

TREATED WHEAT STRAW AS AN ENERGY
FEED SOURCE FOR RUMINANTS

by

MERIOETH WAMUYU WANYAMA, B.S.

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CHAPTER I

INTRODUCTION

Studies on treatment of crop byproducts to improve efficient utilization by ruminants date back to the beginning of this century. Beckman (1922) used the method of soaking straw with NaOH and found increased digestibility. There are many ways better utilization of the bulky highly fibrous plant by products can be achieved, such as mechanical chopping, pelleting, chemical treatment, etc. The major objective of these studies was to improve palatability and hence intake, digestibility and increased productivity of the animals, by use of fibrous plant byproducts.

Ruminants have the unique ability to converting fibrous plant byproducts to high quality food for humans such as proteins, fats, vitamins and minerals. The main fibrous plant byproducts considered of nutritive value to ruminants are the low quality roughages (LQR) from cereal crops, byproducts such as stovers from corn, sorghums, millets, etc., and straw from wheat, barley, rice, etc. and industrial byproducts (Theander 1980). They are characterized by cell walls mainly of cellulose and hemicellulose fiber before it matures and hence easily digestible by rumen microbial cellulase enzymes. However on maturing, the cellulosic cell wall becomes more crystalline and forms polymers with lignin and is therefore limited in availability for utilization by the rumen microbes (Goering and Van Soest 1970; Lindgren et al. 1980; Theander 1981). Efficiency of utilization can be enhanced by treatment methods that are aimed at breaking down the fibrous and lignified

cell matrix mechanically or chemically to a more available and acceptable form both to the ruminal bacteria that bring about fermentation in the rumen and ultimately to the host animal.

Various chemicals and procedures have been tried, both in vivo and in vitro, to increase digestibility of LQR. Chemicals that have been used to treat lignocellulose materials achieving positive results include: NaOH, NH₃, Ca(OH)₂, KOH (Round et al. 1976; Arndt 1980; Said 1981; Zaman et al. 1994). Extensive work has been conducted with NaOH since it has been found to be very effective in the improvement of both dry matter digestibility (DMD) and organic matter digestibility (OMD) (Said 1981; Arndt 1980; McCann 1986; Chandra and Jackson 1971). Other alkalis such as NaHCO₃ and Na₂CO₃ appear promising alternatives since they are easier to handle and cheaper. Little work has been done on the chemical treatment of LQR using either of these carbonates. Some of the chemicals used for treating LQR have given either positive or negative results to the acid-base balance of the animal which is very important to its health and well being (Arndt 1980). In studies with Ca(OH)₂ and Mg(OH)₂, treatment of LQR resulted in an improvement in the acid-base balance of sheep which was associated with increased DM intake (Boukila et al. 1995).

Molasses is a high energy feed which is very palatable. Blackstrap molasses from sugarcane can be used as an additional energy source to LQR and also increase their palatability (Brown 1990). Therefore, molasses also can be regarded as a treatment for the LQR for increasing their acceptability and hence intake and more efficient utilization.

The objective of this study was to evaluate the in vitro and in vivo value of chemically treated wheat straw in terms of dry matter digestibility (DMD), neutral detergent fiber digestibility (NDFD), and acid detergent fiber digestibility (ADFD). Treatment of wheat straw were of NaOH, NaHCO₃, Na₂CO₃ and water, using in vitro and in vivo data parameters for determining the improved value to the animal for each treatment.

CHAPTER II

LITERATURE REVIEW

Composition of low quality roughages (LQR)

The LQR of value to ruminants are generally crop residues or industrial wastes from the Poaceae family (grasses and cereals) and some other byproducts such as cotton , peanut hulls, etc. These LQR are characterized as being high in lignocellulose, and therefore low in available carbohydrates such as cellulose, sugars, starch, and generally low in nitrogen and certain minerals such as phosphorus (Theander 1981). Goering and Van Soest (1970) used a detergent fiber procedure to analyze some roughages and concluded that they were high in lignocellulose content, that is lignin, cellulose, hemicellulose, silica and cutins.

A number of studies have demonstrated that the stage of plant maturity is an important factor in the availability of nutrients to ruminants and therefore the quality of roughages. Lindgren et al. (1980) studied the chemical composition of timothy hay (*Phleum pratense*) at various maturity levels and showed that the protein and extractable non-structural carbohydrates decreased with maturity as the cell wall (cellulose, hemicellulose and lignin) increased to maintain the structure of the plant. Tessema (1988) in his work showed that tropical grasses grow especially fast, characterized by rapid deposition of lignin in the cell matrix resulting in 30-50% loss of quality and quantity (leaves to stem ratio) of the original value of the forages within a

period of just two months. This gives an indication of the declining nutritive value due to lignification, leaching and decreased leaf to stem ratio of LQR as Tessema (1988) concluded. Decline in quality and quantity of LQR may occurs in crops such as straws stovers and hulls which are byproducts of crops that have been in the field for several months until the time of harvest.

Chemical treatment of low quality roughages

Chemical treatment of LQR is aimed at the breakdown of the cell wall structure in order to release cellulose from crystallization with the insoluble and hydrophobic lignin polymer (Theander 1981). Availability of the hemicellulose for ruminal digestion is determined by the degree of acetylation which increases with maturity. Waite et al. (1964) demonstrated that in vivo digestibility is negatively correlated to increased acetylation. Likewise, Bacon et al. (1980) showed that digestibility of barley straw increased with the progress of chemical deacetylation.

For almost a century, many chemicals and treatment methods have been employed to improve the utilization of LQR by ruminants. Beckmann (1922) used the soaking method with NaOH to improve digestibility of wheat straw and succeeded. However, the procedure resulted in extensive loss of nutrients in the soaking solution and rinsing of the alkali from the LQR. This method was improved upon by Wilson and Pigden (1964) by spraying NaOH onto LQR, which successfully improved in vitro digestibility without the leaching effect.

Treatment with alkalis

Sodium hydroxide has been widely studied and has shown better results in the treatment of LQR than other chemicals. Said (1981) experimented with sheep and compared NaOH treated maize stover with star grass (*Chloris gayana*) hay and found them to be of equal nutritive value in terms of performance, although there were lower intake levels of the treated stover. Anderson and Ralston (1973) found that treatment with KOH gave slightly lower effects on in vitro DM digestibility than treatment with NaOH. However, Round et al. (1976) compared KOH with NaOH for treating maize cobs and found them to be of equal strength in delignification of LQR.

There are indications that the response to NaOH treatment depends on the type of roughage. Summers and Sherrod (1975) treated sorghum stover, forage sorghum hay, cottonseed hulls and peanut hulls with NaOH and found that digestibility of peanut hulls, cotton seed hulls and forage sorghum hay was not improved. Response to treatment is also more noticeable in the poorer LQR as Chandra and Jackson (1971) realized after they found increased digestibility on treating sorghum stover with NaOH, unlike earlier work on forage sorghum hay which is of higher quality. Orskov et al. (1988) compared two varieties of straw (low quality and high quality) with or without ammonia treatment. The results of feeding lactating cows showed differences in actual digestibility and potential degradability, and milk yield to be significant, with the treated straw and especially the lower quality straw. Experiments with NaOH treatment of rolled barley and oats, which are a

high quality feed, showed decreased *in vivo* concentrations of several amino acids, starch and NDF in the rumen (McNiven et al. 1995).

Digestibility of DM for LQR treated with NaOH and NH₃ has been found to vary *in vitro* versus *in vivo*. Maize cobs treated with NaOH showed *in vitro* DM digestibility increased 44% using 5% NaOH but the *in vivo* DM digestibility increased only by 11% (Berger et al. 1979). Studies by Rexen and Thomsen (1976) on *in vitro* and *in vivo* digestibility of barley straw treated with 1% to 10% NaOH revealed that *in vitro* digestibility was positively correlated to increased level of the alkali; however, the *in vivo* digestibility did not increase beyond 4-5% NaOH. The *in vivo* digestibility could be limited by the sodium content in NaOH affecting the metabolism of other minerals such as phosphorus, magnesium and calcium consequently affecting rumen pH (Arndt 1980; Kristensen 1981).

Arndt (1980) treated cotton byproducts with NaOH, nitric and sulfuric acids, fed growing lambs and fistulated steers and found increased *in vitro* digestibility of OM, DM and cell wall. However, the acid treatments resulted in a decreased pH of the urine while for NaOH, an increase in percentage of the diet caused increased sodium balance. This may increase the animal's requirement for potassium, chlorine and magnesium. These digestibility results tended to agree with similar work by McCann (1986) with cotton byproducts treated with NaOH, in which he found significant improvement *in vivo* and *in vitro* for the digestibility of most feed components. Yang (1976) treated mesquite wood with NaOH and found no significant difference

between the treated and the control in the digestibility of CP, digestible energy or organic matter.

Haddad et al. (1994) compared $\text{Ca}(\text{OH})_2$, NaOH, NH_4OH and urea at different levels and combinations and reported that the largest in vitro and in vivo NDF digestibility observed was with 5% NaOH combined with 2.5% $\text{Ca}(\text{OH})_2$. The authors also concluded that $\text{Ca}(\text{OH})_2$ apparently enhanced fiber digestion at low pH in vitro. In other studies, Offer and Offer (1992) found that $\text{Ca}(\text{OH})_2$ greatly enhanced the nutritional characteristics of malt distillers grain by increasing the apparent digestibility, intake and performance of sheep. There was also an improvement in the acid-base balance of sheep when $\text{Ca}(\text{OH})_2$ and $\text{Mg}(\text{OH})_2$ were used for treatment, this was hence associated with increased DM intake (Boukila et al. 1995). Other experiments with $\text{Ca}(\text{OH})_2$ showed that using it in ensiled hay could prevent molding (Zaman et al. 1994). Sherrod et al. (1978) treated sunflower stalks, sorghum stubble and cotton byproduct with 6% sulfur dioxide and found a significant increase in DM digestibility of all three LQR.

Ammonia and other treatments

Ammonia has been a popular treatment for LQR with considerable success. Work done by Oji et al. (1977) showed increased cellulose digestibility of corn stover at treatment levels of 3% to 5% NH_3 . Kaingis' (1981) experiment on in vitro digestibility of maize stover, wheat straw and rice straw treated with ammonia showed increased DM, organic matter (OM) and crude protein (CP) digestibility. Males et al. (1981) compared ensiled

ammoniated wheat straw with grass hay while feeding cattle and found that the treated straw produced an average daily gain similar to that of the hay.

Turner et al. (1990) reported that in vivo and in vitro DM digestibility with ammoniation of LQR depended on the forage type with the least digestible forages showing the highest response. Ammonia treatment has been shown effective on a wide range of LQR with the straws of wheat, rye and triticale giving the best results (Waiss et al. 1972; Sundstol et al. 1978). Ammonia has major advantages over other chemicals used for treatment because it adds nitrogen to roughages thereby enhancing rumen microbial performance. However, NH_3 has been shown to be less effective than NaOH in the breakdown of the cell wall and hence delignification of straw (Westgaard, 1981).

Use of carbonates on low quality roughages

Work on bicarbonates in the past seems to have concentrated on supplementation as a dietary buffer but not solely for chemical treatment of LQR. Several studies have concluded that NaHCO_3 improves acid-base balance, buffering capacity of rumen fluid, increased fiber digestion and changes the molar proportions of volatile fatty acids (Goering and Van Soest 1970; West et al. 1986). Ndwiga et al. (1990) reported that NaHCO_3 gave higher silage intake and reduced ruminal acidity in dairy heifers on corn silage when compared with Na_2CO_3 or NaOH.

Supplementation with NaHCO_3 increased ruminal fluid isobutyrate and ruminal ADF, NDF and CP digestibilities in defaunated sheep (Hsu et al.

1990). On the contrary, Harris, Jr. et al. (1983) reported results from a trial supplementing ensiled LQR with NaHCO_3 to lactating cows and concluded that the NaHCO_3 was detrimental to feed intake and milk yield when fed in a diet with pelleted bagasse but was beneficial in corn silage diets in terms of milk yield. However, the authors concluded that bicarbonate was generally beneficial in diets with high moisture such as silages or brewer's yeast.

Molasses as a diet condiment

Molasses is an important energy feed source that is high in palatability and digestibility. Research on the utilization of molasses indicates that cane production in the tropics could easily compete with temperate large scale corn production in terms of a feed energy source (Preston et al. 1974). Research in Wisconsin replaced 10% of corn with blackstrap molasses in a concentrate mixture for milking cows and concluded that the molasses had 88.8% of the feeding value of corn (Cooper 1952).

Brown (1993) reported that supplementation of ammoniated tropical grass hay with molasses to yearling cattle resulted in increased apparent OM digestibility but decreased the NDF digestibility; however, total feed intake was increased. Part of this observation tends to agree with the report by Petit et al. (1994) who found decreased energy and fiber digestibility by steers supplemented with 15% cane molasses. Molasses supplementation up to 25% of the DM of untreated hay has shown increased in vivo OM digestibility by cattle while ammonia hay treatment increased OM and NDF digestibilities (Brown 1990).

Molasses from an improved sugar beet processing referred to as Holly Sugar Concentrate (HSC) is another palatable source of energy that could be used as an energy supplement. Preston and Bartle (1994) estimated the maintenance net energy and net energy for gain values of HSC compared to cane molasses for feedlot steers and found the values to be almost the same on a DM basis with HSC being slightly higher.

Other treatments of low quality roughages

The use of live microbial culture to improve in vivo and possibly in vitro digestibility of LQR is a rapidly growing field. Microbial treatments may improve feed intake by improving the rate of fiber breakdown (Wallace 1994). Harris Jr. et al (1982) used enzymes derived from *Aspergillus oryzae* as a treatment when ensiling sugar cane and found no undesirable properties, although there were no significant effect.

Electron irradiation has been used to disrupt the microstructure of LQR to increase the availability of cellulose by several researchers but, Millet (1970) demonstrated that the cost may not be economical. Ben-Ghedalia et al. (1989), in studies on screened manure fiber (from fed diets of different hays and concentrate supplement), using ozone and NaOH as a treatment for delignification and found that the ozone treatment was most effective in the solubilization action on the lignin and degraded the fiber up to 38% compared to 10% by the NaOH.

CHAPTER III

TREATED WHEAT STRAW AS AN ENERGY FEED SOURCE

Abstract

Three in vitro experiments and one in vivo digestion study with lambs were conducted out to examine the digestibility effects of wheat straw treatment with NaOH, NaHCO₃ and Na₂CO₃ compared to water treatment as the control. The in vitro experiments utilized two amounts of straw, 100mg and 200mg, treated with 10.6% Na₂CO₃, 8.4% NaHCO₃ (as the lower levels) 15.5% Na₂CO₃, 12.4% NaHCO₃ (as the higher levels) or 4% NaOH. There were significant increases in DMD (P< .001) for all treatments. The 100mg straw level had the highest DMD (P<.001), 66.9% compared to 52.9% for the 200mg level straw. The treatment using 15.5% Na₂CO₃ and 12.6% NaHCO₃ gave the highest DMD of 63.5% and 60.4% respectively. The 8.4% NaHCO₃ results showed DMD similar to 4% NaOH and 10.6% Na₂CO₃.

In the in vivo experiment, nine wether lambs averaging 32 kg were used in a digestion study where three lambs per treatment were fed three different diets of straw treated with water, 8.4% NaHCO₃ or 4% NaOH, all mixed with molasses to improve the palatability, and were supplemented with steam-flaked sorghum grain based concentrate. The experimental period was 7 days pre-trial and 7 days for data collection. The 4% NaOH treatment of straw gave the highest in vivo OMD (70.0%), NDFD (70.1%) and ADFD (64.3%) digestibilities. NaHCO₃ result did not differ significantly with NaOH,

on the OMD and DMD, but the cell wall breakdown was significantly lower than that of NaOH. However, NaHCO₃ had the higher DOMI (146.2g) compared to only 98.0g by the NaOH treatment. When DMI was used as a covariate, the DMD for the treatments means approached significance (P=.07). However, this adjustment did not alter the NDFD and ADFD since they were already significant even before the adjustment; the regression coefficients for NDFD and ADFD were negative while these for DMD, CPD and OMD were positive with increasing DMI. The control diet had the highest total DMI and DOMI which could be associated with the use of molasses to enhance palatability, whereas the NaOH treatment resulted in the lowest DMI and DOMI.

Introduction

Utilization of LQR is important for efficient use of a critical energy source for livestock that may otherwise go to waste. The LQR are characterized by structural carbohydrates made up of the cell wall which give protection and strength to plants as they mature. Lignin, a hydrophobic polymer, binds the carbohydrates that could otherwise be available to ruminants as an energy source. Chemical treatment is aimed at breaking down the lignin bonds with those carbohydrates such as cellulose and hemicellulose to be free for utilization by the rumen microbes in the ruminant digestive tract (Theander 1981). As long as there will be food grown for human beings, LQR will always be a feed resource that can be converted from waste byproduct

such as straws, stover, cottonseed hulls, etc., into high quality proteins and vitamins by ruminants for man (Preston 1991).

Use of NaOH among other chemicals has been popular for the proven performance of delignification of LQR (Summers and Sherod 1975; Chandra and Jackson 1971; Arndt 1980; Said 1981). All these studies show that NaOH enhanced the DMD, OMD and performance of the livestock. However, Arndt (1980) reported that increased percentage of diet treated with NaOH may lead to increased animal requirements for potassium, calcium, chlorine and magnesium. Therefore the need to explore further for chemicals that will be as effective, cheaper and easier to handle when treating; also palatable and easily digestible by livestock.

Carbonates and bicarbonates have been used successfully in studies mostly as a buffer supplement to other feeds. (West et al. 1986; Ndwiga et al. 1990; Hsu et al. 1990). A literature review discloses that no work has been conducted on Na_2CO_3 and NaHCO_3 for treatment of LQR; given the desirable effects it has on the acid-base balance and buffering of the rumen fluid (Goering and Van Soest 1970) among other things, it was thought a worthwhile venture to examine their effects for treating LQR.

Molasses is a highly palatable feed byproduct that can be used as a supplement to LQR of low palatability to enhance their intake and possibly (to some extent) digestibility by providing readily available energy to the rumen microbes. Brown (1990) in his studies with molasses suggested that supplementation of up to 25% of DM of untreated hay showed an increase in vivo OMD in cattle.

The studies in this thesis were conducted to compare the in vitro effects of Na_2CO_3 and NaHCO_3 with NaOH treatment of LQR (wheat straw) and treatment with water as a control on digestibility. I also studied the the in vivo effects of treatment of wheat straw with H_2O , NaOH , and NaHCO_3 (all mixed with molasses) for lambs on digestibility and intake of DM by examining parameters of energy digestibility such as DM, OM, NDF and ADF and CP.

Experimental Procedures

In vitro digestion of wheat straw

Three in vitro runs were conducted whereby in the first run there were 4 treatments; wheat straw treated with (1) water, (2) 10.6% Na_2CO_3 , (3) 8.4% NaHCO_3 (lower levels) and (4) 4% NaOH . The second and third runs had the above treatments plus 12.4% NaHCO_3 and 15.5% Na_2CO_3 , (higher levels); this resulted in six treatments.

Sample preparation

The alkalis were weighed on a molar equivalent basis for comparison; 4g NaOH (0.1M), 10.6g Na_2CO_3 (0.1M), 8.4g NaHCO_3 (0.1M), 12.4g NaHCO_3 (0.15M) and 15.9g Na_2CO_3 (0.15M) were mixed with 100ml distilled water. Wheat straw (100g) was placed in aluminum trays and then mixed thoroughly with the alkaline solution using one part solution and one part straw. The mixtures were then oven-dried at 56° C until the moisture content stabilized in about 3 days. Grinding of the samples was done using a 1mm screen in a Willey mill. The samples were then considered ready for the

1mm screen in a Willey mill. The samples were then considered ready for the in vitro digestion. In vitro tubes (50 ml plastic centrifuge tubes) were dried in the oven at a 100° C for 5 hours, allowed to cool in a desiccator and then weighed. The ground straw was weighed at 100mg and 200mg levels and placed into triplicate in vitro tubes.

In vitro procedures

Rumen fluid was collected from cannulated steers at the New Deal Texas Tech University research facility. The fluid was collected from two steers; one was fed a concentrate diet and the other a roughage diet. The rumen fluid was put in thermos flasks to maintain anaerobic conditions and the warm temperature, and then brought to the laboratory. The rumen fluid from the two steers was mixed in a ratio of 1:1. Cheese cloth in four layers was used to strain large particles from the fluid and then the rumen fluid was placed into separatory funnels which allowed separation of the fluid from smaller particles. The separatory funnels with the rumen fluid were placed in the water bath for about 15 minutes, maintained at 39° C (in a water bath) and gassed with CO₂ to provide anaerobic conditions. McDougall buffer solution (Table 1) was kept at 39° C and CO₂ was used to remove oxygen. The rumen fluid was mixed in a 1:3 ratio of rumen fluid to buffer. Then 30ml of the rumen fluid, buffer combination and 1ml of a 1% urea solution were measured into the in vitro tubes, stoppered with rubber stoppers fitted with gas release valves and the tubes placed in a 39° C water bath mixed gently to ferment for 16 hours. The 16 hours period was chosen as the estimated

Table 1. Reagents used to make one liter of McDoughall buffer solution as modified by Bartle et al. (1986).

CHEMICAL	AMOUNT g/l
NaHCO_3	9.800
Na_2HPO_4	3.700
KCl	.570
NaCl	.470
Na_2SO_4	.200
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.120
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$.043
CaCl_2	.140

maximum rate of microbial digestion of straw fiber. After 16 hrs there may not be significant differences in digestion, where by 36 hours digestion may level off. Six tubes were used as blanks; fermentation was stopped in 3 tubes using 1 ml of 5% mercury chloride at the start and 3 tubes at the end of the fermentation period.

After the fermentation period, the tubes were removed from the water bath and further fermentation stopped by adding 1 ml of a 5% mercury chloride solution and shaking gently to mix. Tubes were then put in the centrifuge for 14 minutes at a setting of 31,000G. The supernatant was collected in disposable tubes and put into the freezer for later ammonia determination. The remaining liquid was removed by a vacuum aspiration pump into disposing bottles for later supervised waste disposal of the mercury waste. The remaining solid pellet in the in vitro tubes was dried at 100° C for 16 hours. After drying the tubes were weighed and apparent dry matter digestibility (DMD) was calculated.

Determination of ammonia

The ammonia procedure used to analyse for residual NH₃-N was similar to the phenol hypochlorite procedures by Fawcett and Scott (1960). The supernatant liquid was thawed at room temperature and then 20 micro liters of the supernatant was measured into 10 ml test tubes in triplicate, after the standards of the same volume had been measured in duplicate. The levels used for standards were 0, 5, 10, 20, and 40 mg NH₃-N /dl. Phenol color

reagent was added to the tubes followed immediately by sodium hydroxide-hypochlorite reagent both at 4 ml level and mixed thoroughly. The tubes were put in a water bath at 37° C for 15 minutes. A Beckman DU-50 Spectrophotometer was used to determine the ammonia at 625 nm wavelength by first programming it to analyze and calibrate the standards and then the samples.

Digestion study

Wheat straw was mechanically chopped using a Hay Buster E-1,000 to 3-6 inches in preparation for chemical treatment. It was mixed with a solution of H₂O for the control, 4% NaOH or 8.4% NaHCO₃ (as solution percentages) treatments using one part solution and two parts straw. The straw and the solutions were well mixed using a mechanical paddle mixer (Marion). Metal drums (208 litres) lined with polyethylene bags were used to store the treated straw. The drums were tightly packed with the treated straw in the polyethylene bags, sealed and stored at room temperature for continued treatment.

Animal diets

To improve palatability, the treated straw preparations were mixed with cane molasses in a 1:11 ratio of molasses to treated straw (wet weight). The treated straws constituted 70% of the total diet-as fed. Supplementation with a steam-flaked sorghum grain based concentrate was provided as 30% of the total diet (Table 2).

Table 2. Diet composition (on as fed basis) of wheat straw and concentrate supplement fed to sheep during a digestion study of treated wheat straw with NaHCO₃, NaOH and H₂O.

INGREDIENTS	DIET AMOUNTS (%)
WHEAT STRAW	92.00
CANE MOLASSES	8.00
SUBTOTAL	100.00
CONCENTRATE SUPPLEMENT:	
STEAM FLAKED SORGHUM GRAIN	33.35
COTTONSEED MEAL	54.00
CANE MOLASSES	1.33
UREA	3.33
CALCIUM CARBONATE	1.67
DICALCIUM PHOSPHATE	1.33
TRACE MINERAL PRE-MIX ^a	.83
SALT	.83
VITAMIN A PREMIX ^b	.83
VITAMIN D PREMIX ^c	.83
VITAMIN E PREMIX ^d	1.67
SUBTOTAL	100.00

^a Trace mineral premix: Zn, 8426 ppm; Cu, 1984 ppm; Mn, 9259 ppm; Co, 51 ppm; I, 2801 ppm.

^b Vitamin A premix: 300,000 IU vitamin A acetate/lb.

^c Vitamin D premix: 50,000 IU D₃/lb.

^d Vitamin E premix: 800 IU /lb.

Feeding

Nine black-face wether lambs reasonably uniform in breed, age and weight (32 kg +/-3 kg) were placed in metabolism stalls for a preliminary feeding period of nine days. They were fed a diet of the treated straw and a concentrate supplement until they were consuming a level of about 60:40 of straw to concentrate; the final diet amounts were 250g of treated straw to 175g of concentrate supplement.

There were three treatments in all; the nine lambs were divided into three groups of three lambs each. Group one was fed the water-treated straw, group two was fed the 8.4% NaHCO₃ treated straw, while the third group was fed the 4% NaOH treated straw. However, all the groups were offered the same amount of the concentrate supplement.

Sample collection

A preliminary nine-day period was used to adjust the lambs to the diet and the digestion stalls. Each day the feed offered to the sheep and the feed refused was weighed and recorded. Feed refused was mixed well and a subsample of 20% was taken. Total feces was collected each day from each lamb at the same time right after feeding the sheep, weighed, mixed well and a sample of 20% taken daily. Water was offered free choice. The collection period was 7 days. Fecal samples collected from each lamb were well mixed and a sub-sample of 200g was taken from the fecal aliquot.

Both the feed and fecal samples were individually placed in pans, labeled, weighed and then put into a forced air oven at 55-60° C for 48 hours,

after which they were weighed, left to equilibrate overnight and then weighed again. The samples were ground through a 1mm screen with a Willy mill and then placed in airtight plastic bags in preparation for analysis.

The dry matter, organic matter, ash and protein were analyzed according to the procedures in the AOAC (1980). Dry crucibles were weighed and then 1.5 g of each sample was weighed and placed in the crucibles. They were placed in the oven at 100° C overnight and allowed to cool in the desiccator. Sample weight was then taken to determine the dry matter (DM) of the samples (the dry weight taken at 56° C was also used to calculate the final DM). The crucibles and dry samples were then placed in the muffle furnace at 600° C for 6 hrs to determine the organic matter and ash values. Acid detergent fiber (ADF) and Neutral detergent (NDF) fiber were analysed by a private lab Servi-Tech (on 1816 E. Wyatt Earp), Dodge City, KS.

Statistical analyses

In order to determine the variations and treatment effects, analysis of variance (ANOVA) by the General Linear Model (GLM) procedure of Statistical Analysis System (SAS; 1985) was used for the in vitro experimental data. The design was a 3 x 2 x 4 factorial for the first run and 2 x 2 x 6 for the second and third runs. That is, the experimental data were analysed using runs (n= 3 or 2), in vitro level of wheat straw (n= 2) , and number of treatments (n= 4 or 6)

ANOVA and an analysis of covariance (ANCOVA) of SAS (1985) were conducted in order to determine the variations and the treatment effects on the digestibility parameters of the digestion study with lambs. A simple one-factor ANOVA was used to establish the variations between the treatments. Also, dry matter intake (DMI) was used as covariate with the digestibility parameters in an ANCOVA.

Results and Discussion

In vitro experiments with four wheat straw treatments

Sodium hydroxide has been found to have some of the highest DMD results on treatment of LQR. However, the results observed here during the in vitro digestibility trial showed that NaOH DMD did not differ (significantly) from the 10.6% Na₂CO₃ and 8.4% NaHCO₃ DMD. Kristensen (1981) reports that 80- 85% of the NDF in NaOH treated straw may be digestible among other improvements such as ADFD, OMD, etc., but he also concludes that high sodium content may be hazardous to the animal health. The results reported here show that the carbonates, which enhance buffering of the rumen and acid-base balance (Goering and Van Soest 1970) of the ruminants, could be a promising alternative to NaOH whose chemical nature is corrosive and is not as easily available and to work with as the Na₂CO₃ and NaHCO₃.

The SAS analysis of variance set up to show whether there were any differences between the DMD among all treatments in experimental runs one, two and three showed significant results (Table 3). The results also show that the treatment with 10.6% Na₂CO₃, 8.4% NaHCO₃, or 4% NaOH all

gave higher DMD compared to the control (DMD) (Table 4). The DMD result for NaHCO₃ was 50.1% (P<.0001), which was lower than the 10.6% Na₂CO₃ treatment. (P=.07). The 4% NaOH treatment gave a DMD of 51.8% which was somewhat higher than the 8.4% NaHCO₃ treatment (P=.01) but similar to the 10.6% Na₂CO₃ (P> 0.05).

The two levels of straw used in vitro (100mg and at 200mg) resulted in different (P<.001) DMD, with the 100mg straw level giving the higher DMD (Table 5) (Figure 1). The interaction between the straw treatments and the level of straw used (Table 6) approached significance (P=.06). The level by run interaction (Figure 1) was significant (P=.0001) and the treatment by run interaction (Figure 2) was significant (P=.0001); these interactions could be associated with various environmental factors relating to the different times that the runs were carried out. One could speculate that experimental run one may have been affected due to the fact that the rumen fluid was collected when there were in situ rumen bags in the donor steers' rumen unlike when rumen fluid was collected for the other two runs. However, other factors such as efficient anaerobic processing, temperatures or even incorrect buffer pH could have influenced the results

Table 3. Analyses of variance for in vitro dry matter digestion runs 1, 2 and 3.

SOURCE	DF	MS	P	R-SQUARE
MODEL	23	699	.0001	.988
TRT	3	169	.0001	
LEVEL	1	1636	.0001	
RUN	2	6343	.0001	
TRT *LEVEL	3	10	.0573	
TRT* RUN	6	62	.0001	
LEVELS * RUN	2	389	.0001	
TRT *LEVEL * RUN	6	9	.0388	
ERROR	48	4		
TOTAL	71			

Table 4. Effects of wheat straw treatment on in vitro DMD during runs one, two and three including four treatments.

TREATMENT	DMD	DUNCAN'S MRT*
WATER	45.1%	C
8.4% NaHCO ₃	50.1%	B
10.6% Na ₂ CO ₃	51.3%	B A
4% NaOH	51.8%	A
SEM (n= 18)	0.47	

*Duncan's multiple range test; means with the same letter are not significantly different.

Table 5. Effects of wheat straw level on in vitro DMD.

LEVELS	DMD	DUNCAN'S MRT
100g	54.4 %	A
200g	44.8 %	B
SEM (n =36)	0.33	

Table 6. Wheat straw level by treatment interaction on DMD during runs 1, 2 and 3.

TREATMENT	100mg	200mg
CONTROL	49.8%	40.5%
10.6% Na ₂ CO ₃	55.7%	47.0%
8.4% NaHCO ₃	54.3%	45.9%
4% NaOH	57.7%	45.9%
SEM (n = 9)	0.67	0.67

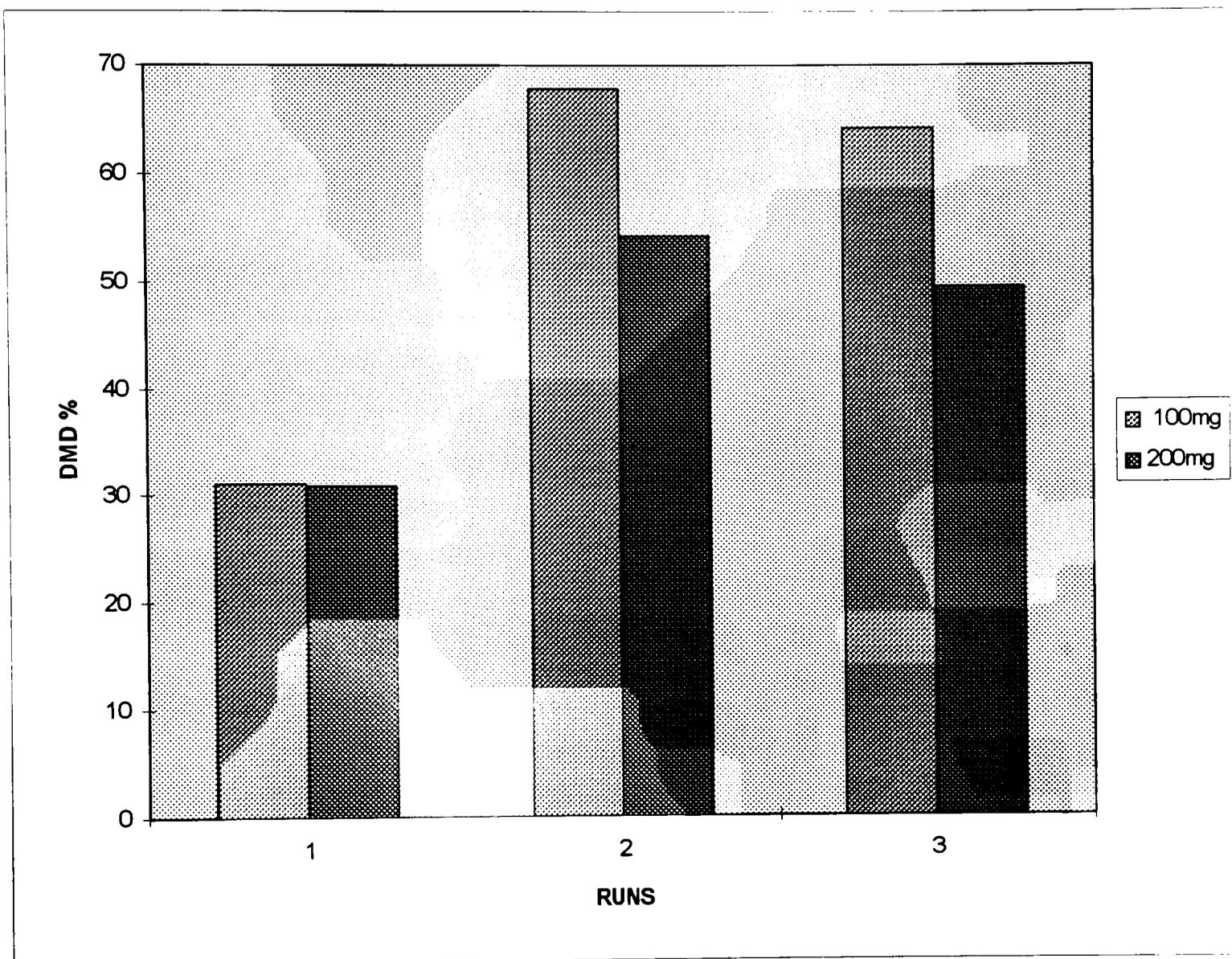


Figure 1. Effects of level of treated straw (100mg and 200mg) in the in vitro digestion trial with 3 runs. The overall effects and the run by level interaction were both significant.

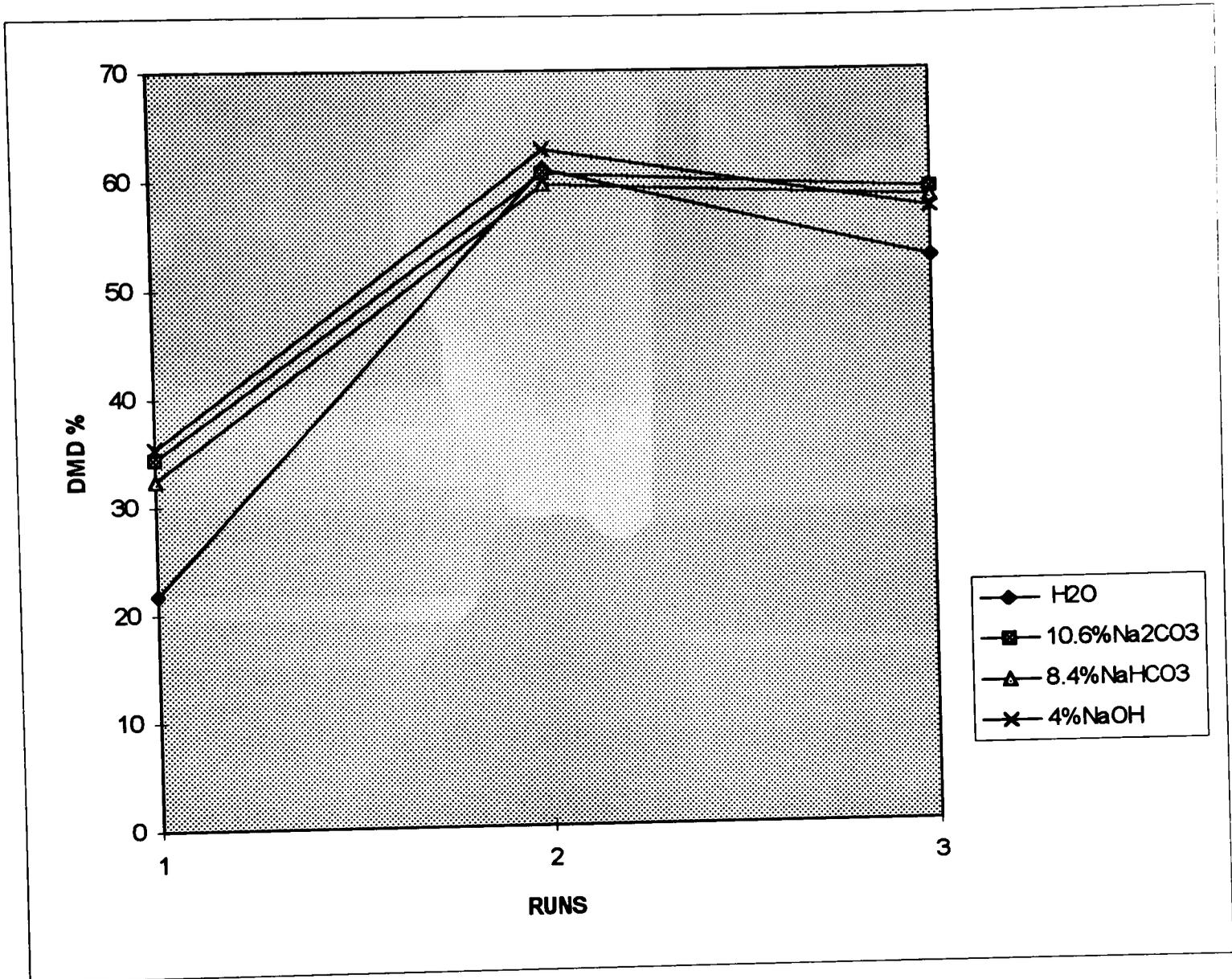


Figure 2. Run by treatment interaction on in vitro DMD for the H₂O, Na₂CO₃, NaHCO₃ and the NaOH treatments.

In vitro experiment with six wheat straw treatments

Results of the above four treatments showed similar trends in significance with the six treatments in runs two and three. However, the higher levels of the carbonates had the highest DMD, but only 15.5 % Na_2CO_3 was significantly higher than all other treatments. Treatments and levels significantly affected the DMD (Table 7). The control treatment had the lowest DMD of 56.8% ($P < .0001$) compared to the other five treatments (Table 8). The other treatments also differed significantly ($P < .0001$) among themselves, except for the 10.6% Na_2CO_3 and 4% NaOH which were not different ($P = .85$) in the DMD. However, 15.5% Na_2CO_3 and 12.6% NaHCO_3 showed higher results in DMD compared to all other treatments with the former being the highest.

There was a major difference ($P < .0001$) between the two levels of straw used (Table 9) with the 100mg level showing a higher DMD (66.9%) compared to the 200mg level (52.9%). For all the treatments, the the 200mg level always had lower DMD. The interaction between the wheat straw treatment and the level (Figure 3) was significant ($P < .005$), this could be because the treatments might have had different effectiveness within the two levels reacted with. The two runs (Figure 4) were significantly different ($P < .0001$) with run two showing higher DMD (62.0%) compared to run three (57.9%). However, the run by level interaction ($P = .27$) and the treatment by level by run interaction ($P = .16$) were not significant. The 15.5% Na_2CO_3 treatment, in spite of giving the highest DMD in the two runs, was considered too high and bulky for effective use as a treatment

of LQR. Sodium bicarbonate was considered a better choice among the lower and higher carbonate levels because the results in DMD were almost the same. The lower level of NaHCO_3 would be economical and more readily available (over the counter) especially in developing countries.

The supernatant derived from the in vitro studies was used for $\text{NH}_3\text{-N}$ determination using the Beckman DU-50 spectrophotometer, but the data collected gave no variation in $\text{NH}_3\text{-N}$ levels across the treatments. Visual evaluation of the data led to the conclusion that there were no differences across the treatments.

Table 7. Analysis of variance for in vitro digestion of wheat straw with different treatments (runs 2 and 3).

SOURCE	D	MS	P	R-SQUARE
MODEL	23	185.49	.0001	.9716
@TRT	5	56.18	.0001	
LEVEL	1	3501.3	.0001	
RUN	1	306.32	.0001	
TRT*LEVEL	5	9.85	.0056	
TRT* RUN	5	20.69	.0001	
LEVEL * RUN	1	3.23	.2702	
TRT *LEVEL*RUN	5	4.37	.1564	
ERROR	48	2.59		
TOTAL	71			

@TRT means treatment.

Table 8. Effects of alkali treated wheat straw on in vitro DMD for runs 2 and 3 including six treatments.

TREATMENT	DMD	DUNCAN'S MRT*
H ₂ O	56.8%	C
8.4% NaHCO ₃	59.0%	B
10.6% Na ₂ CO ₃	59.8%	B
4% NaOH	60.0%	B
12.6% NaHCO ₃	60.4%	B
15.5% Na ₂ CO ₃	63.5%	A
SEM (n= 12)	.46	

Table 9. Effects of wheat straw level on in vitro dry matter digestibility during runs 2 and 3 including six treatments.

LEVELS	DMD	DUNCAN'S MRT
100mg	54.4%	A
200mg	44.8%	B
SEM (n=36)	.27	

*DUNCAN'S MRT; means with the same letter are not significantly different

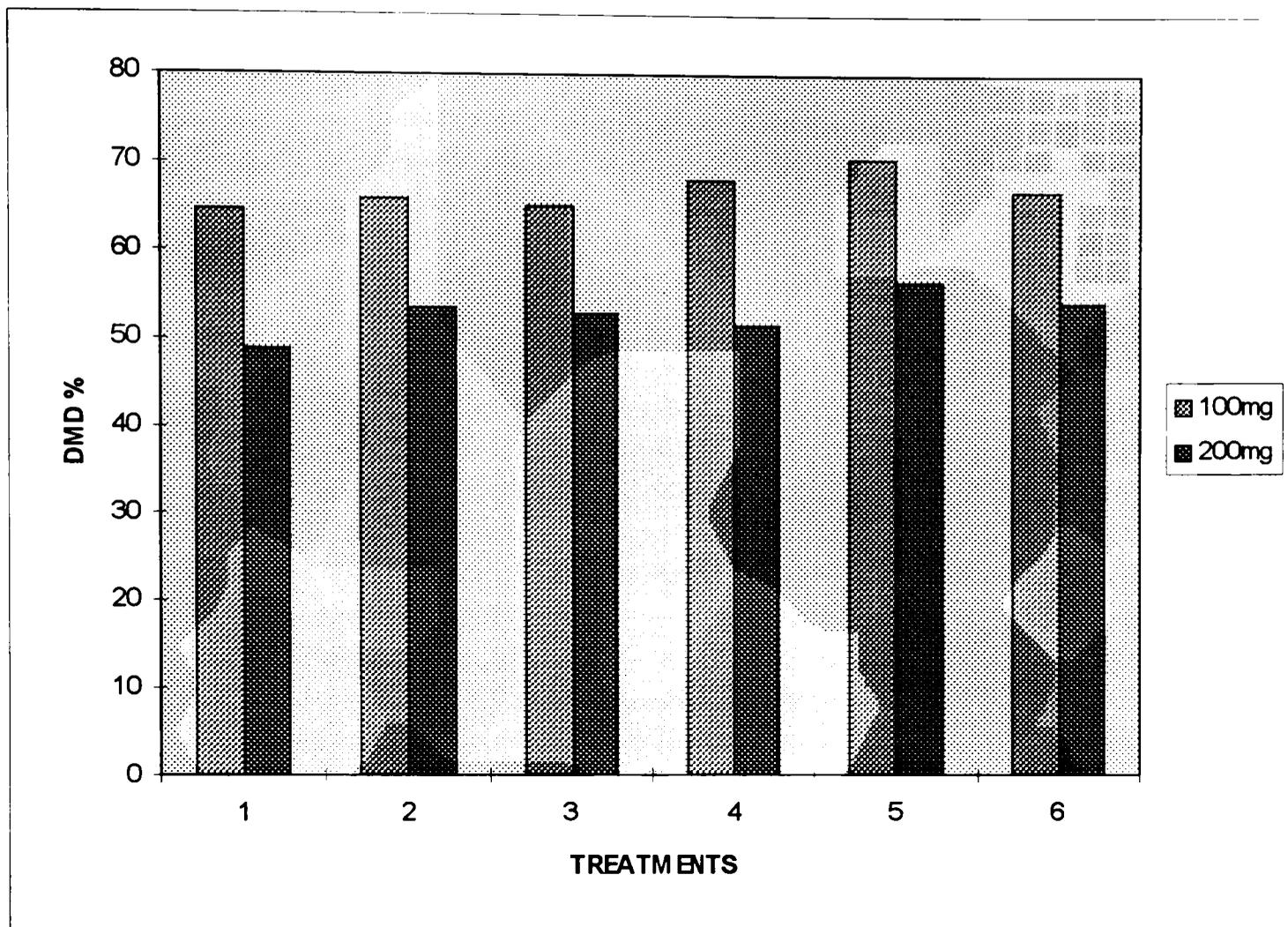


Figure 3. Treatment by level interaction on wheat straw in vitro DMD of six treatments during runs 1, 2 and 3.

Treatments:

1 = H₂O

2 = 10.6% Na₂CO₃

3 = 8.4% NaHCO₃

4 = 4% NaOH

5 = 15.5% Na₂CO₃

6 = 12.6% NaHCO₃

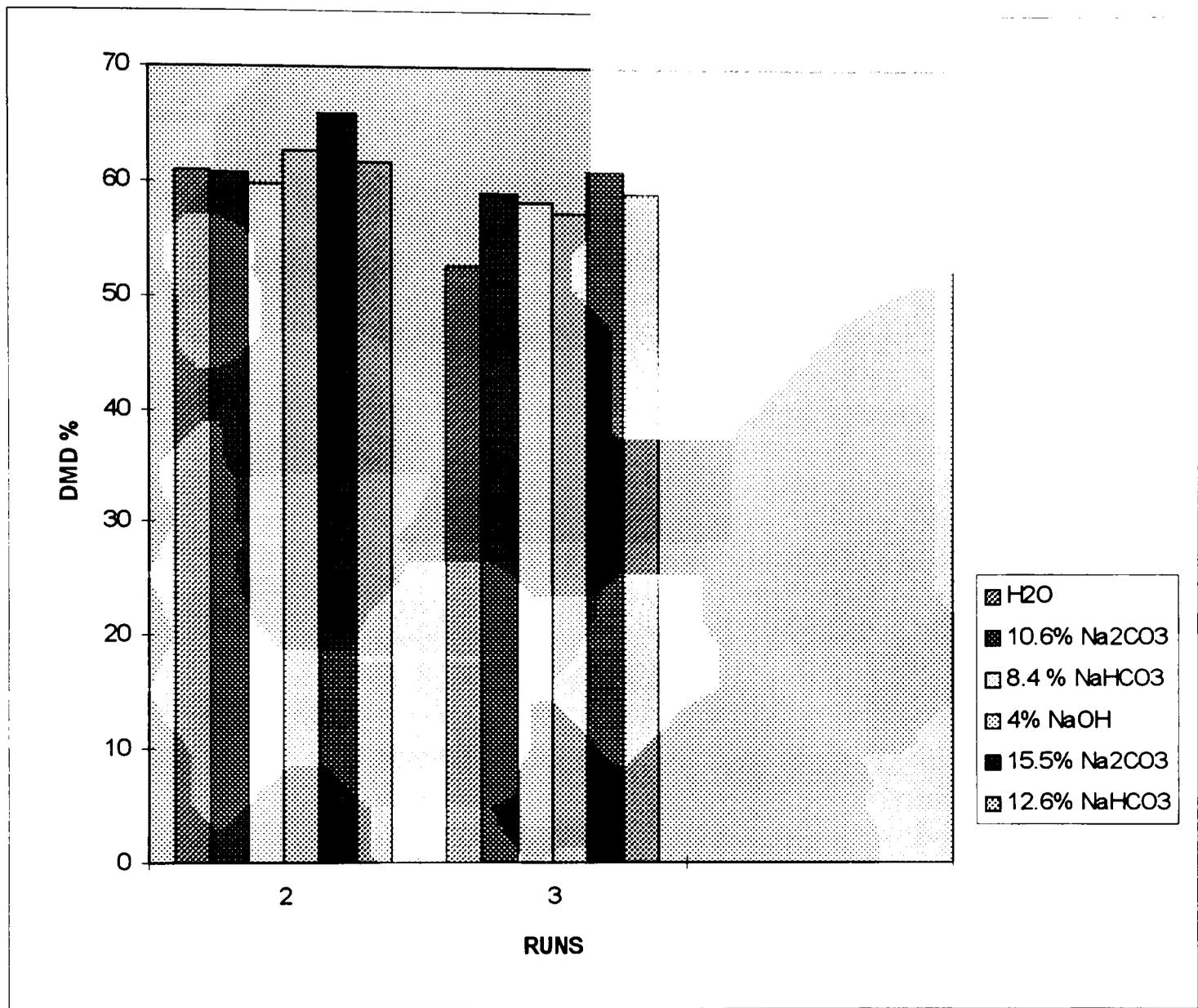


Figure 4. Treatment by run interaction on in vitro DMD of treated wheat straw with six treatments.

In vivo results

The control treatment had higher DMI (272gm) than NaHCO₃ (252gm) or NaOH (150) treated straw. Low intake of NaOH treated straw agrees with other studies of treated LQR with NaOH such as by Arndt (1980) and McCann (1986). The high DMI in the control group could be associated with the molasses use to enhance the palatability in all treatments. It therefore seems that molasses may have had the most positive effects with the H₂O treatment. This would be in agreement with reports by Brown (1993) on increased intake of ammoniated tropical hay on supplementation with molasses.

Although the DMD did not show significance (Table 10) among the treatments, the NaOH treatment gave the highest digestibility results (66.3%), with major differences especially from the control (Table 11). Said (1981), Kristensen (1981), Sankat et al. (1988), Arndt (1980), Chandra and Jackson (1971) all reported an increase in DMD on treatment of LQR with NaOH. DMD did not show significant effects (P= .20), but when DMI was accounted for as a covariate treatments approached significance at (P=.07) in the type III sums of squares (SS) (Table 10). The results of DMD after adjustment for DMI were 55.7%, 59.6% and 69.4% for the water, 8.4% NaHCO₃ and 4% NaOH, respectively, (Table 11). The results presented here might have failed to show statistical significance probably due to the few animals used per group.

Table 10. Analysis of variance and analysis of covariance for digestibility parameters.

ANOVA SOURCE	DMD		OMD		DOMI		CPD		ADFD		NDFD	
	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
MODEL	75	.16	51	.08	3103	.50	1827	.25	614	.01	746	.01
ERRORS	29		13		4059		1040		63		74	
TOTAL												
	*DF											
ANCOVA SOURCE												
MODEL	3	.12	48	.03	10190	.0001	2781	.02	420	.03	420	.02
ERROR	5		7		5		310		63		51	
TYPE I SS												
TRT	2	.18	51	.03	3113	.0001	1828	.05	615	.02	609	.01
DMI	1	.08	43	.05	24346	.0001	4689	.01	31	.50	41	.04
TYPE II SS												
TRT	2	.07	73	.02	233	.0008	102	.73	346	.05	327	.04
DMI	1	.08	43	.05	24346	.0001	4690	.01	31	.51	41	.40

*DF degrees of freedom

Table 11. Dry matter intake and digestion coefficients as affected by wheat straw treatment adjustment.

PARAMETERS	H ₂ O	NaHCO ₃	NaOH	*REG CF.
DMI total	272gm	252gm	150gm	
DMDuntd ^a	56.3% A	60.7% A	66.3% A	
adtd ^b	55.8%	59.6%	69.4%	.043
OMDuntd ^a	63.0% A	64.0% A	71.0% A	
adtd ^b	62.4%	63.4%	73.1%	.027
DOMIuntd ^a	159.5gm A	146.2gm A	98.4gm A	
NDFDuntd ^a	44.5% B	51.9% B	74.8% A	
adtd ^b	45.8%	52.6%	70.1%	-.03
ADFDuntd ^a	36.3% B	45.5% B	64.3% A	
adtd ^b	37.4%	46.1%	62.6%	-.02
CPDuntd ^a	67.5% A	53.6% A	19.6% A	
adtd ^b	54.1%	45.8%	40.9%	.028

* Regression coefficient against DMI

^a values unadjusted for DMI as a covariate.

^b values adjusted for DMI as a covariate.

The OMD was highest (Figure 5) on the NaOH treatment and was 13% point higher than the control in spite of failing to reach statistical significance (Table 10). Authors mentioned above also report significance in OMD on treatment with of LQR with NaOH. The mean separation test of Duncan's multiple range test (Table 11) showed that the NaOH treatment OMD was 71.0%, but not significantly higher than the NaHCO₃ at 64.0% and the control at 63.0 %. The regression coefficient for DMD, OMD, CPD and DOMI were all positive while those for NDF and ADF were negative. Treatment with NaHCO₃ did not come out too well on the OMD but had much higher DOMI than NaOH which was a significant factor because of the high intake compared to NaOH. This means that the lambs may eventually get adequate energy from the higher intake with NaHCO₃ than with NaOH even though the NaOH has higher OMD and DMD. Treatment with NaOH had the lowest (98.4gm) DOMI compared with the control and NaHCO₃ which both had almost similar values (159.5gm and 146.2gm, respectively). This was as a result of the low total DMI by the NaOH treatment group. The control group had the highest DOMI (Figure 6), these observations are similar to those reported by Brown (1990), where he suggested that supplementation of untreated hay with molasses showed an increased in vivo OMD in cattle.

The NDFD and ADFD were highest with the NaOH treatment showing a 68% difference in the NDFD with the control and a 15% difference with the NaHCO₃ using unadjusted means. Kristensen (1981) reported 80-85% of NDF in NaOH treated straws may be digestible but the digestibility of the neutral detergent soluble are much lower therefore the high values on NDFD

with the NaOH treatment show similar trends. The ADFD results were slightly lower in these results reported in this thesis. As mentioned above (Kristensen 1981). NDFD had significant main effects (Table 10; $P = .01$) even without adjusting for DMI. On adjusting for DMI, the significance among the treatments declined slightly ($P = .04$) for NDF. The ADF digestibility also showed unadjusted differences ($P = .01$), the significance of which decreased somewhat for with the means adjusted for DMI ($P = .06$).

Dry matter intake (DMI) was used as a covariant with the digestion parameters using DMD in the procedure of GLM of SAS. The differences between all the treatments in DMD approached significance (Table 10; $P = .07$) when adjusted for the differences in DMI. However, OMD showed significant differences between the treatments with ($P = .04$) or without ($P = .01$) adjusting for DMI (Table 10). The unadjusted DDMI means by Duncan's MRT of 159.5g, 146.15g and 98.4g for water, NaHCO_3 and NaOH treatments, respectively.

Digestibility of CP (Figure 5) was surprisingly highest in the control group. The CP present in the diet would have mainly come from the supplement since straw has insignificant levels. The NaOH and NaHCO_3 treatments seem to have affected the CPD negatively as shown by the lower digestibilities (Table 11). In studies with rolled barley treated with NaOH, McNiven et al. (1995) reported a decreased concentration of several amino acids, starch and NDF and digestibilities of N and starch were reduced in the small intestines. Results presented here could have been similarly affected. One could speculate the possibility that the alkalis have denaturing effects on

the proteins. The CPD treatment effect was not significant at ($P = .25$) without adjusting for DMI but approached significance ($P = .07$; Table 10) on adjusting for DMI. The CPD on adjustment for DMI were 54.1%, 45.8%, and 40.9% for water, NaHCO_3 and NaOH respectively, compared to the variable and nonsignificant unadjusted means (Table 11) of 67.5, 53.6 and 19.6 for water, NaHCO_3 and NaOH , respectively.

Differences in DMI between the treatments, strongly influenced digestibility. Therefore it was found necessary to adjust for intake effects in order to establish the effects of the treatment on adjusted parameters. This is clearly shown by the NaOH treatment where the DMI were low making the treatment effects on CPD and DMD not significant but when adjusted for DMI, the effects were significant to approaching significance.

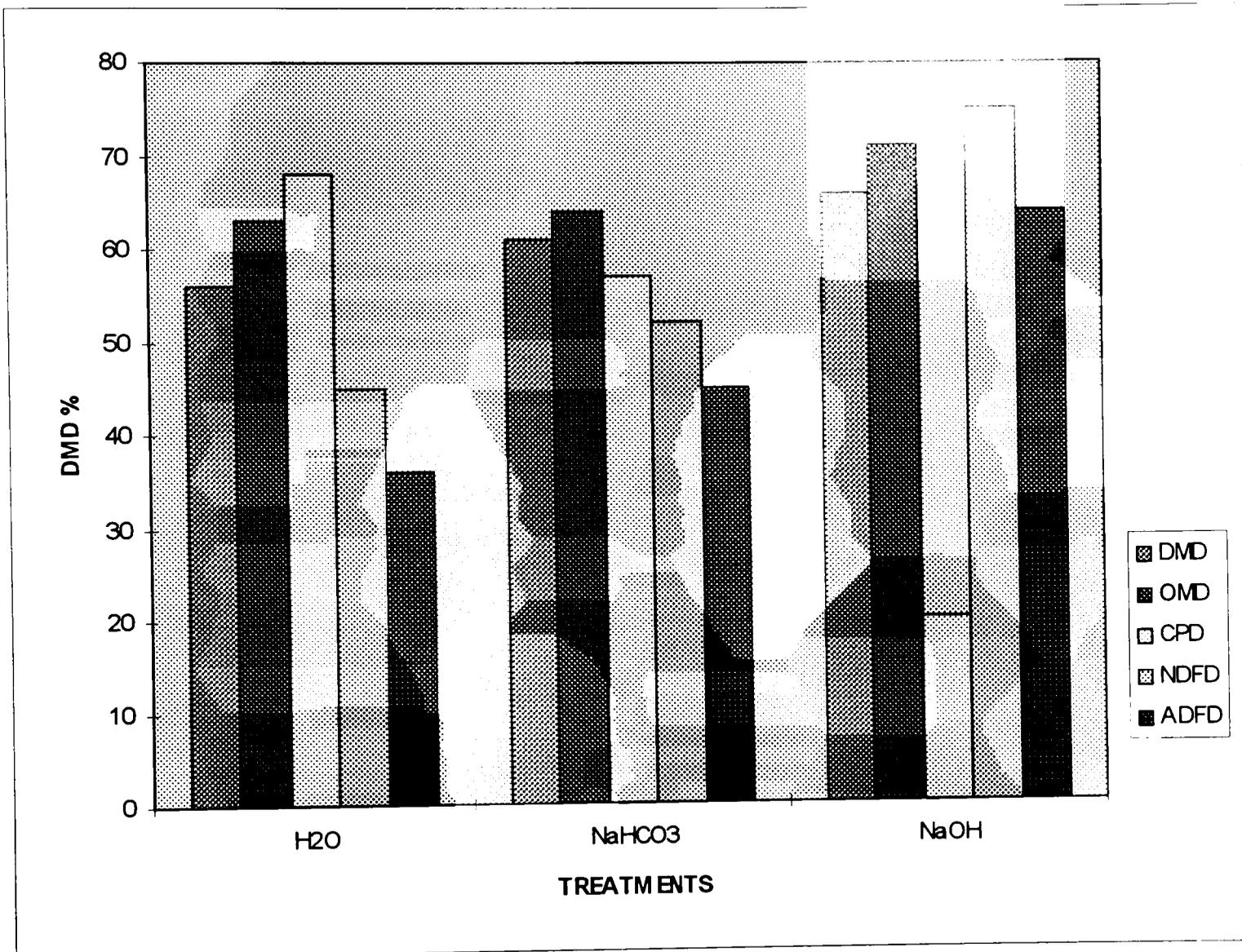


Figure 5. In vivo digestibility parameters of wheat straw as affected by treatment with H₂O, NaHCO₃ and NaOH (without adjusting for DMI).

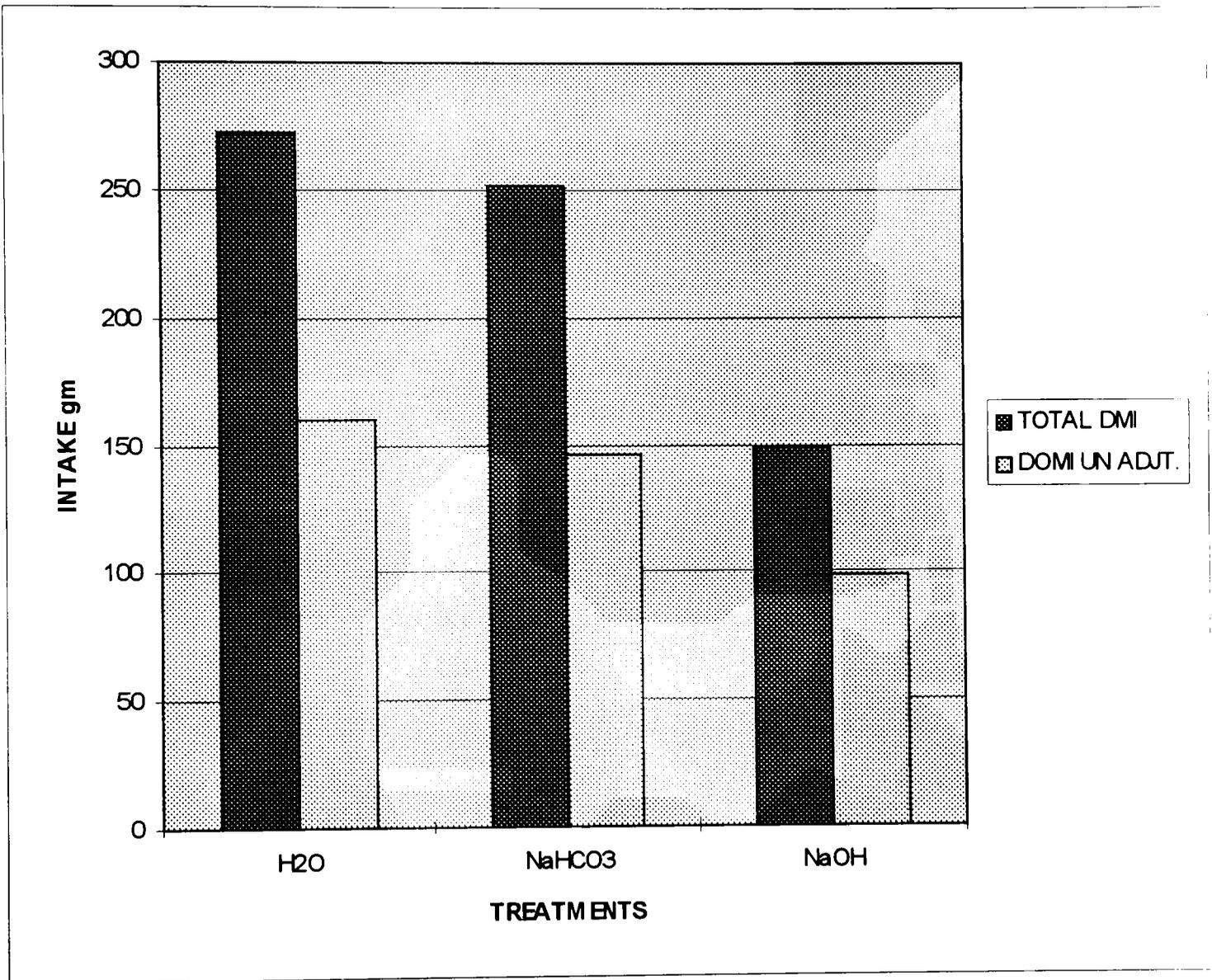


Figure 6. Comparison of DMI and DOMI across treatments of wheat straw with H₂O, NaHCO₃ and NaOH fed to wether lambs during a digestion study.

Other notations

It is important to note here that the first run of the in vitro experiments lacked a blank at the end of the fermentation period (16 hours); this value was estimated by calculating the regression coefficient between wheat straw level and residual DM and extrapolating to zero straw addition (Table 12). The value calculated as the blank estimate of the other 2 runs was surprisingly similar to the ones of the actual blanks.

The 16 hour fermentation period was selected for the in vitro experiments since the estimated maximum rate of microbial digestion of the straw fiber would occur near this time beyond which there may not be significant differences in digestion.

Table 12. In vitro tubes blanks reading for time zero and time sixteen during digestion and the calculated regression coefficient for run one at time sixteen.

RUN	Time 0 blanks	Time16 blanks	*INT. EST.
1	0.1041	No blanks	0.0530
2	0.1640	0.1760	0.1645
3	0.1255	0.1167	0.1136

*regression coefficient estimate at 16 hours for run 1, 2 and 3 blanks.

Summary and Implications

In the in vitro results, there were no significant differences between 10.6% Na₂CO₃, 8.4% and 12.6% NaHCO₃ and 4% NaOH but the 15.5% Na₂CO₃ had higher DMD than all other treatments. This level of Na₂CO₃ was thought to be too high and bulky to be used for LQR treatment during the in vivo trial. Therefore, NaHCO₃ at 8.4% seemed a better choice for use in the in vivo digestion study with sheep. It was decided upon based on similar results in DMD among the four out of six treatments.

Dry matter intake and DOMI were highest in the control treatment with the NaOH having the lowest values in the in vivo experiment. The NaHCO₃ treatment also had a similarly high intake. This information showing NaHCO₃ having a high DOMI is important for its potential as a treatment agent for LQR because of relatively high DMI, DMD and cell wall breakdown. Unlike NaOH, the NaHCO₃ treatment showed high palatability by the lambs. The in vivo experimental results show OM and DM digestibilities were significantly increased with 4% NaOH and the 8.4% NaHCO₃ and these two treatments did not differ significantly statistically. The OMD, NDFD and ADFD reported in this thesis with NaOH treatment agree with reports by other authors (Arndt 1980; Said 1981; Westgard 1981; Haddard 1994). However, Kristensen (1981) and Arndt (1980) both agree on the health risk for the livestock using a diet treated with NaOH because of the effects of high sodium levels, which interfere with the metabolism of other minerals and alter the acid-base balance. There is also the possibility that

NaOH may interfere with protein digestibility as shown in the results discussed here (Figure 4) and conclusions by McNiven (1995) that NaOH treated rolled barley resulted in decreased amino acids in the rumen and lowered digestion of N in the small intestines. The results on 8.4% NaHCO₃ showed effectiveness in DMD, NDFD and ADFD to be relatively higher than the control. More studies need to be done with a larger number of animals, longer straw treatment periods and more levels of the carbonates for more conclusive comparison between carbonates and NaOH.

In conclusion NaHCO₃ and Na₂CO₃ results show potential use in treatment of LQR. These carbonates are more commonly found and easier to handle than NaOH. NaHCO₃ treatment resulted in very high DOMI compared to NaOH. The low palatability of LQR treated with NaOH impairs DOMI even considering its positive effects on digestibility.

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