pH and ADIN levels remained stable.

Growth Trial - Phase I

One pig died as a result of hypothermia, caused by a broken water line. Health of the pigs during Phase I of the growth trial was otherwise good.

Feeding of diets containing SO_2 treated HMB did not influence ($P>0.05$) average daily feed intake (ADFI), growth rate or feed:gain ratio (Table IV-4). Diet three, the SO_2 treated HMB based ration mixed once and then stored over the 28 d feeding trial, had a negative ($P<0.01$) influence on animal thiamin status as measured by % TPP effect. Chemical analysis revealed that this was the only diet deficient in thiamin according to NAS-NRC (1979) recommended requirements.

A negative correlation existed between % TPP effect and feed intake ($r=-0.94$; $P<0.07$) for the three pigs, fed the thiamin deficient diet, which were blood sampled for thiamin determination. Percent TPP effect increased ($P<0.001$) with time ($r=0.92$, $n=9$) for pigs fed diet three. This trend was consistent with the thiamin destruction rate

in diet three. Chemical analysis revealed that 31 and 61% of the initial thiamin in this diet was destroyed at 1 and 7 d, respectively after mixing (Figure IV-1). Thiamin levels did not diminish ($P>0.05$) significantly in this diet between day seven and 28 of the storage period. No significant trend existed between % TPP effect and time for any other treatment groups.

Growth Trial - Phase II

Pigs fed the SO_2 treated HMB from 28 to 85 kg had a 9.8% lower feed intake and 9.0% lower rate of gain than pigs fed the HMB control diet. Treatment did not appear to influence feed:gain ratio. All pigs completed the trial and appeared to remain healthy.

Measures of thiamin adequacy are presented in Table IV-5. The mean thiamin level of the SO_2 treated HMB based diet was lower ($P<0.001$) than that of the control diet based on ensiled HMB. Pigs fed the SO_2 treated HMB were thiamin depleted ($P<0.001$) at slaughter as indicated by higher % TPP effect and lower pork chop thiamin levels than measured for pigs fed the control.

Postmortem examination revealed an 83 and 0% incidence of cardiac hypertrophy in pigs fed SO_2 treated and ensiled HMB diets, respectively. There were no liver abnormalities observed in pigs from either treatment group. Duodenal gastric mucosa were characterized by hyperemia and petechial hemorrhaging in two of the six pigs slaughtered from the SO_2 treatment group while those of pigs fed the control appeared to be normal.

D. DISCUSSION

Diet preservation during Phase I

Increased levels of EtOH, acetate, furfural, ADIN and moisture in spoiled portions of diet three indicate a loss of antimicrobial efficacy, with time, by SO_2. A one hundred fold increase in EtOH concentration provides evidence of yeast proliferation, because the major fermentation by yeasts is an ethanolic one (Woolford 1984). Similar chemical changes were measured in SO_2 treated HMB which spoiled (Chapter II).

The lack of difference ($P>0.05$) in NH_3-N concentration, between preserved and spoiled portions of diet three, suggests no appreciable loss in protein quality via microbial proteolysis. However, this comparison is confounded by the fact that NH_3-N levels increased ($P<0.001$) with storage time even in the 475 kg bagged portion of diet three which remained preserved ($r=0.91$; $n=10$). This resulted in an NH_3-N level at day 28 of almost twice that measured on day one of the feeding trial. A similar but nonsignificant ($P>0.05$) trend in NH_3-N concentration over time was observed in previous work with SO_2 treated HMB (Chapter II). It is unlikely that the increased NH_3-N level in this dietary treatment had any significant influence on ration palatability or pig performance because: (1) NH_3-N level never exceeded 0.01% of the ration DM nor did it influence ($P>0.05$) ration pH, and (2) the finished ration retained its initial appearance and smell throughout the 28 d feeding period. Increased ($P<0.05$) lactate levels observed in the preserved portion of diet three with time ($r=0.67$; $n=10$) imply enhanced activity of lactic acid bacteria. This trend is consistent with previous observations made for SO_2 treated HMB which remained preserved over a 9 mo storage period (Chapter II).

Growth Trial - Phase I

The performance of pigs fed diets containing either HMB or SO_2 treated HMB is in general agreement with results of other studies, conducted with growing-finishing pigs, in which diets included either ensiled or organic acid treated HMB which did not spoil (Cole et al. 1970; Perez-Aleman et al. 1973; Bowland and Corbett 1973; English et al. 1973; Cole et al. 1975; Cole et al. 1980). Weltzien (1986) reported increased ($P<0.05$) ADFI for pigs fed HMB compared to those fed a dry barley based diet. As a result, there was a tendency for improved growth rate and feed:gain ratio but differences were nonsignificant. Weltzien suggested that daily grinding, mixing and feeding of the HMB, coupled with daily removal of uneaten feed may have been responsible for the higher ADFI of pigs fed this diet since the dry barley diet was added to feeders on a weekly basis. However, Weltzien's study indicated no difference ($P>0.05$) in ADFI of pigs fed dry barley when added daily vs. weekly to feeders. Weltzien (1986) also suggested that the ensiling process may have enhanced grain palatability. The results of English et al. (1973) indicated that the ADFI of pigs fed diets based on propionic acid treated HMB was

similar ($P>0.05$) to that of pigs fed a dry barley based diet when both were mixed and fed with the same frequency.

The % TPP effect measurement of Brin's (1970) transketolase assay was used to determine pig thiamin status because it is recognized as being a sensitive, specific and reliable measure in humans and rats (Tanphaichitr and Wood 1984) as well as in pigs (Peng and Heitman 1973). The merit of the assay in evaluating thiamin status is that the coenzyme TPP, derived from dietary thiamin, is essential for the activity of the transketolase enzyme (Brin 1970). Thus, a higher % TPP effect indicates greater thiamin deficiency. Thiamin determination of whole blood was not used because present methodology is unsatisfactory in revealing the small decrease in blood thiamin which occurs during a thiamin deficiency (Sauberlich 1984). Furthermore, Schrijver et al. (1982) have determined that erythrocytes contain 80% of the thiamin present in whole blood.

It is not surprising that diet three was the only diet that negatively influenced ($P<0.01$) animal thiamin status as measured by % TPP effect, because this diet averaged 43% of the NAS-NRC (1979) recommended thiamin level for growing-finishing swine. Pigs fed the thiamin deficient diet three had a 5.4% lower ADFI, 6.7% lower growth rate and 1.9% poorer feed:gain ratio than the respective mean figures of pigs fed thiamin adequate diets, although these differences were not significant. Reduced appetite and growth rate are clinical signs of thiamin deficiency NAS-NRC (1979), but certainly not unique to a deficiency of this vitamin. Correlation between % TPP effect and feed intake among three pigs fed diet three ($r=-0.94$; $P<0.07$) indicated that reductions in ADFI were largely explained by increasing thiamin deficiency. Direct evidence of increasing ($P<0.001$) severity of thiamin depletion were provided by mean % TPP effects for pigs fed diet three of 1.9, 10.5 and 19.4% at day one, 14 and 28, respectively ($r=0.92$; $n=9$). Peng and Heitman (1973) reported that % TPP effects of 23.3% (also based on pentose utilization) for growing-finishing swine were accompanied by reduced feed intake and rate of gain. In humans, % TPP effects of 15 to 25% indicate marginal thiamin deficiency which may precede the appearance of clinical signs of thiamin deficiency (Brin 1970).

Thiamin in all diets containing SO_2 treated HMB would have been destroyed by SO_2 and its bisulfite ion, but primarily the latter because the bisulfite form of SO_2 was favoured at the diet pH of circa 5.4 (Green 1976). The rapid destruction of thiamin in

diet three at a 16°C storage temperature is to be expected considering that after storage for 2 wk at -18°C and 24 hr at 24°C, Til et al. (1972a) observed dietary thiamin losses of 3.5, 28.9 and 30.7% from 0.0, 1.0 and 2.0% (wt/wt) additions of $Na_2S_2O_5$, respectively (SO_2 equivalence of 0.66 and 1.32%). Thiamin levels likely did not significantly ($P>0.05$) diminish in diet three after seven days of storage because the 0.14% level of SO_2 and/or bisulfite may have been depleted due to binding with dietary thiamin, protein, sugars, aldehydes and lignin (Eckoff and Okos 1983), as well as by oxidation to sulfate (Lozeman et al. unpublished). To have supplemented even higher levels of thiamin to ensure that requirements were met in diet three would have been economically feasible. Supplied as thiamin mononitrate, the cost of supplementing twice NAS-NRC (1979) recommended thiamin levels would cost less than $0.01 per pig per day. Such a precaution might be of no benefit however, considering the rapid rate at which SO_2 destroys thiamin.

Considering the inadequate thiamin level of diet three and the increased severity of thiamin depletion observed with time in pigs fed this diet, one would have expected a more adverse effect on pig performance. Pigs fed diet three may have deterred clinical thiamin deficiency by utilizing tissue thiamin stores accumulated during the 35 d period from birth to the start of the experiment. Heinemann et al. (1946) concluded that pigs do have the ability to store thiamin in tissues because 56 d were required for pigs fed a thiamin deficient diet to lose their appetites. Van Etten et al. (1940) implied that the inclusion of tallow in pig diets may reduce the animal's need for thiamin. This may be explained by the fact that as the proportion of dietary energy from fat increases and that from carbohydrate decreases, the thiamin dependent oxidative decarboxylation of pyruvate to acetyl coenzyme A is reduced, a reaction which is essential in the utilization of carbohydrates for energy (Lehninger 1982). The work of Ellis and Madsen (1944) indicated that on the basis of the time required for reduced appetite and growth rate, pigs fed thiamin-free diets with 2, 11 and 28% tallow showed evidence of thiamin deficiency, on average after 25, 28 and 33 d of feeding. Based on the: (1) minimal amount of dietary energy from carbohydrates replaced by 2% tallow inclusion, and (2) results of Ellis and Madsen (1944), we would conclude that the thiamin nutriture of pigs was probably not influenced by the inclusion of tallow in the experimental diets.

Data collected for pigs fed diets four and five were examined on the basis of

performance for palatability and/or toxicological effects of SO_2 in the diet since neither treatment was confounded by thiamin deficiencies. The similar ($P>0.05$) performance of pigs fed these diets compared to pigs fed either dry or HMB control diets suggests that short-term feeding of 1.27 g of S pig^{-1} day^{-1} from SO_2 (67 mg of S from SO_2 kg^{-1} of body weight (bw d^{-1}) had no palatability or toxicological effects. Similarly in a 48 wk trial with pigs, Til et al. (1972b) reported that 0.35% (wt/wt) $Na_2S_2O_5$ (2.83 g of S from SO_2 pig^{-1} d^{-1}; 28 mg of S from SO_2 kg^{-1} of bw d^{-1}) in diets heavily supplemented with thiamin had no ill effects on pig health or performance. At 0.83% $Na_2S_2O_5$ (6.7 g of S from SO_2 pig^{-1} d^{-1}; 65 mg of S from SO_2 kg^{-1} of bw d^{-1}) pigs remained healthy but performance was reduced slightly and mild inflammation of stomach gastric mucosa was observed in several animals. Til et al. (1972b) concluded that the effects of high sulfite levels on performance were due to less palatable diets. Since pigs fed SO_2 treated HMB, at 47% of the diet (DM basis), consumed a diet with an SO_2-derived S concentration lower than that of the 0.35% $Na_2S_2O_5$ treatment fed to pigs in the trial of Til et al. (1972b), one would expect no effect on diet palatability or animal performance, as indeed observed for pigs fed the thiamin adequate diets containing SO_2 treated HMB. However, from a toxicological perspective (i.e. mg of S from SO^2 kg^{-1} of bw d^{-1}) pigs ingested equal amounts of added S from SO_2 as those fed the 0.83% $Na_2S_20_5$ treatment in the trial of Til et al. (1972b), this might suggest similar predisposition to stomach inflammation.

Growth trial - Phase II

Thiamin status criteria (Table IV-5) indicate that the major cause for reduced performance of pigs fed a diet containing SO_2 treated HMB from 28 to 85 kg was thiamin inadequacy. The good health of the thiamin depleted pigs is in contrast with other reports concerning pigs fed thiamin deficient diets in which vomiting (Ellis and Madsen 1944; Heinemann et al. 1946; Miller et al. 1955; Peng and Heitmann 1973), stiff gait and poor movement coordination (Peng and Heitmann 1973) were observed.

Mean dietary thiamin levels were 2.65 and 0.21 mg kg^{-1} DM for the HMB control and SO_2 treatments, respectively. These levels were 217 and 17% of the thiamin levels recommended for growing-finishing swine (NAS-NRC 1979). Previous research with pigs has established that: (1) tissue thiamin levels are proportional to the amount of

thiamin ingested, and (2) additional thiamin over and above daily requirements contributes to tissue storage (Hughes 1941; Ellis and Madsen 1944; Heinemann et al. 1946). Therefore, the reduced pork chop thiamin levels observed in pigs fed the SO_2 treated HMB diets, in this study, is consistent with these results.

Mean % TPP effects measured one day prior to slaughter were 1.8 and 40.3 for the control and SO_2 treatment fed pigs, respectively. Seventy five days earlier (i.e. end of growth trial - Phase I) mean % TPP effects from pigs fed the same treatments were 1.58 and 0.31, respectively. These changes indicate that pigs fed the HMB control remained thiamin adequate, but those fed the SO_2 treated HMB went from adequate thiamin nutriture in short-term feeding (10 to 28 kg) to thiamin depletion in long-term feeding (28 to 85 kg) according to % TPP effect guidelines for humans (Brin 1970) and those reported for pigs (Peng and Heitmann 1973). A subclinical sign common to either thiamin or copper (Cu) deficiency is cardiac hypertrophy (NAS-NRC 1979). Sulfur, as sulfide can react with copper (Cu) and/or molybdenum to induce a Cu deficiency (NAS-NRC 1980). Unlike ruminants, pigs do not have the capacity to reduce sulfate to sulfide in the foregut (Dick et al. 1975). Therefore, a Cu deficiency would not be expected with feeding SO_2 treated HMB to pigs since the added S, unless bound with feedstuff components, is primarily in the form of molecular SO_2, its ions or sulfate, not sulfide (Green 1976; Lozeman et al. unpublished; Chapter II).

It is possible that the ingestion of high levels of S from SO_2 reduced feed intake. This is probable because pigs fed SO_2 treated HMB at 75% of the diet (DM basis) in Phase II of this experiment daily consumed, on average, 2.8 g of added S kg^{-1} of DM. Til et al. (1972b) stated this to be the "adverse effect" level in pigs fed diets containing $Na_2S_2O_5$, reporting reduced diet palatability and mild inflammation of stomach gastric mucosa. The occurrence of hyperemia and petechial hemorrhaging of duodenal gastric mucosa in two of six pigs fed the SO_2 HMB was not surprising since pigs consumed on average 98 mg of S from SO_2 kg^{-1} of bw d^{-1}, and Til et al. (1972b) demonstrated that 65 mg of S from $Na_2S_2O_5$ kg^{-1} of bw d^{-1} caused similar changes in pigs. The irritant effect of high dietary S on the digestive tract, in both monogastrics and ruminants, is believed to be caused by its conversion to hydrogen sulfide by gastrointestinal flora (NAS-NRC 1980).

In conclusion, feeding of SO_2 treated (1% wt/wt) HMB to weaner pigs at 47% of the diet (DM basis) was not detrimental to performance. The reduced performance of

growing-finishing pigs fed a diet containing a 75% dietary inclusion level (DM basis) of SO_2 treated HMB was due primarily to thiamin depletion. Reduced diet palatability and toxicological effects on the digestive tract due to the high dietary inclusion level of SO_2 treated HMB may have also contributed to the reduced performance of growing-finishing pigs fed the SO_2 treatment. While SO_2 effectively preserved a HMB based diet for 18 d at 16°C, its value in preserving swine diets based on high moisture grains may be limited due to the rapid rate at which it destroys dietary thiamin.

Table IV-1 Formulation and chemical composition of diets in Phase I of growth trial

Diet no.	1	2	3	4	5
Treatment	Dry barley	Ensiled HMB†	SO_2 treated HMB	SO_2 treated HMB	SO_2 treated HMB + thiamin‡
Mixing frequency	28 days	daily	28 days	daily	daily
Ingredients (% dry matter)					
Barley	47.0	47.0	47.0	47.0	47.0
Soybean meal	32.80	32.80	32.80	32.80	32.80
Oat groats	13.80	13.80	13.80	13.80	13.80
Tallow	2.0	2.0	2.0	2.0	2.0
Calcium phosphate	1.65	1.65	1.65	1.65	1.65
Calcium carbonate	1.10	1.10	1.10	1.10	1.10
Iodized salt	0.55	0.55	0.55	0.55	0.55
Vitamin-mineral premix§	1.10	1.10	1.10	1.10	1.10
Chemical analysis (Dry matter basis)					
Dry matter (%)	88.7	78.8	81.0	77.0	77.0
Crude protein (%)	26.0	26.1	26.4	25.0	25.0
Sulfur (%)	0.31	0.32	0.45	0.46	0.46
Thiamin (mg kg^{-1})	3.02	3.02	0.51	1.27	1.96

†High moisture barley.

‡Thiamin premix to supply 2 mg thiamin as thiamin mononitrate per kg of diet.

§Provided the following per kg of dry matter diet: 120 mg zinc, 13 mg manganese, 250 mg iron, 10 mg copper, 0.11 mg selenium, 5500 IU vitamin A, 550 IU vitamin D_3, 25 IU vitamin E, 13 mg riboflavin, 50 mg niacin, 30 mg calcium pantothenate, 30 ug vitamin B_{12}, 550 mg choline.

Table IV-2 Formulation and chemical composition of diets in Phase II of growth trial.

Diet no.	2	4
Treatment	Ensiled HMB†	SO_2 treated HMB
Mixing frequency	daily	daily
Ingredients (% dry matter)		
Barley	75.0	75.0
Soybean meal	20.5	20.5
Calcium phosphate	1.7	1.7
Calcium carbonate	1.1	1.1
Iodized salt	0.5	0.5
Vitamin-mineral premix‡	1.2	1.2
Chemical analysis (Dry matter basis)		
Dry matter (%)	73.0	72.5
Crude protein (%)	18.2	18.6
Sulfur (%)	0.21	0.49
Thiamin (mg kg^{-1})	2.65	0.21

†High moisture barley.

‡Provided the following per kg of dry matter diet: 65 mg zinc, 7 mg manganese, 90 mg iron, 6 mg copper, 0.12 mg selenium, 3000 IU vitamin A, 300 IU vitamin D_3, 14 IU vitamin E, 7 mg riboflavin, 27 mg niacin, 15 mg calcium pantothenate, 18 ug vitamin B_{12}, 300 mg choline.

Table IV–3 Fermentation products (dry matter basis) of a diet† containing SO_2 treated high moisture barley, after 18 d of aerobic storage at 16°C

Item‡	Preserved	Spoiled	Significance
Ethanol (mg g^{-1})	0.02 (0.009)§	2.87 (1.43)	*
Acetate (mg g^{-1})	0.49 (0.06)	0.77 (0.19)	NS
Lactate (mg g^{-1})	4.03 (0.11)	4.24 (0.32)	NS
pH	5.4 (0.03)	5.3 (0.03)	NS
Furfural (mg g^{-1})	0.38 (0.09)	0.63 (0.11)	NS
Ammonia N (ug g^{-1})	83.63 (5.65)	68.54 (2.78)	NS
ADIN (% of total N)¶	3.54 (0.08)	5.31 (0.33)	**
Moisture (%)	17.10 (0.20)	18.03 (0.03)	**

†Diet 3 in Phase I of growth trial which contained SO_2 treated high moisture barley at a 47% dry matter inclusion level.

‡Means of preserved and spoiled diet are based on four observations each.

§Values in parentheses represent standard error of mean.

¶ADIN = Acid detergrent insoluble nitrogen.

* $P<0.05$, ** $P<0.01$, NS $P>0.05$.

Table IV-4 Effect of treatment on pig performance and thiamin status in Phase I of growth trial

Diet no.	1	2	3	4	5		
Treatment	Dry barley	Ensiled HMB†	SO_2 treated HMB	SO_2 treated HMB	SO_2 treated HMB + thiamin		
Mixing frequency	28 days	daily	28 days	daily	daily	SEM‡	Sig
No. of pigs	9	9	8	9	9		
Initial wt. (kg)	10.2	10.4	10.3	11.2	10.6		
Final wt. (kg)	26.7	26.5	26.0	28.9	26.5		
Avg. daily feed (kg)	0.92	0.91	0.88	0.97	0.88	0.05	NS
Avg. daily gain (kg)	0.59	0.58	0.56	0.63	0.57	0.03	NS
Feed/gain	1.57	1.57	1.58	1.52	1.55	0.04	NS
Dietary thiamin ($mg.kg^{-1}$ DM)§	3.02^{a}	3.02^{a}	0.51^{d}	1.27^{c}	1.96^{b}	0.09	*
TPP effect (%)¶	ND//	1.58^{b}	10.5^{a}	0.31^{b}	1.97^{b}	1.30	**

†High moisture barley.

‡Standard error of the mean.

§Dry matter.

¶Percentage increase in activity of the erythrocyte transketolase enzyme due to the addition of thiamin pyrophosphate (TPP). Each value represents the mean of three pigs which were blood sampled at 0, 14 and 28 d.

//Not determined.

* $P<0.05$, ** $P<0.01$, NS $P>0.05$.

$^{a-d}$Means in the same row with different letters are significantly different ($P<0.05$).

Table IV-5 Influence of feeding SO_2 treated high moisture barley on thiamin status of growing-finishing pigs at day 75 of Phase II growth trial.

Diet no.	2	4			
Treatment	Ensiled HMB†	SO_2 treated HMB			
Mixing frequency	daily	daily	No. of pigs	SEM‡	Sig
Dietary thiamin (mg kg^{-1} DM)§	2.65	0.21		0.09	***
TPP effect (%)¶	1.8	40.3	9	1.72	***
Cardiac hypertrophy (% incidence)	0	83	6		
Pork chop thiamin level, mg 100 g^{-1} fresh meat//	2.90	0.22	6	0.14	***

†High moisture barley.

‡Standard error of the mean.

§Dry matter.

¶Percentage increase in activity of the erythrocyte transketolase enzyme due to the addition of thiamin pyrophosphate (TPP).

//Longissimus dorsi muscle.

*** $P<0.001$.

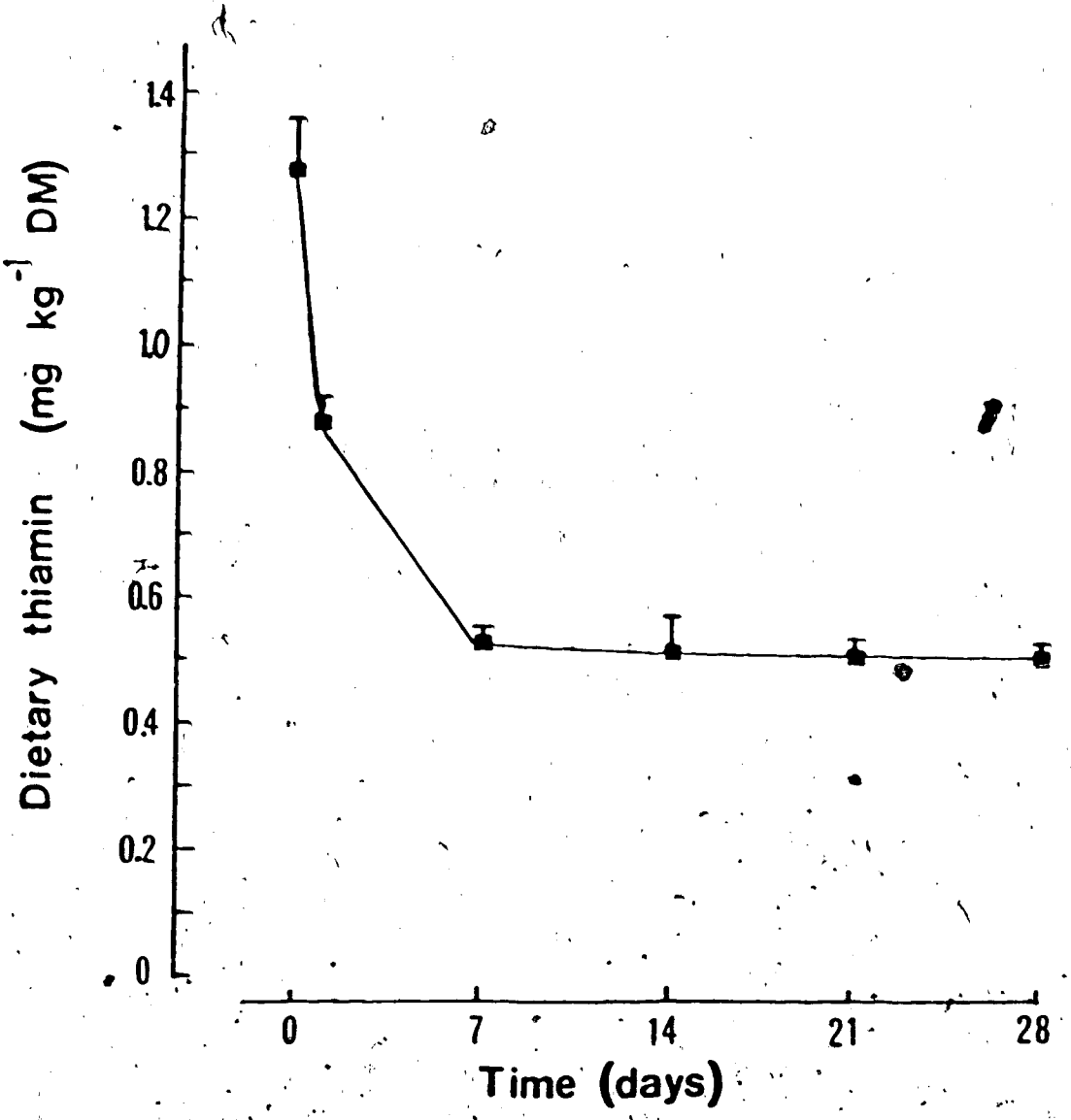

Figure IV–1 Rate of thiamin (mean ± SE, n=2 for each mean) destruction in a preserved diet containing SO_2 treated high moisture barley, stored aerobically at 16°C

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V. GENERAL DISCUSSION AND CONCLUSIONS.

In Experiment I, a 1% (wt/wt) SO_2 application rate did not effectively preserve 69 t of HMB (30 to 32% moisture) stored in a near anaerobic upright bag. However, the treated grain was preserved successfully for 9 mo with small scale (700 kg) aerobic storage. Likewise in Experiment II, it was observed that a diet (19% moisture) containing SO_2 treated HMB at 47% of the diet (DM basis), remained well preserved for 18 d of 16°C aerobic storage in 25 kg bags.

These three storage methods differed markedly in scale, storage temperature, and air permeability of the storage container. These differences appear to be major factors affecting the efficacy of SO_2 as a GP.

Since moisture migration is more of a problem with large bins of grain (Muir 1973), HMB stored in the 69 t bins would be expected to be the most susceptible to moisture migration. Indeed, moisture migration was halted once the grain was transferred from the 69 t upright bag and stored in 700 kg unsealed containers. Mathison et al. (1985) also observed no spoilage of HMB (22–25% moisture), treated with similar levels of SO_2, when stored aerobically in 300 kg to 26 t lots. The failure of SO_2 to satisfactorily preserve HMB under large scale storage conditions suggests a need for aeration of bins to prevent moisture migration (Campbell 1972; Sauer 1972; Jones et al. 1974; Bothast et al. 1975). As a guideline, Agriculture Canada (1983) recommends aeration of bins holding more than 30 t of grain to prevent moisture migration.

For the 69 t upright bag (Chapter II), a mean temperature of 35°C was measured at the bin centre for the first 30 d of storage. In contrast, storage temperatures for the 700 kg (Chapter II) and 25 kg (Chapter IV) portions of SO_2 treated HMB never exceeded 20°C. However, the higher storage temperature of the 69 t bulk was due primarily to a 28°C grain temperature at harvest, followed by a temperature rise normally associated with fermentative activity. These differences in storage temperature between the 69 t vs. the 700 and 25 kg portions of treated grain would have been important in terms of predisposition to moisture migration. Additionally, the amount of molecular SO_2 bound with components of the HMB such as thiamin and lignin, would have differed between the different lots of grain because the amount of bound SO_2 increases with increasing temperature (Ingram and Vas 1950; Hearne and Tapsfield 1956; Sawyer and Crosby 1980). The extent of molecular SO_2 lost through

these binding reactions would have been critical since bound SO_2 gives little or no preservative action (Green 1976). In this regard, Mathison et al. (1985) reported that SO_2 treated HMB could be held at room temperature for up to 2 wk after removal from storage without any visible signs of spoilage. However, they also observed that heating occurred if a 19.6% moisture diet containing SO_2 treated HMB at 91.4% of the diet (DM basis) was stored in excess of 3 or 4 d during hot summer temperatures. While SO_2 absorption by grain is enhanced with increasing moisture levels (Eckoff and Okos 1983; Lozeman et al. unpublished) so is binding, presumably because of higher levels of water activity (A_w) (Hearne and Tapsfield 1956; Eckoff and Okos 1983).

As the small scale storage units (700 kg boxes, 25 kg bags) were not sealed, they would have been considerably more aerobic than the sealed 69 t near anaerobic upright bag. Considering that SO_2 is readily lost by oxidation and volatilization in aerobic storage (Hearne and Tapsfield 1956; Green 1976), it is surprising that the 700 and 25 kg lots of treated grain remained preserved for as long as 7 mo and 18 d, respectively. This time period difference for effective preservation is likely explained by the fact that the rate of SO_2 loss via oxidation and volatilization is dependent on SO_2-permeability of the storage container, as well as on product moisture and temperature (Sawyer and Crosby 1980; Eckoff and Okos 1983). The successful preservation achieved by Mathison et al. (1985) with aerobically stored HMB (22 to 25% moisture) with at least 0.75% (wt/wt) SO_2, also suggests that the loss of molecular SO_2 via oxidation or volatilization should not be a concern.

The work of Lozeman et al. (unpublished) with $^{35}SO_2$ treated HMB (22% moisture) stored in sealed containers at room temperature, indicated that the loss of molecular $^{35}SO_2$ from binding exceeded its combined losses via oxidation and volatilization. The nonsignificant loss of total S in SO_2 treated HMB between bin opening and 7 mo thereafter in this study (Chapter II), indicated that the volatile loss of SO_2 was negligible, but such quantitative analysis would not have detected molecular SO_2 lost from binding and oxidation. Qualitative analysis as per Eckoff and Okos (1983) would have differentiated between total S, free and bound SO_2 in HMB, thus revealing the extent of molecular SO_2 lost via binding, oxidation and volatilization.

On the basis of observations made in both this study and that of Mathison et al. (1985), as well as those of Lozeman et al. (unpublished), it would appear that the loss of

molecular SO_2 via binding is more of a determinant of its efficacy as a GP than losses via oxidation and volatilization. Thus, a minimal amount of aeration in the 69 t upright bag for purposes of lowering grain temperature to prevent appreciable binding and moisture migration likely would have enhanced the storage life of SO_2 treated HMB stored in the 69 t upright bag.

The sulfites, SO_2 and its salts ($NaHSO_3$, $KHSO_3$, $Na_2S_2O_5$, $K_2S_2O_5$, Na_2SO_3), have a wider antimicrobial spectrum than that of propionate (Tilbury 1980), while propionic acid (Sauer and Burroughs 1974) and NH_3 are highly valued as fungicides. Furthermore, Tilbury (1980) stated that as a food preservative, the persistence of propionate in a food is superior to that of the sulfites, presumably because of its low chemical reactivity, or lack of binding with food components. A further concern regarding the efficacy of SO_2 as a GP is that moulds and yeasts can develop an immunity to SO_2 (Green 1976; Tilbury 1980). The fact that lactate and NH_3-N levels increased ($P<0.05$), in well preserved SO_2 treated HMB, with increasing storage time, in both the 700 and 25 kg portions of grain are indicative of a loss of antimicrobial efficacy. But, whether this occurred because of molecular SO_2 loss and/or the development of microbial resistance was not determined.

The poor persistence of molecular SO_2 due to binding, oxidation and volatilization, as well as the risk of microbes developing resistance to the chemical, casts doubts on its reliability as a GP. However, SO_2 may be effective in: (1) checking microbial growth during ambient air drying of grain, as has been demonstrated by Eckoff et al. (1979; 1983) and (2) in reducing ensiled losses of HMG (i.e. anaerobic storage) since this (Chapter II) and previous research (Knodt et al. 1952; Petrosyan 1967; Mathison et al. 1979) demonstrated that SO_2 reduces the extent of fermentation.

The comparable performance and health status of dairy cows (Chapter III) and weanling pigs (Chapter IV) fed diets containing SO_2 treated HMB vs. those fed diets based on dry or ensiled HMB was in general agreement with the results of other trials conducted with either ensiled or organic acid treated HMB (Marx 1973; Ingalls et al. 1974; Kennelly et al. 1984; Cole et al. 1970; English et al. 1973; Cole et al. 1980). However, there is no other research reported on the feeding value of SO_2 preserved grain for dairy cows or swine. The results obtained with dairy cows were consistent with those reported by others in which SO_2 treated silage (Dufour et al. 1954) or beet pulp with

added $Na_2S_2O_5$ were fed to lactating dairy cows (Weigand et al. 1972), also with no detrimental effect on performance or health.

A number of characteristics common to both the dairy and weanling pig trials should be noted considering that the feeding of SO_2 treated HMB had no adverse effect ($P>0.05$) on animal performance. Added S from SO_2 at 0.20 and 0.14% (DM basis) for the dairy and swine diets respectively were well below the maximum tolerable level of 0.35 to 0.40% as sulfate for ruminants (NAS-NRC 1980; Kandylis 1984), and the adverse effect of 0.28% S from $Na_2S_2O_5$ established by Til et al. (1972b) for swine. These trials were also similar in that diets containing less than 50% SO_2 treated HMB (DM basis) were fed for a maximum of 6 wk. While the thiamin status (as measured by % TPP effect) of weanling pigs fed diet three (which was rendered thiamin deficient through the destruction of thiamin by SO_2) was negatively influenced ($P<0.01$) there was no indication that cows or pigs fed SO_2 treated HMB developed an acute thiamin deficiency.

Feeding of SO_2 treated HMB to feedlot cattle (Chapter III) and growing-finishing pigs (Chapter IV) significantly reduced performance. A number of factors were common to both of these trials. Added S from SO_2, at 0.30 and 0.28% for beef and swine diets, respectively, approached the maximum tolerable level of sulfate S for ruminants (NAS-NRC 1980; Kandylis 1984), and in the swine diets equaled the level of 0.28% at which adverse effects on performance were reported by Til et al. (1972b). In both trials, diets containing at least 75% SO_2 treated HMB (DM basis) were fed for at least 75 d. Additionally, animals fed diets containing SO_2 treated HMB were severely thiamin depleted on the basis of: (1) a 12.5% incidence of polioencephalomalacia observed for steers, and (2) % TPP effect, cardiac hypertrophy and the low pork chop thiamin levels measured for pigs. ADG of growing-finishing pigs fed diets containing SO_2 treated HMB was reduced in proportion to their reduced DMI, both parameters measured about 10% below those measured for pigs fed the HMB control. There was no apparent treatment influence on feed:gain ratio. Despite the similarities between the feedlot cattle and growing-finishing pig trials, the primary factor responsible for both the reduced performance and health of steers fed SO_2 treated HMB was probably the feeding of spoiled grain. This we concluded from the fact that the distinguishing feature of this trial, from those of Mathison et al. (1985), in which feeding of SO_2 treated HMB to

feedlot cattle had no adverse effects, was the feeding of spoiled SO_2 treated HMB.

To be considered as a GP, one criterion that SO_2 would have to meet is that it have either no or very minimal toxicological effect (Michell 1972). The feeding of SO_2 or $Na_2S_2O_5$ treated feedstuffs to dairy and beef cattle to date, when not confounded by excessive chemical application (Stallcup 1955 cited by Mathison et al. 1979), uneven chemical application (Btatzler et al. 1956), or spoilage (Chapter III) has had no readily apparent physiological or toxicological effect (Knodt et al. 1952; Luedke et al. 1959; Weigand et al. 1972; Mathison et al. 1979; 1984; 1985). Luedke et al. (1959) concluded that high doses of S from SO_2 or $Na_2S_2O_5$ would produce anorexia and because of this, an acute toxicity would be unlikely. It does appear that a toxicological level of SO_2 treated HMB was reached in the growing-finishing pig diets (Chapter IV) since hyperemia and petechial hemorrhaging of duodenal gastric mucosa were observed. Til et al. (1972b) made the same observations when $Na_2S_2O_5$ was fed to pigs at 0.28% of the diet. The irritant effect of high dietary S on the digestive tract, in both pigs and cattle would likely be caused by its conversion to hydrogen sulfide by gastrointestinal flora (NAS-NRC 1980). Of further concern, is that the elevated dietary S level caused by feeding SO_2 treated HMB could overload the urinary excretion system (Keener et al. 1953; NAS-NRC 1978). The feeding of a buffer, such as in Experiment II (Chapter III), may help in this regard. Additionally, the high S level could interfere with the metabolism of Se, Cu and Mo. Metabolic interference with Se would be of concern for both cattle and pigs fed high S diets (Whanger 1970; NAS-NRC 1980). Data of Mathison et al. (1984), indicate that the feeding of SO_2 treated silage to steers for up to 66 d does not influence animal Se status as measured by blood Se levels. Metabolic Cu and Mo interference would only be of concern in ruminants fed diets containing SO_2 treated HMB, since monogastrics do not have the capacity to reduce sulfate to sulfide in the foregut (Dick et al. 1975). Sulfide is the reactive form of S in the Cu-Mo-S relationship in ruminants (NAS-NRC 1980).

Another criterion that SO_2 would have to meet, to be considered as a GP, is that it leave no residue in animal tissues or products (Michell 1972). Sulfide and sulfites in milk and meat, respectively would have been the obvious potential residues for animals fed SO_2 treated HMB in experiments II and III; however, neither milk or meat was tested in this regard. The nonsignificant difference between treatments in sensory evaluation of milk (Chapter III) might indicate the absence of increased sulfide levels in

milk from cows fed SO_2 treated HMB. In feeding beet pulp containing $Na_2S_2O_5$ to lactating cows, Weigand et al. (1972) reported no significant increase in milk sulfide levels. We would not expect sulfite residue in meat from cattle or pigs fed the SO_2 treatments because of sulfite oxidase (EC 1.8 3.1). This enzyme, in mammalian tissue, oxidizes sulfide, metabisulfite and sulfite to sulfate so that S in excess of metabolic needs is excreted as free or esterified sulfate (Siegel 1975; Gray 1980).

Relating SO_2 to propionic acid and to NH_3, chemical costs (F.O.B. Edmonton) are approximately \$0.98, \$1.90 and \$0.53 kg^{-1}, respectively. Safe storage of 30% moisture barley for 6 mo would require (wt/wt) applications of 1.25% propionic acid (BP Oil Ltd., Celanese Canada as cited by Jones et al. 1974) and about 1.0% for SO_2 and NH_3, resulting in chemical costs of \$0.52, \$0.21 and \$0.12 $bushel^{-1}$, respectively. As a comparison, custom grain drying in Alberta was recently reported to range from \$0.13 to \$0.34 $bushel^{-1}$ (Alberta Agriculture 1986). While this comparison shows favourable economic incentive for continued investigation of SO_2 as a GP, a number of factors introduce uncertainty for further assessment. Its chemical reactivity with food constituents, influenced by factors which include temperature, moisture and oxygen permeability, can lead to poor persistence of molecular SO_2, and as previously mentioned, there is the risk of fungi developing resistance to the preservative.

The highly reactive nature of SO_2 with food components, which include carbohydrates, proteins, thiamin, folic acid, Vitamin K (Gray 1980) as well as lignin (Ben-Ghedalia and Miron 1984), is causing considerable doubt as to its safety as a food preservative, primarily in regards to the toxicological consequence of some of the reaction products (i.e. hydroxysulfonates) which occur (Gray 1980). Considering that the use of SO_2 is prohibited in foods considered to be major sources of thiamin in the human diet (Gray 1980), its use as a preservative for HMG is questionable. Grain is a major source of thiamin for pigs, which depend entirely on dietary sources of thiamin. The concern regarding the adverse effect of SO_2 on grain thiamin level is strengthened also when one considers the rapid rate at which SO_2 destroys thiamin (Til et al. 1972a; Chapter IV).

Jones et al. (1974) has stated that due to their corrosive nature on metal equipment, as well as safety precautions required to prevent inhalation of fumes and acid contact to the hands and eyes, the use of organic acids as GP has not been extensive. Additionally, an application system which ensures even preservative application at the required application rate, while maximizing operator safety, is still lacking (Harrison

1985). This situation, coupled with the fact that SO_2 is also corrosive to most metals (C-I-L 1984; Mathison et al. 1985), and its fumes more obnoxious than those of propionic acid (C-I-L 1984; Woolford 1984), would also mitigate against its widespread use by farmers.

On the basis of this research we cannot recommend the use of SO_2 as a GP, nor the feeding of SO_2 treated HMB to livestock. The present concern as to the safety of sulfites as food preservatives, both in terms of the toxicological consequence of some of the reaction products which occur and the risk to sulfite-sensitive individuals (Food and Drug Administration 1986), leads to the conclusion that future research in examining the potential of SO_2 as a GP is not advisable.

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