MAIZE SILAGE

A study of the nutritive value of ensiled ZEA MAYS L.
for growth in young cattle

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ABSTRACT

An experiment is described in which the nutritive value of New Zealand produced maize silage, harvested at the dent-glaze stage of maturity, was investigated. Of central interest in the study was the adequacy of the protein content of the maize silage for supporting growth in young cattle.

Following a six-week standardisation period, 15 Friesian steers, eight months of age, were each allocated to one of three treatments in an experiment of randomised block, covariance design. While maize silage either alone (MS) or supplemented with biuret (MSB), formed two of the treatments, it was considered necessary to include a ration of better known nutritive value as a form of control. Hay and barley meal (HB) was selected for this purpose.

During a six-week comparison period, ration physical and chemical analyses were made; dry matter (DM) and component digestibilities and intakes, nitrogen (N) utilisation, and liveweight gain by the steers were measured. Identical recordings were made during the standardisation period for use in covariance analyses. Throughout the study emphasis was placed on animal performance (liveweight gain) as the prime criterion of nutritive value, the other parameters measured being considered to characterise that nutritive value as components of it.

Mean rates of liveweight gain during the comparison period were similar for all three groups (0.44, 0.52 and 0.51 kg/day for the HB, MS and MSB treatments respectively). Liveweight gains adjusted for DM intake, however, significantly favoured the MSB treatment in comparison with the HB ($P < 0.05$) and MS ($P < 0.10$) treatments.

The possibility that this superior efficiency of the MSB treatment was a result of unaccounted bias is discussed. Notwithstanding the reasonably convincing evidence presented in favour of the reliability of the finding, the possibility that it was merely an artifact could not be unequivocally excluded.

The apparent equivalence of the MSB treatment to, if not superiority over the HB treatment, was difficult to reconcile with the recorded DM digestibilities and digestible energy intakes (62, 14.7 megacals./day and 67, 18.1 megacals./day respectively, for the MS and HB treatments). Enhanced efficiency of utilisation of metabolisable

energy mediated via increased post-ruminal digestion is suggested as a possible explanation for this unusual finding.

If the efficiency advantage of the MSB over the MS treatment was truly a nutritional effect, it can only be ascribed to the presence of biuret in the former ration. While evidence presented would suggest some utilisation of the supplemental biuret by the rumen microbes, the extent is clearly indicated by the N retention results to be limited.

It was found that New Zealand produced maize silage, harvested at the dent-glaze stage of maturity (33% DM) had physical and chemical compositions closely approximating those of the American produced material. When fed as a sole ration to young growing cattle, levels of DM intake (2.6-2.7% of liveweight) potentially conducive to rapid rates of liveweight gain (i.e. greater than 0.75 kg/day) may be expected. The present results would however suggest that the digestibility of the gross energy (61%) may impose a ceiling of 0.75 kg/day on potential rates of gain. The possibility of bias in the estimation of both intakes and digestibilities, resulting from the use of the oven-drying technique in determining silage DM contents, is discussed. It would seem that both the DM digestibilities and digestible energy intakes recorded in the present study, may be consistently low.

It is concluded that maize silage with a 9-10% crude protein content can support growth rates in young cattle of 0.5 kg/day, at least in the short term. With the intake and digestibility data recorded in the study described being of a level sufficient to support greater rates of liveweight gain, it would seem reasonable to suggest, assuming no vitamin or mineral deficiencies, that protein availability to the steers fed maize silage was limiting growth rate. The present study does support a response to N supplementation (biuret) although the finding is adopted with caution. The evidence is inconclusive.

It is therefore suggested that the need exists for a further more specific study of the adequacy of the protein content of maize silage for growth in young cattle. A longer period of maize silage feeding, and the inclusion of a treatment containing a natural protein supplement would be considered essential.

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PREFACE

Progress in pasture management has resulted in more efficient utilisation of available grass by the grazing animal and concomitantly, increased production of milk, meat and wool. The point has now been reached, however, where the amount of grass actually grown is limiting further increases in animal productivity. Possible means of lifting this annual herbage production would include the use of nitrogen fertilizer, irrigation, or high yield forage crops.

While the use of both nitrogen and irrigation on pastures can be clearly beneficial, their potential seems limited by the very morphology and physiology of the pasture plant. Without the limitations imposed by the grazing animal, the use of high yield forage crops may have the greater productive potential.

Maize (Zea mays L.) is one of the most productive forage crops known to New Zealand and when used as silage, provides an animal feed at quite low cost, comparable with grass silage. Its characterisation in New Zealand however, is still in initial stages.

While extensive work on maize silage has been reported in the United States, extrapolation of their findings to our vastly different conditions is difficult. Emerging from their work, however, with singular clarity, is the confused understanding of the protein adequacy of maize silage for growing animals.

The study described herein was designed to investigate the nutritive value of New Zealand produced maize silage as a sole ration for young growing cattle, with emphasis on the adequacy of its protein content.

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CHAPTER ONE

REVIEW OF LITERATURE

An extensive literature on the nutritive value of maize silage has evolved from the many years of its use as a livestock feed in the United States. The present review is in no way considered to be exhaustive. Rather, it outlines the characteristics of maize silage, with discussion centred on the state of knowledge which led to the present study. Reasons for various decisions made in the design of the experiment are offered, and where possible, techniques used and procedures followed are discussed and justified.

Where useful evidence is reported from experiments with lactating cows, it is used in this review, despite the confinement of the present study to cattle growth. However, relevant information available since the completion of this work will be discussed in relation to the results in Chapter Four.

The review is approached in six sections, the first dealing with the physical and chemical characteristics of maize silage, to provide a background against which to compare the New Zealand produced silage used in the present study. The second section considers the state of knowledge of the feeding value of maize silage, highlighting the confusion surrounding its protein adequacy for growing cattle. The factors affecting that feeding value are discussed in section three, where reasons for many of the managerial decisions involved in the production of the maize silage are provided.

In planning this work it was necessary to decide whether to feed maize silage alone, and if fed alone, what deficiencies might be expected. Consideration is given, in section four, to the central question of protein supplementation, justification being provided for the use of non-protein nitrogen to examine this question in the present study. Possible requirements for supplementary energy, vitamins and minerals are also discussed.

The evidence involved in the choice of both experimental design and the procedures followed in the measurement of growth and nitrogen balance, is discussed in section five; the final section, six, providing a brief summary of the review.

1.1 CHARACTERISTICS OF MAIZE SILAGE

In this section the physical and chemical characteristics of maize silage are summarized. The influence on these characteristics, of varying production conditions, including variety and cultural aspects, is discussed in section 1.3.

1.1.1 Physical Composition

As far back as 1925, Hopper reported from a study of 18 maize varieties in the United States, that ear content as a percentage of total dry matter (DM) averaged 53% at maturity. Since that time, however, the advent of higher grain yielding hybrids has increased the ear content of mature maize. In a comprehensive analysis of the growth stages of hybrid maize, Hanway (1963) reported an ear content figure of 63% (of DM) for physiologically mature maize plants, while Bryant and Blaser (1968) returned a figure of 75% of the DM for an early maturing hybrid.

Maize silage is generally harvested a little before physiological maturity at 30-35% DM, and a complete physical analysis of hybrid maize (120 day maturity) at the dent-glaze stage of maturity (30-35% DM content) is presented in Table 1.1.

TABLE 1.1: Physical analysis of maize plants at ensiling (30-35% DM content)

Plant Component	Percentage of Total Shoot DM*	
Grain	47	
Cob, silks	9	Ear = 63%
Husk, shank	7	
Stalk	18	
Leaf sheaths	6	
Leaves	13	Leaves = 19%

* Based on the data of Hanway (1963). Supporting data contained in Loomis (1937), Johnson et al (1966a) and Bryant and Blaser (1968).

Figure 1.1 shows, in generalized form, the changes in the proportions of these components with advancing plant maturity.

1.1.2 Chemical Composition

A summary of analyses of the proximate composition of maize

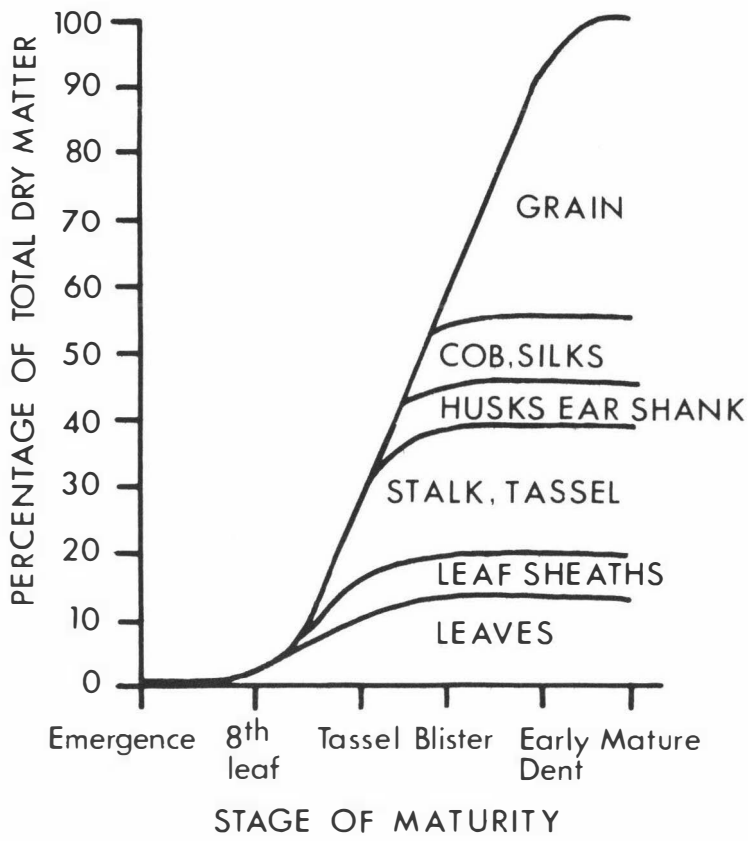

FIGURE 1.1: Changes in physical composition of hybrid maize plants (120 day maturity) with advancing maturity (Based on Harway, 1963).

silage made at the dent stage of maturity is presented in Table 1.2. For comparative purposes, the results of analyses of New Zealand, spring grown, mixed pasture are presented (see Davey, 1964).

TABLE 1.2: Proximate composition of maize silage made at the dent stage of maturity (approx. 30% DM). The pasture analysis results of Davey (1964) are included for comparison.

Source of Data		Morrison 1957*	NRC 1970	Huffman & Duncan 1960**	Bryant 1970***	Davey 1964****
Dry Matter Content		28.5	27.9	29.1	38.0	17.2
Crude Protein	DM Basis	8.1	8.4	9.4	8.1	15.2
Ash		5.6	-	5.0	4.7	9.4
Ether Extract		3.2	-	3.4	-	4.3
Crude Fibre		22.1	26.3	21.3	-	29.3
NFE		61.1	-	60.8	-	41.9

* Average of 237 separate analyses.

** Average of 17 years' crops at Michigan State University.

*** New Zealand produced maize silage.

**** Mixed New Zealand ryegrass-clover spring pasture.

Scrutiny of Table 1.2 reveals two characteristics of maize silage when compared with pasture. Both its crude protein and ash contents are considerably lower. These characteristics are discussed later in the review (see sections 1.4.1 and 1.4.4). However, further analyses of the crude protein and ash contents of maize silage are presented in Table 1.3.

In comparing the mineral contents of maize silage and grass (see Table 1.3), the maize silage appears similar in magnesium and possibly sodium, but low in calcium, phosphorus and potassium. Consideration of the NRC (1970) requirements for these minerals by growing steers, reveals that while the potassium content may be lower than in grass, it is still far in excess of estimated requirements. However, calcium and phosphorus contents of maize silage may be inadequate for rapid gains of young growing steers (see section 1.4.4).

The changing physical composition of the maize plant with advancing maturity (see figure 1.1) could reasonably be expected to

TABLE 1.3: Further chemical analysis of maize silage - crude protein and ash fractions, and carotene content (% of total maize silage DM). The pasture mineral analyses of Wilson et al (1969) are included for comparison.

Source of Data	Johnson et al (1967)	Morrison (1957)	NRC (1970) [Coppock (1969)][a]	Huffman & Duncan (1960)	Wilson* et al (1969)
True Protein	4.9				
NPN Protein (N x 6.25)	3.8				
Ca	-	0.32	0.28	0.26	0.53
Mg	-	0.18	0.18	0.26	0.22
P	-	0.25	0.21	0.24	0.46
S	-	0.14	0.11[a]	-	-
K	-	0.95	0.95	1.17	3.32
Na	-	0.04	0.03[a]	-	0.11
Carotene		0.005 (46.3ppm)	-	0.002 (18.2ppm)	-

* Analysis of New Zealand mixed spring pasture.

For information on the other sources of data see Table 1.2.

alter chemical composition concurrently. Such changes have been recorded and are presented in Figure 1.2. Clearly the chemical composition of maize silage will depend on stage of maturity at harvest.

1.2 FEEDING VALUE OF MAIZE SILAGE

Typical maize silage harvested at 30-38% DM (i.e. Dough-dent to Glaze stages of maturity) would have a DM digestibility of 68% (Hillman, 1969) and a total digestible nutrient (TDN) content of 70% (Alexander et al, 1963; Colovos et al, 1970), or 67% digestible organic matter (DOM) (DM basis). Assuming a DM intake level of 2.5 kg/100 kg live weight (11.35 kg DM for 454 kg animal), sufficient energy would be furnished (7.95 kg TDN) to meet the maintenance requirements of the 454 kg cow (3.18 kg TDN) plus 15 kg of 4% fat corrected milk (FCM) (@ 0.31 kg TDN/kg 4% FCM). With a digestible crude protein value of 5.7% (DM basis) (Coppock, 1969), the maintenance protein requirement could be adequately met (0.27 kg), leaving 0.38 kg digestible crude

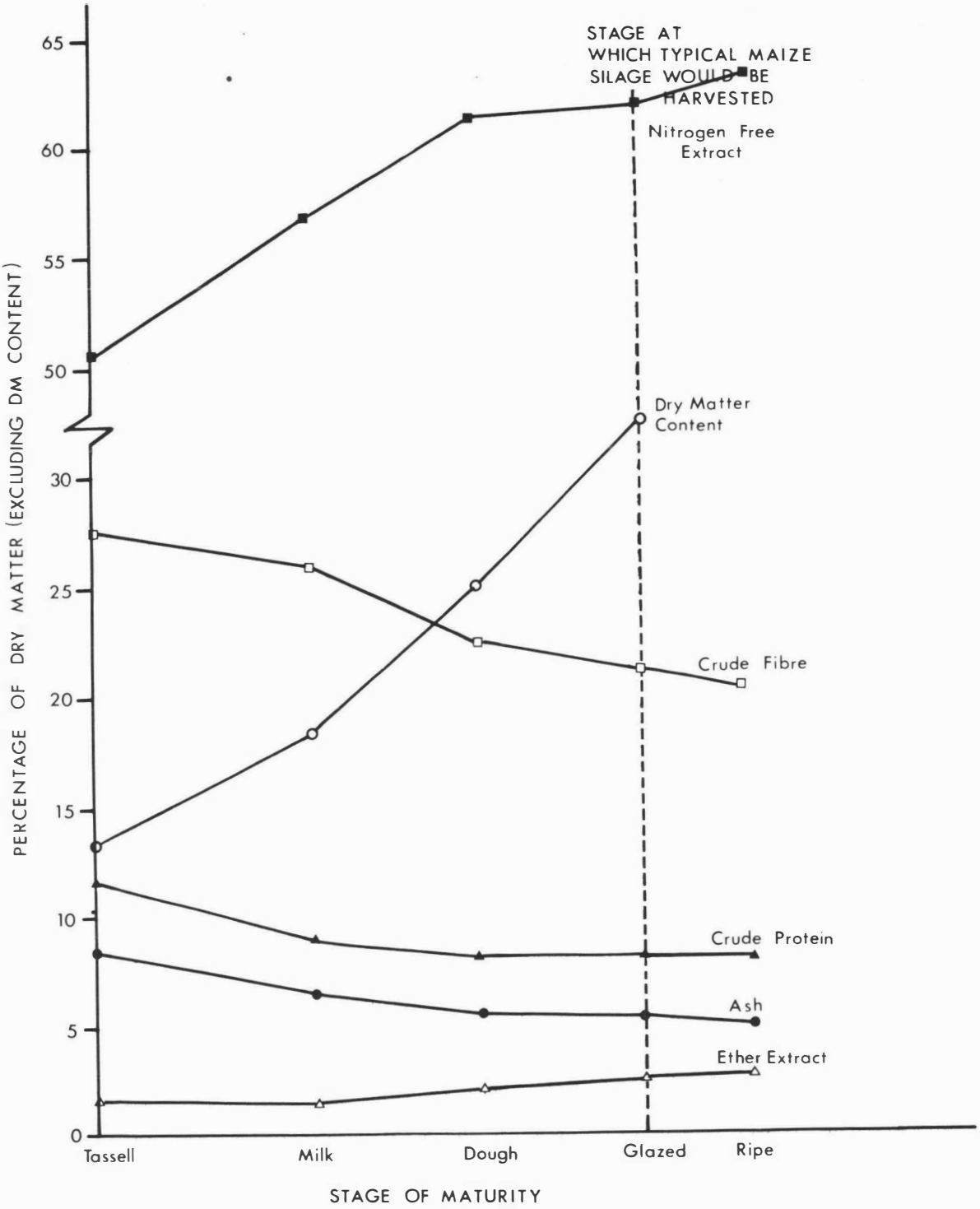

FIGURE 1.2: Changes in Chemical composition of the maize plant with advancing maturity (Adapted from Hopper, 1925)

protein, sufficient to produce only 9 kg 4% FCM. (All requirements based on Morrison, 1957).

While the limitations of this feeding standard approach are freely acknowledged, the calculations do demonstrate that although maize silage is energetically capable of supporting reasonably high levels of milk production, it is clearly deficient in protein for this purpose.

For the growing steer, similar figures are presented in Table 1.4.

TABLE 1.4: Energy and protein adequacy of maize silage for growing cattle

Liveweight	Requirements*		Intakes**			
	TDN	Dig.Cr.Prot.	TDN		Dig.Cr. Prot.	
kg	kg	kg	kg	% of requirement	kg	% of requirement
136	1.77	0.24	2.38	134	0.19	79
181	2.18	0.29	3.17	145	0.26	90
227	2.59	0.32	3.98	154	0.32	100
272	2.95	0.36	4.76	161	0.39	108
318	3.63	0.38	5.57	153	0.45	118
363	3.99	0.41	6.36	159	0.52	127

* Based on Morrison (1957) to promote gains of 0.34 kg/day (0.75 lb/day)

** Assumed DM intake of 2.5 kg/100 kg liveweight, maize silage TDN value of 70% and digestible crude protein (Dig.Cr.Prot.) value of 5.7% (DM basis).

Consideration of table 1.4 would indicate that maize silage may supply considerably more energy to the growing steer than is required for a growth rate of 0.34 kg/day. However, up to 227 kg liveweight it seems possible that growing cattle may be incapable of ingesting sufficient protein from maize silage to gain at this rate. Above 227 kg liveweight, sufficient digestible crude protein may be furnished for faster rates of growth. However, the writer considers that the assumed digestible crude protein content of maize silage (5.7% of DM) taken from the review of Coppock (1969), may be generous, values as low as 4% being reported in the literature (Morrison, 1957; Conrad and Hibbs, 1961; Colovos et al, 1970).

While for milk production maize silage is undoubtedly deficient in protein, for the growing steer the situation seems less clear. The

question is further investigated in the section on supplementation of maize silage (see 1.4.1).

1.3 FACTORS AFFECTING THE FEEDING VALUE OF MAIZE SILAGE

A number of factors involved in the production of maize silage are known to affect its nutritive value. These would include fertilizer rates and plant population density, maize variety, stage of maturity and DM content at harvest, and additives used at ensiling. Knowledge of the extent and direction of these influences is briefly reviewed and reasons are given for various procedures followed in the production of the present maize silage. Consideration is also given briefly to silage yield where it may also be affected by these factors.

1.3.1 Fertilizer Rate

Nitrogenous fertilizer levels in particular have received the attention of researchers, not only through the possibility of increased DM yields, but also because of postulated influences on the crude protein content and nutritive value of resultant silage.

The literature is not all in accord. Zimmerman et al (1962) using either no nitrogen (N) fertilizer or 247 kg N/ha failed to alter either the silage DM yield or its crude protein content, although yearling steers did inexplicably gain better on the high N fertilizer silage. In contrast, Cummings et al (1965) with three levels of N fertilizer (0, 225 and 900 kg/ha) increased the crude protein level of resultant silages 0, 18 and 39% respectively from 6.19 to 7.28 to 8.58% of DM. Information on animal response to these particular silages was sparse and confounded by protein supplement feeding. Vandersall et al (1962) however, using levels of N fertilizer similar to the first two of Cummings et al (1965), reported more efficient milk production from the high-N silage, despite supplemental protein feeding.

Some of the most comprehensive evidence available comes from the work of Alexander et al (1963). By doubling NPK fertilizer rates (62-54-54 to 125-108-108 kg NPK/ha) 33% and 11% increases in DM yield and crude protein content respectively, were recorded, the latter being increased from 6.3% to 7%. Nitrogen free extract (NFE) and ash contents were decreased, while crude fibre and ether extract levels remained unchanged. The high fertilizer level also increased the digestibility

of the crude protein causing the digestible crude protein content to be increased by 21% (2.94 to 3.57% of DM), a result very much in keeping with the work of Jahn (1964) who recorded a 16% increase (5.5 to 6.4% of DM) in digestible crude protein content, using similar levels of fertilizer.

The weight of evidence favours beneficial effects from high N fertilization not only in terms of DM yield, but also in terms of crude protein content and digestibility, an important consideration in the production of maize silage (see section 1.2). For these reasons 250 kg N/ha was used in the production of the maize silage for this nutritive value study.

At such high levels of N fertilization, silage nitrate levels may be elevated (Zimmerman et al, 1962; Cummings et al, 1965; Owen, 1967), as in drought conditions (Loomis, 1937), the significance of this toxic substance being discussed in section 1.4.3.

1.3.2 Plant Population Density

It is known from agronomic studies that as plant population density is increased, total DM yields generally rise until plant competition prevents further increases and may even effect a yield decrease (Stafijcuk, 1962). The literature contains reports of maize crops grown at population densities ranging from 16,700 to 111,000 plants/ha (Dzinic, 1960; Alexander et al, 1963; Bryant and Blaser, 1968; Hunter et al, 1970). Dry matter yields increased up to the 98,000 plants/ha of Bryant and Blaser (1968), and in the work of Hunter et al (1970), over a range of 48,000 to 72,000 plants/ha, yield response was linear. In this latter study, the leaf area index (LAI) at 72,000 plants/ha (2.9) was below that considered by the authors to be necessary to give maximum grain yield (3.5-4.0). It was suggested that 96,000 plants/ha would be required to achieve this desired LAI. On the basis of the work of Bryant and Blaser (1968) this figure appears not to be too high.

In recent reviews, Owen (1967) and Hillman (1969) suggested that increasing plant population would result not only in decreased crude protein content, but also in decreased crude protein and dry matter digestibility. Supporting evidence for this contention can be found in the results of Stafijcuk (1962) and Alexander et al (1963). However,

the results of the latter group also show that these effects are substantially reduced with high levels of nitrogen fertilization.

In summary, it could be considered undesirable for plant population to be so high as to reduce the grain content of the resultant silage. Bryant and Blaser (1968) found no evidence of this occurring in plant populations up to 98,000 plants/ha, although high plant density was possibly responsible for the poorer silages made from stands of 111,000 plants/ha in the work of Dzinic (1960). It is therefore likely that any small losses in digestible nutrient content with increasing plant population will be more than compensated for by increased digestible nutrient yield at the higher plant populations (Stafijcuk, 1962; Alexander et al, 1963).

In the present study the moderately high sowing density of 80,000 seeds/ha was chosen so as to achieve near maximum yields without risk of prejudicing silage quality.

1.3.3 Maize Variety

For some time maize varieties used for grain production have been considered most desirable for silage making because of the high net energy content for productive purposes of the grain in comparison with the stover (Morrison, 1957). In his 1967 review, however, Owen questioned this premise and cited evidence from work comparing the value of extremely high grain, dwarf maize varieties with normal hybrids for milk production. There were no differences. The writer, however, criticises this evidence as differences other than grain content between two such diverse varieties could have been influencing the milk production results.

The ready passage of shelled corn undigested through the alimentary tract of ruminants (e.g. 23% - Becker and Gallup, 1929) may have lead to a belief that the same waste would occur with grain consumed in maize silage. However, this has not been the case, and both Becker and Gallup(1929) and Huffman and Duncan (1959) recorded undigested grain losses of 8.47% and 2.7% for silage harvested at the glaze and early dent stages of maturity, respectively.

Nutritive value studies with silages of differing grain contents have shown varying animal responses. However, Hayden and Perkins (1923) and Huffman and Duncan (1956), comparing silages with

29 and 39%, and 5 and 47% grain content (DM basis) for milk production, reported small but consistent advantages in favour of the higher grain varieties. Moreover, Stafijcuk (1962) found a high positive correlation between the proportion of heads in the dry matter and the nutritive value of the silage, and Nevens (1933) reported higher DM intakes, liveweight gains, and efficiencies of gain for growing cattle on maize silage with higher grain content (47 vs 35% ear). Also producing evidence in support of high grain content maize silage, Huber et al (1965) compared three silages with ear contents of 34, 47 and 51% (DM basis). Milk production increased with increasing ear content of the silage, as did overall DM content (25 to 33% DM). In this study as in that of Huffman and Duncan (1956) silage dry matter content could have confounded results.

This criticism cannot be levelled at the work of Dunn et al (1955) who made normal and grainless maize silage from the one crop. When grainless maize silage replaced approximately half the hay in a ration fed to milking cows, milk production was maintained, but when maize meal replaced the grainless maize silage to a similar level of TDN intake, milk production increased. Moreover, the grain in the normal maize silage was found to have equal productive value to the maize meal, clearly demonstrating its nutritional importance in maize silage.

This evidence led to the selection of a high grain maize variety for the present study, the three-way hybrid, Wisconsin 575.

1.3.4 Stage of Maturity and Dry Matter Content at Harvest

Stafijcuk (1962) reported a high positive correlation between maize plant maturity and DM content of the crop. It therefore seems appropriate to consider these two factors together.

As has been established in section 1.3.3, the presence of large quantitites of grain in the silage is likely to add to its feeding value. By definition, the greatest yield of grain occurs at physiological maturity about 35% DM (Coppock and Stone, 1965), when in fact, as can be seen in figure 1.1, overall DM yield is likely to be at a maximum (Hanway, 1963; Johnson et al, 1966a). The importance of not harvesting too early, in terms of DM yield, is demonstrated by Nevens et al (1954). In 25 days from the early milk stage of maturity, DM yields increased by up to 63%. Once DM content passes 35%, however, total DM yields are

are likely to decrease (Johnson et al, 1966a).

As can be seen in figure 1.2, from tassel to physiological maturity the chemical composition of the maize plant alters considerably. While NFE and ether extract contents increase, ash, crude fibre, crude protein, and also carotene contents all decrease. Evidence supporting this early work (Hopper, 1925) has been reported by Huffman and Duncan (1960), Huber et al (1965), Owen (1967), Owens et al (1967) and Johnson and McClure (1968).

Also changing with advancing maturity is the composition of the crude protein fraction itself. From a comprehensive study of the effects of maize plant maturity, Johnson et al (1967) showed that the percentage of total N as true protein increased with advancing maturity from the blister to just prior to the frost stage (i.e. up to 44% DM), (see figure 1.3), tending to diminish the effect of the decreasing crude protein content. Moreover, the losses of true protein during ensiling were considerably less at more advanced maturities. This is probably a reflection of the reduced fermentation activity with more mature silage, lactic and acetic acid production, especially the latter, being reduced (Johnson et al, 1967).

At the same time however, the apparent digestibility of the crude protein will decrease (Noller et al, 1963; Johnson and McClure, 1968) and may reduce the digestible crude protein content to low levels at advanced maturities.

It has been suggested that stage of maturity has very little effect on DM digestibility (Nehring and Laube, 1959; Owen, 1967; Coppock, 1969; Hillman, 1969). From in vitro digestibility studies, Johnson et al (1966b) have shown that while leaf cellulose digestibility may decrease slowly with maturity, stalk cellulose digestibility decreases until about 15 days post-tassel, with little change thereafter. From this point on, vegetative growth probably ceases and grain development is the major process taking place (see figure 1.1). In fact, the rapid deposition of starch in the developing grain, with concurrent dilution of the plant crude fibre content, forms a basic difference between grain crops, and pasture grasses and legumes, precluding the likelihood of marked decreases in DM digestibility with advancing maturity of maize.

Available evidence would support this contention and the results of studies on the DM digestibility of silages made at varying maturities

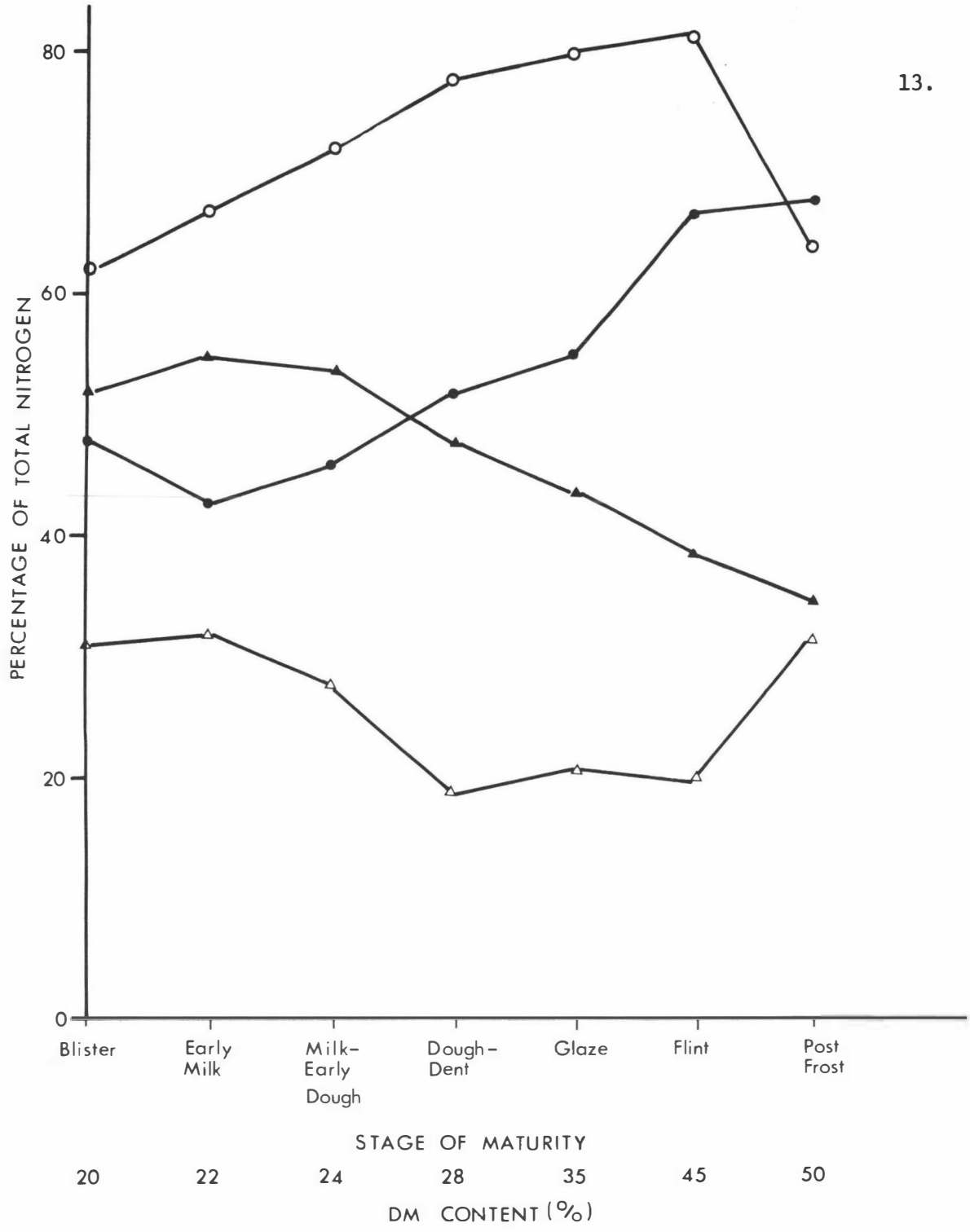

FIGURE 1.3: Effect of maturity on N distribution in maize plants and maize silage (Adapted from Johnson et al, 1967)

O true protein in plants ● true protein in silage

▲ NPN in silage △ NPN in plants

are summarized in table 1.5.

TABLE 1.5: The effect of stage of maturity at harvest on the DM digestibility of maize silage.

Stage of Maturity	Blister	Early Milk	Milk-Early Dough	Dough-Dent	Glaze	Flint	Very Mature
DM %	20	20-24	25-27	28-30	33-35	38-47	47+
Johnson & McClure (1968)	67.3	67.6	70.3	71.2	68.2	68.2	68.8
Colovos et al (1970)			68.2	71.1	68.8	66.7	
Noller et al (1963)			72.3	69.7	68.7		
Huber et al (1965)			68.2	68.4	68.0		
Bryant et al (1965)		66.7		68.6			
Byers & Ormiston (1964)				62.7			56.7

As has been suggested, the variations in digestibility post-blister and pre-frost appear small. The published data would suggest DM digestibility to be approximately two percentage units higher from the milk- early dough to dough-dent stages (25-30% DM), than at earlier or later stages of maturity.

In contrast to this insubstantial influence of maturity on digestibility, Dzinic (1960) found that silages made at earlier maturities (milk stage) were less readily eaten and of lower nutritive value than those made later. Evidence in support of this contention is strong. Over a range of silage DM contents, 21 to 73% DM, Huber et al (1965), Bryant et al (1965), and Owens et al (1967) reported increasing DM intakes and milk production with increasing DM content. Only one of these studies, however, contained silages over 40% DM content. Silages in the range 54-73% DM content, in the work of Owens et al (1967), were ground prior to ensiling, this procedure being considered by the writer to cast doubt on the validity of the results. Moreover, Huber et al (1967) reported that silage with a DM content of 45% was less well consumed and produced less milk than those in the range 30-37%. Similar evidence of poorer intakes with very high DM silages is reported by Noller et al (1963); Colovos et al (1970) supporting the finding of Johnson and McClure (1968) that DM intakes appear to be highest at the glaze stage of maturity (35% DM). In the last mentioned

work silages ranged from 20 to 71% DM content.

While it is known that increasing the DM content of ensiled grass will improve DM intakes by ruminants (Moore et al, 1960; Neumark et al, 1964; Harris et al, 1966), the beneficial effect of advancing maize plant maturity (up to the glaze stage) may not be mediated solely through this factor. In their work on maize silage maturity, Huber et al (1965) noted that the productive advantage of the more mature silage (33.3% DM compared with 25.4 and 30.3% DM) was diminished in the presence of a grain supplement, implicating the value of the extra grain (see section 1.3.3) with the more mature silage. There seems little doubt that both factors, DM content per se and the value of higher grain content are involved.

It should also be noted that at the extremely late stages of maturity when DM contents are very high (over 40% DM), Byers and Ormiston (1964) and Owens et al (1967) have shown that harvesting losses caused by collapsing plants can be much elevated (25% compared with 5% of total DM). Moreover, Huber et al (1967) reported losses in the silo to be over 100% greater for the very mature silages, and at the other extreme, Miller and Clifton (1965) showed seepage losses to start at less than 30% DM. Yet another source of nutrient loss which may increase with maturity is the expulsion of undigested grain in the faeces. It seems, however, that this increase in loss with maturity is small (Becker and Gallup, 1929; Huffman and Duncan, 1959).

The evidence would indicate that harvesting maize for silage at or near the mature seed stage (dent to glaze stage of maturity, 30-35% DM), when the grain consitutes 45-55% of the DM, will maximise both grain and overall DM production and minimise DM losses. Levels of DM intake are likely to be highest at this stage of maturity, and despite a slight reduction in DM digestibility, animal productivity is likely to be maximised provided the digestible crude protein content is not limiting. On the basis of these conclusions, the maize silage used in the present study was harvested at the dent-glaze stage of maturity, 30-35% DM content.

1.3.5 Buffers and Other Additives

Klosterman et al (1960, 1961) showed that maize silage treated with limestone and urea additives at ensiling, supported more efficient

and frequently faster gains in fattening cattle, when compared with untreated maize silage. Protein supplementation of both rations precluded the possibility of an N effect per se. The lactic acid content of the limestone-urea silage had been increased 78% (DM basis) by the treatment. It was concluded that the higher feeding value of the treated silage was due to its higher organic acid content. Not all evidence is in accord.

The buffing effects of limestone, Ca SO_4 and urea in increasing silage organic acid content, have been widely reported (Byers et al, 1964; Simkins et al, 1965; Cummings et al, 1966; Johnson et al, 1967; Johnson and McClure, 1968; Condon et al, 1969; Schaadt and Johnson, 1969). However, it is questionable whether this is entirely desirable. While Schaadt and Johnson (1969) have shown increased proportions of propionic acid in the rumens of sheep fed limestone treated silage, and have implicated this in increased growth efficiency, Johnson et al (1967) demonstrated concurrent reductions in the true protein content of silages treated in this way. Nitrate content of silages may also be reduced with limestone treatment (Cummings et al, 1966), but the writer considers in contrast to the authors cited, that this may be of no real benefit. It will be shown in section 1.4.3 that nitrate levels in normal maize silage are nutritionally of little moment, while its reduction to NO_2 and other nitrogen oxide gases in the silo, aided by limestone treatment, may totally destroy the carotene present. In attempts to prevent this occurrence Meiske et al (1963, 1965) added sodium bisulphite preservative to high nitrate silages and successfully raised silage, plasma and liver carotene levels.

Both Kesler et al (1964) and Simkins et al (1965) have reported reduced intakes of animals fed limestone treated silages. This is not entirely surprising in light of the intake depressing effects of high levels of lactic acid in silages reported by Emery et al (1961), Harris et al (1966), McCullough (1966) and Walker (1968b) and contradicts the reported higher intakes of Klosterman et al (1961). Also contradicting the reports of Klosterman et al (1960, 1961) is the work of Nicholson and Cunningham (1964). They reported decreased DM intakes, liveweight gains and efficiencies of gain when yearling heifers were fed limestone treated maize silage.

In view of the inconclusive nature of the evidence available on

the value of buffer addition at ensiling, it was decided not to use any such material in the production of silage for the present study. It is acknowledged however, that limestone, $CaSO_4$ and urea additives would provide useful sources of N, Ca and S, elements which will be established in sections 1.4.1 and 1.4.4 to be of marginal adequacy in maize silage.

Finally, the use of enzyme additives such as cellulase, to render the less digestible fractions more digestible during ensilage, is an interesting concept. Cellulase additions, however, have resulted in no appreciable degradation of the crude fibre fraction (Owen, 1967).

1.4 SUPPLEMENTATION OF MAIZE SILAGE

Requirements for protein, energy, vitamin and mineral supplementation will be considered in this section. It is necessary, however, to first ascertain whether, given any of the listed supplements, maize silage can be fed alone or whether some less readily identifiable factors are required in the ration.

The use of maize silage as a major feed component over an extended period of time appears to have no deleterious effects. Porter (1950) raised heifers from birth through two lactations, and Converse and Wiseman (1952) took cows through to the third generation, milking some for eight years on maize silage as the only roughage. Protein rich concentrates were fed and a marginal carotene deficiency was the only problem reported. Brown et al (1966) also milked cows for two lactations on maize silage as the only roughage, milk production remaining extremely high and averaging 23-24 kg/day.

Waugh et al (1955) found improved DM intakes and milk production when lucerne hay replaced part of the maize silage in a milking ration. Moreover, Bryant (1970) reported slightly improved gains and intakes with increasing quantities of grass fed with maize silage. However, in both these sets of work, the hay or grass provided the only or major source of protein supplementation, confounding the general effect of roughage supplementation with specific protein supplementation.

In work where protein concentrates have been fed, hay supplements have consistently increased DM intakes but have either unaltered or decreased milk yields (Rumsey et al, 1963; Brown et al, 1965). Moreover, Huffman and Duncan (1954) reported increasing levels of milk production as maize silage replaced clover hay in a milking ration

at a slightly lower TDN level.

Both the evidence cited and practical applicability of the results, led to a decision to feed maize silage as the only forage in the present study. Consideration is given to specific supplementation requirements in the following sections.

1.4.1 Protein or Nitrogen (N) Supplementation

Reference to table 1.4 shows that maize silage may contain sufficient protein for growth rates greater than 0.34 kg/day depending on the age and body weight of the cattle. In fact, Thomas et al (1967) and Coppock (1969) report growth rates in heifers fed maize silage alone, of 0.83 and 0.87 kg/day, further confusing knowledge on its protein adequacy for growth.

What is clear, however, is that the protein content of maize silage is inadequate for effective utilisation of the energy available. Conrad and Hibbs (1968) found that 5.0 g digestible N was required for efficient utilisation of every megacalorie of DE consumed (or 31 g dig. protein/ megacalorie DE), a lower requirement than was previously thought necessary (38-40 g dig. protein/megacalorie DE). A 200 kg steer consuming 5.0 kg maize silage DM or 3.5 kg TDN (15,500 Kcal DE or 15.5 megacalories), would therefore require 480 g digestible protein for its efficient utilisation. Allowing a digestible crude protein content for maize silage of 5.7%, as used in previous calculations, 285 g, or little more than half the required digestible protein would be ingested. It would appear that the suggestion of Coppock (1969), that maize silage may furnish ample protein for growth, is valid only for sub-optimal levels of gain, highest growth performance in theory requiring protein supplementation.

As can be seen from figure 1.2, maize silage crude protein contents fluctuate according to plant maturity at harvest. The graph shows a crude protein content range from 11.7 to 8.2% over the blister to ripe seed stages of maturity, although during summer-autumn drought it may not fall at all, Pfander et al (1957) and Huffman and Duncan (1960) recording figures of 11.8 and 14.9% respectively for drought injured maize silage at the dent stage of maturity. Also, high protein maize hybrids do exist. Nevens et al (1955) reported an Illinois High Protein variety to have a crude protein content of 11.8% when nearly

mature, although DM yields were poor. Clearly, the magnitude of responses to protein supplementation will vary with the maturity of the maize plant at ensiling, and it is possible that immature silages containing 11% CP may in fact contain adequate protein for rapid growth. Goodrich et al (1961) reported no responses to increased protein levels over 10% of the ration DM with 270 kg steers gaining at 1.1(kg/day. Similarly Armstrong (1968) concludes that growth responses to protein levels over 11.0% are unlikely in cattle over 200 kg liveweight. However, it will be assumed that typical maize silage harvested at the dent stage of maturity will have a crude protein content of 8-9% of the DM.

As feed protein is becoming both scarce and expensive in New Zealand, it was decided to investigate a potentially cheaper form of N, non-protein nitrogen (NPN), as a source of supplemental N for maize silage. While it was realised that good quality pasture may provide a moderately cheap source of supplemental protein, its lack of availability at certain times of the year, and the fact that it would supply nutrients other than protein to the ration, precluded its use. Prior to the feeding of NPN, however, consideration had to be given to published evidence on the ability of cattle to successfully utilise this form of dietary N.

1.4.1.1 Non-Protein Nitrogen (NPN) Utilisation by Ruminants.

While in 1879, Weiske first showed that the ruminant could utilise NPN (Conrad and Hibbs, 1968), it was not until the longer term growth work of Hart et al (1939) that it was unequivocally demonstrated that NPN could be utilised in place of at least part of the dietary protein. In the intervening years, a vast and confused literature has evolved from innumerable feeding trials, and a lack of astute thinking. This mass of evidence has been recently reviewed by Armstrong and Trinder (1966), Armstrong (1968), Chalupa (1968), Conrad and Hibbs (1968), Waldo (1968), Oltgen (1969) and Smith (1969). It is not therefore the writer's intention to review once more the general field so adequately covered by these authors. Emerging from these reviews, however, is the fact that animal performance on NPN has varied considerably, and frequently been inferior to performance on natural protein diets. Explanations for this in the reviews have tended to be inconclusive, and its relevance to the use of NPN in the present study necessitates its further discussion.

It would appear that two major factors are involved. Either the

rumen microbial population is unable to effectively utilise the ammonia released by the rapid hydrolysis of NPN in the rumen (Oltjen et al, 1962; Clifford and Tillman, 1968) or, the microbial protein synthesised in the rumen and digested and absorbed from the lower tract; the major source of amino acids (AA) available to the ruminant on high NPN diets, may have limitations in terms of its amino acid content (Chalupa, 1968; Oltjen, 1969).

The dearth of literature on the essential amino acid requirements of ruminants led the writer to survey, in unpublished work, relevant data on the requirements of non-ruminants, rats, chicks, pigs and humans (ARC, 1963; Allison, 1964; Hegsted, 1964; ARC, 1966), and develop an amino acid requirement pattern considered likely for ruminants. Work by Black et al (1952) demonstrating the uniformity of somatic AA metabolism between ruminants and non-ruminants tended to justify this approach.

Comparison of this pattern with the essential amino acid content of rumen bacterial protein (protozoa disappearing from the rumen with high NPN diets - Virtanen, 1969) recorded in a number of studies (Loosli et al, 1949; Duncan et al, 1953; Walker, 1957; Purser and Buechler, 1965; Schelling et al, 1967), and adjusted for individual amino acid availabilities (Loosli et al, 1949), revealed tryptophan as likely to be first limiting. Blood plasma essential amino acid patterns tended to confirm this finding (Oltjen and Putman, 1966; Schelling et al, 1967; Clifford and Tillman, 1968). While some of the evidence from limiting amino acid studies reported the literature would support this contention (Ellis et al, 1959; Barth et al, 1959; McLaren et al, 1962), apparently contradictory evidence can be found (Bergen et al, 1968; Freitag et al, 1968; Klopfenstein et al, 1966; Purser et al, 1966; Schelling and Hatfield, 1968; and Devlin and Woods, 1965).

While the writer considers the essential amino acid tryptophan to be implicated in the reported inferior performances of ruminants fed NPN, the extreme contradictory nature of the literature would tend to indicate that microbial protein amino acid composition is probably not the major cause of depressed animal performance on NPN. For this reason supplemental amino acids were not considered for the present study.

The other factor implicated in the poor performance of ruminants on NPN was the ability of the rumen microbial population to efficiently

utilise the ammonia released in the rumen. This would depend on the degree of adaptation of the rumen microbes to the unusual feed form, microbial requirements for specific amino acids and carbon skeletons, and the energetic adequacy of the ration.

Adaptation responses to NPN feeding have been investigated by a number of workers and their occurrence confirmed (Smith et al, 1960; Barth et al, 1961; McLaren et al, 1965b; Virtanen, 1966 and 1969). Duration of the response has fluctuated but has been recorded as continuing up to 6 months, clearly precluding the use of change-over designs in experiments utilising NPN. This was one of the reasons why a continuous design was employed in the present study and could well account for depressed animal performance on NPN in some short term work.

The requirement of certain rumen microbes for specific carbon skeletons (e.g. branched chains and amino acids) (Barth et al, 1950; Allison et al, 1962; Gossett et al, 1962; McLaren et al, 1965a; Rook et al, 1965; Cline et al, 1966; Hungate, 1966; Freitag et al, 1968) has also been implicated in disappointing responses to NPN feeding. However, in 1949, Loosli et al unequivocally demonstrated that the ten essential amino acids could be synthesised in the rumen, and it is now clear that symbiotic microbial relationships supply the specific carbon skeletons normally derived from dietary protein (Virtanen, 1966; Ørskov and Oltjen, 1967), provided the carbohydrate or energy fraction of the ration is adequate, both qualitatively and quantitatively (Conrad et al, 1967; Clifford and Tillman, 1968).

One energetic limitation immediately apparent with NPN feeding is that imposed by the condition of anaerobiosis in the rumen. Bauchop and Elsden (1960) showed quite clearly that in anaerobic systems the dry weight of microbial protoplasm synthesised was directly proportional to the amount of ATP produced, and in such systems, only 4 moles of ATP are produced from the fermentation of one molecule of glucose, compared with the 38 moles ATP under aerobic conditions. Even where some protein is included in the ration as in the present study, Bergen et al (1967) and Smith (1969) have suggested that up to 80% of this may be broken down and require resynthesis.

The writer, in unpublished work, has used the technique of Walker (1965, 1968a) and the purified diet data of Virtanen (1966, 1969) and Oltjen and Putnam (1966) to estimate the potential microbial

protein yield per 100 grams of carbohydrate fermented in the rumen. It was calculated to be 18.0 g protein/100 g CHO fermented, a figure very similar to Walker's more recent calculations as reported by Hume and Bird (1970). The in vivo protein synthesis studies by Conrad et al (1967), Hogan and Weston (1967), Hume (1970a, 1970b) and Hume et al (1970) tend to add credence to the accuracy of the writer's estimated potential. From this calculation, and again using the data of Virtanen (1969), it is concluded that while for milking cows the condition of anaerobiosis may well impose a limitation on milk yield at high levels of NPN feeding, for growing animals with their lesser protein requirements this is unlikely.

This, however, is not the only energetic consideration in the utilisation of NPN. Balch (1967) rather elegantly points out that while protein in a diet is limiting, the response to its addition is rectilinear. Once energy becomes limiting, however, the response to added protein diminishes and the relationship becomes curvilinear. The fall in apparent biological value is associated with the use of protein as an energy source. While protein addition to a ration where energy is limiting will in non-extreme cases still give a response, albeit a smaller one, NPN, because it contains little or no available energy, can give no positive response whatsoever. Clearly, experimental work involving isonitrogenous substitution of NPN for protein will not only alter the quality of the dietary nitrogen, but will also alter the shape of the recipients N response curve. It will in fact lower the point, in terms of N addition, at which the dietary energy level becomes limiting. In effect, the potential for production on the ration is automatically lowered, and it seems likely that this factor is responsible for many of the reported poor responses to NPN feeding. In this light it is interesting to note that NPN addition to rations with excess energy (Bond et al, 1962), or where isonitrogenous and isocaloric NPN substitutions for protein have been made (Balch, 1967), NPN has produced growth rates and efficiencies of gain of a level similar to those resulting from the use of natural protein.

Finally, the quality of the carbohydrate or energy fraction of the ration may also determine the energetic adequacy of the ration. This influences the rate of supply of energy to the microbes following feeding, and for efficient NPN utilisation must be matched to

the rate of supply of ammonia. While Hart et al (1939) found that most efficient utilization of NPN occurred when soluble carbohydrate (CHO) was fed in the ration, Mills et al (1942) showed starch to be the most suitable form of this carbohydrate. The actual quantities of soluble carbohydrate required have recently been estimated (McLaren et al, 1965b; Conrad and Hibbs, 1968), the latter authors finding that for the efficient utilisation of urea in the adapted cow, one kg of readily fermentable carbohydrate must be present in the diet per 100 g of urea fed. Moreover, two thirds of the required soluble carbohydrate had to be in the form of starch. Confirmatory evidence has been presented by Virtanen (1966), who showed that the proportion of starch in purified NPN rations, fed to lactating cows, could not be reduced below 55% of the total carbohydrate (70% of soluble carbohydrate) without an associated drop in milk production.

The question immediately arises, in light of the present study, whether maize silage contains sufficient soluble carbohydrate, including starch, to enable efficient utilisation of supplementary NPN. Provided this last requirement is met there seems little reason why, if maize silage protein levels are deficient for growth, NPN supplementation should not adequately rectify the deficiency.

1.4.1.2 NPN Supplementation of Maize Silage. Johnson et al (1967) have suggested that maize silage may be one of the few forages conducive to the reasonably efficient utilisation of NPN. They argue that it produces in the rumen a lower pH than many feeds, under which conditions the NH_4 ion predominates and is less rapidly absorbed than the free ammonia which is more abundant at higher pH's. Moreover, they suggest that the high lactate content of maize silage may provide a substrate for rapid fermentation with hydrolysis dynamics similar to those of NPN.

However, the maize plant has been shown by Johnson et al (1966b) to contain between 200 and 300 mg soluble carbohydrate per g DM at harvest. It was also shown that 65-70% of this soluble carbohydrate was fermented during ensiling, leaving about 90 mg soluble CHO/gDM. If 5 kg urea are added per 1,000 kg of whole plant maize silage (33% DM) (sufficient to raise crude protein content four percentage units to approximately 12%), and a 90% recovery of urea is assumed (Ryley, 1967), the silage would contain 0.0135 g urea/g DM, and for every 100 g urea, only 670 g (0.67 kg)

soluble CHO; well short of the 1,000 g required for efficient urea utilisation (see section 1.4.1.1). On this basis, Coppock (1969) has suggested in his review that maize silage may contain insufficient soluble carbohydrate for efficient use of urea.

However, Coppock (1969) has overlooked one important factor. That is, that the soluble carbohydrate determination method of Johnson et al (1966b) is unlikely to extract starch, a readily fermentable carbohydrate included in the requirement estimation for efficient NPN utilisation of Conrad and Hibbs (1968).

Most of the carbohydrate fermented during ensiling seems to come from the cold-water-soluble carbohydrate fraction of the stalks of the corn plant (Johnson et al, 1966b), dropping the pH rapidly and preserving the starch in the grain. Moreover, lactic acid is the predominant fermentation product (Barnett, 1954; Johnson et al, 1967) and it has been reported by Watson and Nash (1960) that energy losses of only 2-3% may be sustained in its production. While it would not be included in the soluble carbohydrate fraction of the silage (Johnson et al, 1966b) but rather the ether extract fraction (Barnett, 1954), it may well provide an energy source with appropriate breakdown dynamics for urea utilisation (Johnson et al, 1967). Confirmatory evidence comes from the work of Klosterman et al (1961) where limestone-urea additions at ensiling, which would further reduce the soluble carbohydrate content and increase the lactic acid content of silage, did not detrimentally affect liveweight gain.

In light of this background, studies of the NPN supplementation of maize silage in productive rations should be of interest.

Where NPN, mainly urea, has been fed in maize silage based milking rations, results have varied. Conrad and Hibbs (1961), Van Horn and Jacobson (1971), and Van Horn and Mudd (1971) reported negligible or very poor responses to NPN supplementation. In the latter two cases NPN was added to the concentrate part of the ration, a procedure which tends to depress DM intake (Van Horn et al, 1967; Huber et al, 1968). Moreover, not only were comparison periods short (3-4 weeks), hardly allowing time for animal adaptation, but insufficient attention appeared to be directed towards keeping rations isocaloric as well as isonitrogenous. Where this was done (Van Horn et al, 1967), and urea was added to the maize silage part of the ration, milk yields

compared favourably with natural protein (soy bean) supplementation. In keeping with this, work by Huber et al (1967a, 1967b, 1968) and Polan et al (1968) has shown that NPN can replace up to 38% of the required ration protein without depressing milk production, and can enable the level of protein in concentrate supplements to be reduced from a typical 18% to 10% crude protein. However, Conrad and Hibbs (1961) clearly showed that urea could not replace all of the supplementary protein required for high levels of milk production on maize silage.

With the lower protein requirements for growth, results of NPN supplementation trials have been characteristically different. In 1941, Harris and Mitchell found maize silage rations (5.35% CP), supplemented with starch, corn syrup, brown sugar, salt mixture and citric acid, were unable to support appreciable growth in lambs or even consistently maintain their nitrogen equilibrium. Addition of urea to bring the ration to 11% crude protein produced normal growth rates. However, whether maize silage rations without the supplemental starch would have responded to the urea addition remains unclear. In contrast, Thomas et al (1967) failed to show consistent benefit from urea supplementation of maize silage fed alone to 250 kg heifers growing at 0.83 kg/day. That soy bean meal (SBM) supplements were also of no benefit would suggest an unusually high level of protein in the silage. Tolman and Woods (1966) found urea supplements to be less satisfactory for growth of calves than SBM, a result similar to that of Owens et al (1967), although in the latter case the difference was significant only for the first 40 days, the adaptation response again confounding results. In neither of these studies was an unsupplemented control reported, this factor limiting the interpretation of the results. Where unsupplemented maize silages have been incorporated in the design, Schmutz (1966: Cited Coppock, 1969) lifted growth rates of heifers from 0.86 to 0.95 kg/day with urea, and Ryley (1967) reported increased growth rates similar to those recorded with SBM supplementation. Using maize silage containing 1% urea (wet basis) and shelled corn, Klosterman et al (1970) recorded high growth rates of 1.13 kg/day with cattle. Further high quality supplemental protein (SBM) was of no value whatsoever.

This level of addition of urea, however (1% of fresh weight), is higher than the accepted norm (0.5% of fresh weight). It is generally considered tht NPN intakes should not exceed 0.45 kg/

1000 kg LW (Huber et al, 1968; Hillman, 1969) and cattle eating the silage of Klosterman et al (1970) would have been consuming 0.75 kg urea/1000 kg LW. Whether this level of urea could be utilised effectively without the supplemental starch fed is not known.

It would appear from this not entirely inconsistent evidence that where maize silage is deficient in protein, NPN supplementation can produce responses. Whether, however, extra starch is required to achieve best utilisation of the NPN requires further investigation. It is expected that the present study will cast some light on this question.

1.4.1.3 Choice of NPN Supplement. Sources of NPN considered for the present study were urea and biuret, a reflection of NPN availability in New Zealand.

The palatability (Armstrong and Trinder, 1966) and toxicity (Repp et al, 1955) problems associated with urea feeding, in addition to the problem of matching carbohydrate fermentation to its rapid rate of hydrolysis, led to consideration of the more slowly hydrolysed biuret (Campbell et al, 1963) as a more suitable NPN source.

Artificial rumen studies on the suitability of biuret as a nitrogen supplement by Belasco (1954) showed it to produce only 7% of the cellulose digestion stimulated by urea supplements, bacterial growth also being poor. A similar result was reported by Meiske et al (1955), indicating biuret to be very poorly utilised by rumen microbes.

Despite this, Ewan et al (1958), working with lambs, reported only slightly lower N retention, and Campbell et al (1963), slightly lower liveweight gain of cattle, for biuret supplements in comparison with urea. In neither case was the difference statistically significant. Moreover, working with sheep and lambs, Gaither et al (1955) making the same comparison reported no difference, while Hatfield et al (1959) and Karr et al (1965b) found greater nitrogen retention with biuret than urea. In terms of milk yield (Waite et al, 1968), and lamb and steer growth rates (Meiske et al, 1955; Karr et al, 1965a; Mies et al, 1967; Owens et al, 1967), either no differences, or advantages in favour of biuret were reported. This evidence is in complete contrast to the in vitro work cited earlier and must warrant further discussion.

The finding by Ewan et al (1958) that a 70% increase in N retention, with lambs fed biuret, resulted from innoculation of their

rumen contents with microbes from sheep fed biuret over a prolonged period, would indicate a very low level of biuret utilising microbes in the rumen of animals on normal rations. This is in contrast to the situation with urea feeding where salivary urea is continually being hydrolysed in the rumen on all rations.

A marked adaptation response to biuret feeding is indicated, and Campbell et al (1963), Johnson and McClure (1964), Karr et al (1965b) and Oltjen et al (1969) have reported increasing utilisation of biuret, and animal growth rates up to 40 days on diet; well beyond the time utilisation of urea ceased increasing. This response seems a likely explanation for the very poor utilisation of biuret reported in the early in vitro studies (Belasco, 1954; Meiske et al, 1955).

Both Campbell et al (1963) and Johnson and McClure (1964) have reported very low ammonia production from in vitro incubation of biuret with rumen microbes from adapted sheep. Moreover, while Campbell et al (1963) and Oltjen et al (1969) reported slight rumen ammonia increases in vivo following biuret feeding, Repp et al (1955) under similar circumstances found no changes in blood ammonia levels.

Ammonia, absorbed readily from the rumen, is converted to urea by the liver, very little ammonia normally appearing in the peripheral blood. With urea feeding however, the absorption of ammonia from the rumen can become so great that the liver cannot cope with the load and ammonia spills over into the peripheral circulation. Ammonia levels of about one mg/100 ml of blood have proved fatal to sheep (Repp et al, 1955). With biuret producing only half the ruminal ammonia concentrations of urea (Oltjen et al, 1969), this overloading of the liver appears not to occur and hence the reported non-toxicity of biuret even at many times the lethal dose of urea (Repp et al, 1955; Berry et al, 1956; Hatfield et al, 1959).

Since biuret is tasteless (Armstrong and Trinder, 1966) it might be expected to be associated with more satisfactory levels of intake than the bitter-tasting urea. While Owens et al (1967) reported greater feed sorting and refusals with growing cattle fed urea, in comparison with biuret, Oltjen et al (1969) actually reported greater DM intakes on rations supplemented with biuret (2.5 versus 2.2% of body weight for biuret and urea respectively), indicating biuret to be less seriously afflicted by the palatability and intake problems of urea in productive rations.

In the practical situation, supplemental NPN is generally added to maize silage at ensiling. While it is not the intention of the writer to become involved in the controversy surrounding the nutritional benefits or adverse effects of this practice, it is worthy of note that biuret has a much lesser effect on the ensilage process than urea, tending to be more stable and less prone to nitrogen loss (Karr et al, 1965b; Condon et al. 1969).

Finally, reports on studies of the suitability of biuret as an NPN supplement for maize silage are sparse. Results with growing lambs and steers have indicated slight advantages in favour of biuret (Karr et al, 1965a; Owens et al, 1967).

Biuret's freedom from the palatability and toxicity problems of urea, and its being at least as satisfactory as urea nutritionally, led to its choice for the present study, despite the longer adaptation period required for its effective utilisation.

1.4.2 Energy Supplementation of Maize Silage

Maize silage is generally considered to be a high energy feed (Coppock, 1969; Hillman, 1969). Certainly, consideration of table 1.4 reveals that maize silage contains considerably more energy (TDN) than is required for a growth rate in young cattle of 0.34 kg/day. In fact, on the basis of the Morrison (1957) feed requirements, maize silage would contain sufficient energy for 'rapid growth', provided an intake level of 2.5 kg/100 kg LW could be maintained.

Undoubtedly animal performance on maize silage could be improved by energy supplementation, but this in New Zealand would be extremely expensive and likely uneconomic. For these reasons no energy supplements were used in the present work although the possible value of special types of energy supplement to improve the utilisation of non-protein nitrogen has been mentioned (section 1.4.1.2).

1.4.3 Vitamin Supplementation

Of the essential fat soluble vitamins, vitamin A and D deficiencies have been implicated in maize silage feeding (Hillman, 1969).

The vitamin D content of maize silage has been found to range from 269 to 364 USP vitamin D units/kg DM (Bechtel et al, 1936). Daily ingestion of maize silage at the level of 0.7 to 1.0 kg/100 kg liveweight was found to be effective in curing and preventing rickets in yearlings,

and also supplied sufficient antirachitic material for growth and reproduction in dairy cows (Bechtel et al, 1936). However, green maize leaves have been shown to contain very little vitamin D (Bechtel and Hoppert, 1936) and it is therefore possible that immature silages may have a vitamin D deficiency. It would seem though, that typical maize silage made at the dent or glaze stages of maturity, when the lower leaves have dried, as was the case in the present study, is extremely unlikely to be deficient in this respect.

Carotene or pro-vitamin A contents of maize silage, recorded in the literature, range from 4-156 ppm (DM basis), values for typical farm silages averaging from 10 to 70 ppm (Wiseman et al, 1938; Huffman and Duncan, 1960; Coppock, 1969; Hillman, 1969).

Carotene content is again fairly clearly related to plant maturity, decreasing from 140 ppm at the milk stage to about 18-33 ppm at the dent stage of maturity (Wiseman et al, 1938; Huffman and Duncan, 1960; Coppock, 1969). In brief, the greener the plants at harvest, the higher their carotene content is likely to be. Contrary to popular misconception, even yellow maize varieties contain little vitamin A precursor in the grain (Wiseman et al, 1938).

A number of factors associated with the ensilage process are also known to affect silage carotene content. It has been shown that generation of heat sufficient to brown the silage will almost totally destroy the carotene present (Bechtel et al, 1943), while the addition of calcium carbonate or urea can effect a carotene reduction from the normal 20 ppm to 10 ppm (Simkins et al, 1965; Ryley, 1967). Also, the high plant nitrate levels, resulting from heavy nitrogen fertilization (Zimmerman et al, 1962; Cummings et al, 1965; Owen, 1967) or drought conditions (Loomis, 1937), have been shown to destroy carotene during ensiling (Wright and Davidson, 1964). Sodium bisulphite additions have been found to effectively prevent this destruction (Meiske et al, 1963) by blocking the reduction of NO_3 to the highly destructive NO_2 (Meiske et al, 1965), which occurs during ensiling (Jacobson and Wiseman, 1963). While plasma and liver carotene levels of cows eating the bisulphite treated silages were effectively raised, associated production benefits were not reported (Meiske et al, 1963).

It would appear that the 230 kg growing steer requires about 30 mg carotene/day (Morrison, 1957; NRC, 1970). With an extremely

mature silage, or one where carotene has been destroyed during ensiling leaving only 5 ppm, and with an intake of four kg DM/day, a carotene deficiency is clearly possible. However, with typical mature maize silage containing 20 ppm carotene, sufficient carotene for rapid growth should in theory be ingested.

Despite this, Jordan et al (1961) and Zimmerman et al (1962) have reported decreased liver vitamin A levels in growing steers following 133 and 79 days respectively on maize silage rations. Moreover, Wiseman et al (1938) considered a vitamin A deficiency to be responsible for calf deaths when cows were fed maize silage for five lactations, and Jordan et al (1963) found vitamin A deficiency symptoms in steers fed maize silage over the winter and spring periods. In most of this work silage carotene levels were not unduly low and in the case of the study of Jordan et al (1963), supplemental vitamin A (7000-8000 I.V. daily) was actually fed to no avail. This evidence led Hemken and Vandersall, in their 1967 review, to express the view that cattle fed maize silage convert its carotene to vitamin A poorly.

The poor carotene metabolism in the study of Jordan et al (1963) seemed to be exaggerated in the presence of nitrate and rectified by the administration of the thyroid hormone, triiodothyronine. The knowledge that carotene utilisation is influenced by thyroid activity (Moore, 1957) and that nitrate has an inhibitary effect on thyroid activity (Blomfield et al, 1964), together with the finding of Garner et al (1958) that rats became rapidly depleted of liver vitamin A stores, showing vitamin A deficiencies when fed nitrate containing rations led to an extensive literature on the chronic effects of nitrate in maize silage. The assumption, based on the nitrate-carotene syndrome with rats, was that the nitrate effect was mediated via the thyroid. However, as pointed out by Wright and Davidson (1964) and Goodrich et al (1964), nitrate levels acutely toxic to ruminants would be required to interfere with iodine and thus vitamin A nutrition.

In this light it should be noted that the positive vitamin A response recorded by Jordan et al (1963) on administration of triiodothyronine to steers was only circumstantially linked with nitrate in the silage, and in fact, the authors themselves consider high temperatures rather than nitrate to have been inhibiting the thyroid. Apart from this one piece of evidence of a possible nitrate-carotene

link in ruminants, demonstration of the nitrate-carotene effect appears to have been confined to monogastrics.

With the demonstration by Olson et al (1963) of the potent carotene destructive properties of nitrite in acid conditions, it would seem that with normal dietary nitrate/nitrite levels, the nitrate-carotene effect in non-ruminants is caused by the destruction of carotene in the acid stomach. In the near neutral conditions of the rumen this does not occur (Olson et al (1963) and the rapid absorption of nitrite (Wright and Davidson, 1964) prevents it from reaching the acid abomasum thus avoiding extensive carotene destruction.

A number of workers have produced evidence supporting this thesis of the non-significance of the chronic nitrate-carotene effect in ruminants. Davison et al (1963) fed 0, 20 and 30 g nitrate/day to Holstein heifers either just before or during their first pregnancy. While the highest level of nitrate was fatal to one heifer, those remaining did not differ in rate of growth, length of gestation, birth weight of calves, rate of milk production or liver vitamin A reserves. A similar result with lactating cows fed maize silage (0.78% KNO_3 + 1.25% KNO_3) is reported by Jones et al (1966), and Meiske et al (1965) actually reported elevated liver carotene with dairy cattle fed maize silage with raised nitrate (0.9% NO_3) and carotene levels through sodium metabisulphite treatment.

With growing steers Weichenthal et al (1963) reported no influence on liver vitamin A levels from adding 1% Na NO_3 to the ration. Moreover, both Jordan et al (1961) and Zimmerman et al (1962) found no effect on liver vitamin A content from feeding normal or high nitrate maize silages (0.16 to 0.75% NO_3). In fact, steer growth rates were higher on the high nitrate, than on the lower nitrate maize silages; possibly a reflection of protein content.

The weight of evidence would indicate that the level of nitrate in maize silage required to interfere with vitamin A nutrition cannot be distinguished from the level that would cause acute toxicity. Nitrate has been fed in maize silage rations successfully up to levels of 1.67% (Cummings et al, 1965) and 1.25% (Jones et al, 1966), Wright and Davidson (1964) suggesting a ration containing about 2% NO_3 to be lethal to 50% of ruminants consuming it. Even in maize silages produced with extremely high levels of nitrogen fertilizer (900 kg N/ha), or

treated with preservatives, nitrate levels have not been recorded above 0.9% of DM (Cummings et al, 1965; Meiske et al, 1965). Only with severely drought injured maize silage could nitrate content reach these dangerous levels (Loomis et al, 1937).

There would seem to be no substantiated reason why carotene from maize silage should not be effectively utilised by ruminants. In fact, much evidence would indicate quite satisfactory utilisation. While with lactating cows fed maize silage through two lactations, Brown et al (1966) found quite normal plasma vitamin A levels, Martin et al (1967) reported depleted plasma and liver vitamin A levels in lambs to be returned to normal with maize silage feeding (23 ppm carotene DM basis). A similar result with growing steers is reported by Klosterman et al (1964), Miller et al (1967), also finding maize silage to be quite satisfactory as a carotene source for growing cattle.

The occasional reports of depleted liver vitamin A stores on maize silage (Jordan et al, 1961; Zimmerman et al, 1962) are not surprising in view of the variability in maize silage carotene content, and the finding that it takes five times the vitamin A to maintain liver storage than to prevent the appearance of deficiency symptoms (Jordan et al, 1963).

Consideration of the evidence leads to the conclusion that vitamin A supplementation of maize silage for ruminants should not be necessary unless a unique combination of circumstances prevails. For this reason vitamin A supplements were not used in the present study.

1.4.4 Mineral Supplementation

While Johnson et al (1967) contend that maize silage is inherently deficient in Ca and P, Hillman (1969) extends this list claiming it to be low in S, Ca and Na, borderline in P, and possibly deficient in Co and I. That maize silage feeding will reduce mineral intake in comparison with pasture grazing, is undoubtedly true. However, reports of Na and Co deficiencies are not readily apparent in the literature, although an I deficiency has been implicated in an isolated report of a goitre problem (Hemken and Vandersall, 1967).

As indicated in section 1.1.2, it would seem that P, Ca and also S are the minerals most likely to prove deficient. Moreover, such incipient deficiencies may be precipitated when supplemental NPN replaces a large part of the plant protein in a diet, proteins being

rich in minerals.

Both S and P appear to be important in NPN utilisation (Burroughs et al, 1951; Thomas et al, 1951), but unfortunately a dearth of information exists on the value of phosphorus, as is the case with calcium, supplementation of maize silage. A considerable effort, however, has been directed towards sulphur nutrition, possibly indicating its importance in maize silage feeding.

It seems that sulphur requirements are related to nitrogen level in the ration, an N/S ration of 10/1 being required (Allaway and Thompson, 1966). Maize silage has a typical N/S ratio of 13/1, the addition of 0.5% urea widening this to about 18/1. Responses to S supplementation, however, have been variable.

Both Davis et al (1954) and Jacobson et al (1967) failed to find any response from S supplementation of maize silage rations fed to lactating cows. Since it can be calculated that the N/S ratios of the maize silages fed were 5/1 and 12/1 respectively (i.e. within and near requirement respectively), S supplementation responses could hardly be expected.

With growing calves fed maize silage and urea, Tolman and Woods (1966) found no response to S supplementation. The N/S ratios of the rations fed were unfortunately not reported. While Shively et al (1966) recorded an initial response to S supplementation with growing cattle on a 17/1 N/S ratio, high urea diet, it did not last beyond three months. Goodrich et al (1967) however, reported S supplementation of high urea diets to give consistent responses in both growing cattle and lambs.

Interpretation of this data is difficult. It has been suggested (Allaway and Thompson, 1966) that when the yield of a forage is increased through the use of S fertilisation, an improvement in nutritive value for ruminants will coincide with the increased yields. Beyond this, where maize silage is fed with NPN, efficient utilisation of the N fraction may require S supplementation. However, care must be taken in adding supplemental S as it has been shown to precipitate incipient molybdenum toxicities (Vanderveen and Keener, 1964).

On the basis of the avilable evidence it was decided to supply a mineral supplement containing Ca, P and S for the present study, as it was contrary to the purpose of the experiment to have such factors

limiting the utilisation of the supplemental NPN.

1.5 CONSIDERATION OF EXPERIMENTAL DESIGN

The objective of the present study as outlined in the preface, was to investigate the nutritive value of maize silage for growing cattle, with particular emphasis on its protein adequacy. The ultimate criterion of nutritive value is animal performance. Other measures, such as voluntary intake and digestibility, are considered to be components of, or to help characterise, nutritive value.

In a study with growing cattle, the best measure of productivity would be carcass weight gain. Unfortunately, however, financial circumstances precluded measurement of carcass weight gain, leaving liveweight gain as the major measure of nutritive value in the present study.

Consideration of protein adequacy required study of nitrogen (N) utilisation and necessitated measurement of N balance.

In order to achieve this objective, it was necessary to design an experiment with a minimum of three treatment groups, one fed maize silage alone, another fed maize silage supplemented with nitrogen, and a control group fed a ration of known value to measure the responsiveness of experimental subjects to treatment.

1.5.1 Choice of Design

Liveweight change is a somewhat gross measure, being the result of both changes in body tissue weight and fluctuations in the weight of digestive tract contents (gut fill). Gut fill has been found to account for 10-28% of the total liveweight of adult cattle beasts (Mott and Lucas, 1962), and over the course of one day may cause an average change in measured liveweight of 9-16 kg (Taylor, 1954). Moreover, from day to day, liveweights of individual animals have been shown to vary up to 28 kg (Hughes and Harker, 1950). It is therefore not surprising that coefficients of variation for liveweight gain of up to 65% have been recorded (Hubbard *et al*, 1969).

Clearly to obtain reasonable sensitivity in liveweight gain studies, without using vast numbers of animals, sophisticated design and management techniques are required. For the present study facilities would permit the use of only 16 animals.

Greatest error control in experimentation can be achieved by the

use of change-over designs (Lucas, 1959), with the risk however, that performance in a given period might reflect not only the direct effect of the current treatment, but also the residual effect of preceding treatments (Patterson and Lucas, 1962). It has been clearly shown that liveweight gain response is affected by previous treatment (Joblin, 1968; Almquist et al, 1971). This coupled with the likelihood of an NPN adaptation response, discussed in section 1.4.1.1, rendered change-over designs as being unsuitable for the present study.

The possibility of using monozygous twins was considered since Dick and Whittle (1951) reported such twins to be .5 times as efficient as unrelated animals. However, twin efficiencies in liveweight gain studies have been variable. Hancock (1951) reported a range from 4 to 21 depending on 'between' and 'within set' variability. With three treatments, however, efficiencies would be lowered, and in short term work (three to six weeks) with more than two treatments, Bailey et al (1958) failed to find any advantage in their use. Moreover, it was considered monozygous twins would not be representative of the population to which the results were to apply; a prerequisite of sound experimentation (Lucas, 1959). For these reasons twins were not used.

In experiments with limited numbers of animals, Lucas (1959) has suggested a procedure of balanced allotment, whereby means and ranges in treatment groups are made as like as possible. This technique is open to the criticism of possible bias in the estimation of experimental error. Moreover, Meyer et al (1960) found balanced allotment of growing cattle, eight to ten per treatment, to be less successful in reducing error variance than the technique of randomised blocking, when both were combined with covariance analysis. Randomised blocking not only tends to equate group means but also the variability within groups, without the risk of bias.

Ashton et al (1955a), with growing pigs, found the coefficient of variation of rate of gain, adjusted by covariance analysis, to be reduced by 10% when compared with unadjusted gains. When covariance analysis was used in association with a randomised block procedure, the reduction in coefficient of variation was 25% (Ashton et al, 1955b). The independent covariable in each case was initial liveweight. Working with growing steers, Kincaid et al (1945) effected a 14% reduction in error variance with covariance analysis, using liveweight

gain in a previous period as the independent covariate. Moreover, Ashton et al (1955a) recorded a 20% reduction in error variance of pig N balance data adjusted by covariance analysis.

On the basis of this evidence a randomised block, covariance design was chosen for the present study. Animals were to be blocked on initial liveweight, and gain during a six week standardisation period was to provide the independent covariate. The standardisation period would also provide an opportunity for obtaining the N-balance data necessary for its own covariance analysis. A six week standardisation period was chosen as Bailey et al (1958) found two weeks to be too short, and on the basis of their data, six weeks appeared to be the minimum time compatible with reasonable error variance.

It was estimated that maize silage supplies would also last six weeks, thus allowing a six week comparison period. A larger quantity of maize silage had been made, but the unexpectedly long period of storage (nine months) resulted in greater than expected wastage (see Appendix VI).

With 16 animals available and a minimum of three treatments required it was decided to use 15 animals in three groups of five. Coefficients of variation for growth rate of cattle in New Zealand, over longer periods of time, and with larger numbers of animals have been shown to be in the order of 12% (Carter, 1969). However, in a six week growth study, 24 animals per treatment, and under stringent managerial conditions, Bailey et al (1958) recorded a coefficient of variation of 9 to 10%. Moreover, with covariance analysis of pig and cattle gains, Ashton et al (1955b) and Kincaid et al (1945) both recorded coefficients of variation of 10%.

It seemed that at best a coefficient of variation of adjusted liveweight gain data of 10% might be achieved. With the five animals per treatment, it was calculated that the sensitivity of the design would enable detection of significant differences in daily liveweight gain of 0.15 kg/day at the 5% level, with a probability of 65%, and differences of 0.20 kg/day at the 5% and 1% levels, with probabilities of 93 and 72% respectively (see Appendix II). It was considered that such precision was sufficient to justify the running of the experiment, but extreme care would be needed in measuring the liveweight gain.

1.5.2 Measurement of the Major Response Parameters

Consideration will only be given to the most important measures of nutritive value and N utilisation, namely liveweight gain and N balance. Where necessary, brief reasons for certain techniques used in the measurement of other parameters examined (see 2.4) are mentioned along with the description of the procedure in Chapter Two or alternatively, with the discussion of results in Chapter Four.

1.5.2.1 Measurement of Liveweight Gain. In section 1.4.1.1 the adaptation response to NPN feeding was discussed. On the basis of the evidence outlined it seemed likely that any response to the nitrogen supplementation would increase over time. Moreover, with a covariance experimental design the change from the standardisation to the comparison period rations could be expected to elicit some form of adaptation response. It was therefore considered necessary to weigh the cattle every week and examine liveweight gain on a week to week basis. By proper analysis this procedure would enable removal of a large part of the variation due to time trend, both linear and cyclical (Baker and Guilbert, 1942), from the error variance.

For such a procedure to be meaningful, however, weekly weights as accurate as possible were required. The finding by Taylor (1954) of 9-16 kg fluctuations in liveweight over the course of a day, with steers having a continual supply of feed, indicates a need for standardised weighing procedures. These variations in association with large day to day fluctuations (Hughes and Harker, 1950), due largely to changing gut fill, have led to such techniques as weighing on more than one day, and overnight fasting prior to weighing (Lush, 1928; Patterson, 1947; Taylor, 1954; Whiteman et al, 1954 and Koch et al, 1958).

While Lush (1928) reported a 42% reduction in the error contained in a one day weight of steers with two additional days' weights, Hughes and Harker (1950) showed that the disturbance of weighing may in fact reduce growth performance, a finding also reported by Green et al (1952). Moreover, Patterson (1947) found that while with grazing steers, three-day mean weights reduced the error variance of the measured gain by 7.32%, with stalled cattle the equivalent reduction was only 2.27%. Furthermore, the advantage of three-day means over two-day means was slight. With this evidence in mind it was decided to weigh on two

consequtive days each week, the liveweight for any one week being the mean of those two weighings, and the liveweight gain for a week being the difference between consecutive two-day means.

Overnight fasting has been shown to be effective in reducing the error caused by gut fill fluctuations under some circumstances (Baker and Guilbert, 1942; Taylor, 1954; Whiteman et al, 1954; Koch et al, 1958). However, Hughes and Harker (1950) showed such fasts to have a detrimental effect on growth performance, and found early morning weighings to be equally successful in minimising variability. With grazing cattle, early morning (three to five hours after sunrise) has been shown to be the time of minimum gut fill (Taylor, 1954) and it is probable that a similar situation exists for stall-fed animals. Provided water was continually available (Whiteman et al, 1954), and the animals were weighed at as near as possible the same time each relevant morning, overnight fasting was considered unnecessary.

1.5.2.2 Nitrogen (N) Balance. By accepted definition, N-balance in growing animals is equal to N intake less urinary and faecal N output (Allison, 1955; Rippon, 1959). The oft reported gross discrepancies between N retention and body growth (Wallace, 1959; Duncan, 1966) have led to a questioning of the validity of the technique for examining N utilisation. Justification of its use would require investigation of its validity.

In 1964, Allison and Bird summarised the criticisms of the classical N balance into two aspects: (a) The over-estimation of N intake as a result of loss of unconsumed diet, and under-estimation of N output from excreta loss, may combine to produce an over-estimate of retention, and (b) over-estimation may also result from excretion via unmeasured pathways (e.g. respiration, dermal losses).

While the loss of unconsumed dietary N can only be minimised by meticulous feed measurement techniques, the under-estimation in measurement of N output seems very much dependent on the procedure followed. Both Martin (1966) and Fuller and Cadenhead (1969) have reported negligible losses of N from faeces between voiding and collection, and during cool storage. Urinary N losses, however, from voiding to collection were variable and seemed dependent on the pH of the urine. Martin (1966) found such urinary N losses at near neutral pH to be 10%, declining to 1% at or below pH 2.0; temperatures

below 20°C further minimising losses. This evidence shows the reason for certain of the procedures followed in measuring N balance in the present study (section 2.4.5) and suggests that given certain requirements reasonable accuracy of measurement of faecal and urinary N outputs can be attained.

In seeking unaccounted pathways of N loss, Butterworth (1962), working with chickens in a respirometer, failed to show any loss of N through respiration. The author, like Sanslone and Squibb (1962) and Henry (1965) working with chickens and rats respectively, concluded that faecal and uninary N were the sole sources of N output, measurement and analytical errors being responsible for discrepancies with growth. Reinforcement of this theory was found in the failure of Wegner et al (1940) to detect any loss of N from an incubating mixture of rumen bacteria and urea, and the inability of Magnus (1902: cited Harris and Mitchell, 1941a) to demonstrate any permeability of the lungs to NH_3. However, Robin et al (1959) showed that following intravenous administration of ammonium acetate to dogs, measurable levels of free NH_3 appeared in the expired air. Furthermore, Costa (1960), after recording consistent positive N retentions for dogs, rats and mice at constant body weights (the magnitudes of the retentions placing them outside the range of possible compositional shifts within the body), pointed out that gaseous loss from the mouth could be sufficient to account for the discrepancy without being recorded by the usual methods of gas analysis. Despite this, Martin (1966) measured only one mg N per 24 hours in the expired air of sheep, a value not influenced by increasing levels of rumen ammonia. It would seem that gaseous losses of N are most unlikely to invalidate conclusions drawn about N utilisation based on the N balance technique.

Dermal N losses, resulting from the desquamation of keratinised cells, loss of hair and wool, and the secretions of the sebacious and sweat glands, have been measured with sheep by Harris and Mitchell (1941a) and Martin (1966). The recorded N losses were amazingly consistent (3.1 to 3.6 mg/kg BW/day) and independent of diet fed. In the work of Martin (1966) such losses were sufficient, however, to alter N balance by up to 58%. Clearly in absolute studies dermal losses could confound interpretation. However, their independence from the ration feed, prevents them from invalidating comparative studies

such as the present. No other sources of N loss have been reported.

Technically there seems no reason why the conventional N balance procedure should not be valid in a comparative study of N utilisation. However, caution is required in the interpretation of such data as N balance is a variable changing with time as body protein stores increase or decrease. Briefly, the amount of N excreted is high when the metabolic pool is full. Under these conditions a relatively high intake of N is required to maintain equilibrium. As the body protein pool is depleted, the excretion of endogenous N falls rapidly to a low and slowly decreasing value. Only a relatively small amount of N may then be needed to maintain such a depleted individual in equilibrium (Allison and Bird, 1964). Moreover, as pointed out by Allison (1951), animals tend to drift towards an N equilibrium if the balance is either positive or negative. If an N deficient diet is fed, an animal will deplete its protein pool until it comes into equilibrium with the low protein diet. Failure to recognise such adaptive changes may lead to mis-interpretation of data.

Furthermore, Allison (1951) recorded rapid shifts in N balance with alteration in caloric intake. A sub-maintenence energy intake, especially in the ruminant, could result in a net outward movement of N from the protein pool, the amino acids being required for gluconeogenesis and energy metabolism. The associated increase in urinary N excretion would cause a rapid shift to a negative balance.

Despite these interpretive difficulties, provided animals are in a similar physiological state, following a period of standardisation, the risk of erroneous conclusions must be low.

1.6 SUMMARY

Maize silage, produced from hybrid grain maize grown in the United States, has been shown to contain 45 to 50% grain on a DM basis when harvested at the dent to glaze stages of maturity (30-35% DM). Both DM yields and digestible DM consumption by cattle seem to be maximised by harvesting at this stage of maturity.

The major characteristic of the chemical composition of maize silage is its low level of crude protein when compared to New Zealand mixed pasture. Despite its high dry matter digestibility (68%), yielding a 70% TDN value, digestible crude protein contents of 5%

and below appear inadequate for efficient utilisation of the energy fraction. Whether, however, maize silage protein levels are sufficient to support reasonable growth rates in cattle seems less clear. The experiment reported in the following chapters is designed to cast further light on this aspect through the use of NPN (Biuret) supplementation, and measurement of N utilisation by the N balance technique. There appears to be no convincing evidence why the NPN should not be utilised effectively in a maize silage ration, assuming that it is protein deficient for rapid growth of cattle. This is considered a major but integral part of the overall nutritive value study, in which liveweight gain is used as the ultimate criterion of that value.

As it was necessary to have only protein as being possibly limiting to growth in the experiment, other reported deficiencies (e.g. vitamins A and D and minerals) were investigated in deciding the composition of the experimental rations. The weight of evidence would suggest an extremely low likelihood of any vitamin deficiencies developing with maize silage feeding of growing cattle, unless an improbable combination of circumstances prevailed. However, in light of the NPN feeding, the available evidence indicated the need for a mineral supplement, Ca, P and S being considered marginally deficient in maize silage.

Error control consideration, amplified by the limitation on animal numbers (16 steers) imposed by shortage of facilities, led to the choice of a randomised block, covariance design for the nutritive value study. Three treatments were required, maize silage alone, maize silage plus biuret and a control of known value, leaving five steers available for each treatment. It was calculated that liveweight gain differences of 0.20 kg/day could be detected at the 5% level of significance with a 93% certainty; a sensitivity considered sufficient to justify the continuation of the experiment as designed.

CHAPTER TWO

METHODS AND MATERIALS

Only information strictly relevant to the nutrition study outlined in section 1.5 is presented in this chapter. Details of the production and storage of the maize crop, while being considered important aspects of the overall study of maize silage, are referred to in numbered appendices found preceding the bibliography.

The chapter is divided into five sections, the first three providing information on the design of the experiment, the feeds studied, and the animals used, while the fourth section outlines the experimental procedures followed in measuring the various parameters. The final section summarises the major statistical models used in analysing the results.

2.1 EXPERIMENTAL DESIGN

The nature of the work necessitated the use of a design appropriate for continuous experiments, in contrast to change-over experiments, and a randomised block-covariance design was chosen (see section 1.5.1). Animals, blocked on the basis of liveweight, were randomly allocated to treatments on a within blocks basis. A discussion of the suitability of the design used is presented in section 4.1.

As is generally implicit in this type of design, the experiment was divided into three periods of observation; a preliminary period (PP) to allow for animal adaptation to the experimental environment, a standardisation period (SP) for obtaining information on concomitant variables, and a comparison period (CP) in which to compare the treatments. The latter two periods were run under stringent experimental conditions.

A three week preliminary period commenced on 1/12/70, followed by standardisation and comparison periods, each of six weeks, commencing on 21/12/70 and 1/2/71 respectively. The experiment was concluded on 12/3/71.

Further details of the timing of operations are presented in Appendix I.

2.2 EXPERIMENTAL FEEDS

To enable inductive inferences to be made regarding the nutritive value of maize silage for growing animals, it was considered necessary to have as one of the experimental feeds, a control ration of known value (see section 1.5). Hay and barley was selected for this purpose, and the feeds used throughout the experiment are outlined in Table 2.1.

TABLE 2.1: Feeds used during the three major periods of the experiment

Period	Preliminary Period (PP)	Standardisation Period (SP)	Comparison Period (CP)
Feed	Hay and Barley (all animals)	Hay and Barley (all animals)	Hay and Barley (HB) Maize Silage (MS) Maize Silage plus Biuret (MSB)

2.2.1 Hay and Barley (HB)

Ryegrass-clover, meadow hay of good quality was used in the experiment. It was fed in conjunction with barley meal, the amount of meal fed each individual being altered weekly on the basis of metabolic body weight (i.e.: $LW^{0.75}$). In this way, one influence of variation in animal size on growth rate was reduced.

The level of meal feeding was calculated on the basis of NRC feeding standards (NRC, 1970), to promote, along with hay to appetite, an average liveweight gain of 0.75 kg/day. Of a wide range of possible growth rates with the hay and barley combination, this target was chosen as it was considered to be readily obtainable with animals of this age. Moreover, it would provide a realistic standard against which to compare the silage rations.

The calculations on which the level of meal feeding was based are shown in Appendix III. It was estimated that barley meal would consititute 42% of the total DM intake.

2.2.2 Maize Silage (MS)

Details of the production of the maize crop, and yield data, are presented in Appendices IV and V.

The silage was made from the entire hybrid maize plant harvested at the dent stage of maturity (33$\pm$3% DM), with a single row New Holland 717 Maize Chopper.

The finely chopped material was vacuum packed in two elongated stacks, approximately 2.4 m x 12 m x 1 m, designed so that the estimated usage rate from the open face would be sufficient to keep ahead of air entry, thus avoiding wasteful secondary fermentation. The silage was stored for nine to ten months before being used, and the nature of the material in the stacks when opened can be seen in plate 2.1. The maize silage produced would have been considered less than excellent in quality on visual appraisal. However, the light colour of the bulk of the material indicated extensive heat damage to be unlikely, and the pleasant smell precluded extensive clostridium microbial activity. Further details on the ensilage process are presented in Appendix VI.

At all times during the feeding of the maize silage, a mineral supplement (rock salt/bone flour, 50/50) was offered to the animals.

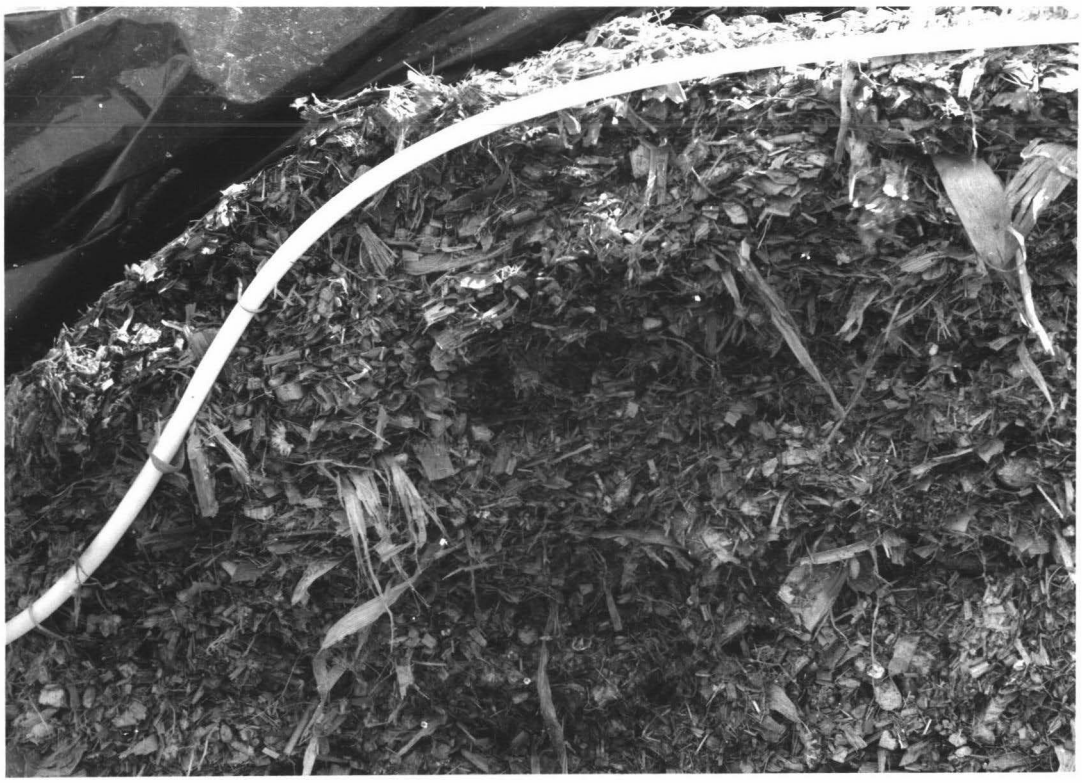

PLATE 2.1 *The maize silage at time of feeding, in the opened face of the stack. The photograph indicates both the fineness of chop and the dry friable nature of the silage. Material outside the white line is wastage, comment on the pattern of this loss being made in Appendix VI.*

This was considered a necessary precaution as it was shown in section 1.4.4 that maize silage may be deficient in Ca, P and S, and a procedural objective in the experiment was to eliminate, as far as possible, limiting factors other than nitrogen.

2.2.3 Maize Silage plus Biuret (MSB)

The maize silage in this ration was identical with that described above (2.2.2), the same mineral supplement also being offered. In addition, however, this ration contained granular biuret at a level sufficient to raise the crude protein content of the maize silage 4.7 percentage units to an estimated 13-14% on a dry matter basis.

Biuret being tasteless (see section 1.4.1.3) posed a feeding problem. Mixing it with the silage would have left doubt as to the amount eaten. However, feeding it with the mineral supplement in a separate bin, meant that an exact amount could be fed daily to each individual; the amount being altered weekly on the basis of the previous week's level of intake (See Appendix VII).

The biuret was supplied by Ivon Watkins-Dow Limited.

2.3 EXPERIMENTAL ANIMALS

The proper selection of experimental subjects is an important aspect of experimentation. It is necessary that the animals be representative of the population to which inductive inferences are to be made, and in this case, it was also important that they have a good growth potential, liveweight gain being the major response parameter measured (see section 1.5.2).

The widespread and increasing use of Friesians for beef production, with their potentially high growth rates, made them a logical choice. Sixteen Friesian steers, off the one farm, nine months of age and 170 kg mean liveweight, were used in the experiment. This number allowed for three treatment groups of five plus one emergency (see section 1.5.1).

The steers were permanently housed in a 16-stall barn for the purpose of individual feeding. They were stalled by means of wide, heavy gauge collars, with chains to loops set in the concrete floor. Water was continually available to each animal.

During the preliminary period, the steers were accustomed to both the barn and the hay and barley diet of the standardisation period by being housed for increasing periods of time, with decreasing time

on pasture. The barley meal was offered first at the rate of 0.45 kg/day, with similar daily increments until the predetermined level was reached for each animal. In this way foot and leg troubles, and digestive upsets, were largely avoided.

During this time, the animals were sprayed twice for lice with Diazinon (Neocidal, Ivon Watkins-Dow Limited), drenched for worms with Levamisole (Nilverm, I.C.I. N.Z. Limited), and treated for ringworm with Tamed Iodine (Ciba Company). They were also allocated to permanent positions in the barn at random.

Following the second week of the standardisation period the steers were blocked into five groups of three on the basis of liveweight (see Appendix VIII), and animals within blocks were allocated at random, one to each of the three comparison period treatments. This was necessary so that three animals from each treatment group could be used in the standardisation period, total urine and faecal collections.

2.4 EXPERIMENTAL PROCEDURES

A number of parameters were measured where it was considered they would both assist in the interpretation of the liveweight gain response data and contribute further information on the characteristics of the nutritive value of the rations studied. These included, physical composition, chemical composition, and digestibility of rations, with voluntary intake and N balance of the steers fed the rations.

2.4.1 Physical Analysis of Rations

The component feeds of the three CP rations, with the exception of the biuret in the MSB ration (see 2.2.3), were fed in separate bins to enable accurate measurement of the quantities of each actually consumed by the steers. The physical composition of each of the rations fed had to be estimated in this way, as diets with such physically diverse components could not have been fed ad lib. to an exactly predetermined composition without grinding and mixing.

Further details of the feeding procedure are provided in section 2.4.4.

A physical analysis was also carried out on the maize itself. Four days prior to harvesting the silage, the crop was sampled (see Appendix V) and the sample plants were each separated into leaf, leaf sheath, stem, grain and remainder. The components were oven dried

(90°C), weighed, and their proportion of the total DM yield calculated.

During the digestibility trial (see section 2.4.3) the maize silage was sampled daily, and the bulked composite sample was later sub-sampled eight times for hand separation of the grain from the rest of the material. Both fractions were oven dried (90°C) and the percentage grain content of the silage dry matter determined. It was compared with the grain content of the crop prior to ensiling.

2.4.2 Chemical Analysis of Feeds

All feeds were sampled daily during the digestibility trials, a fixed percentage of each being taken so as to ensure representative sampling of the total feed used.

These samples were bulked, stored at -10°C and later sub-sampled, the sub-samples being freeze dried, ground through a one mm sieve (Wiley Mill) and stored in air tight jars for chemical analysis. The bone flour, rock salt and biuret, all being both homogeneous and dry, were carefully sampled only once, ground and stored for analysis.

A Proximate Analysis (AOAC, 1965) was carried out on the ground sub-samples of each feed and the compositions of the three rations as fed were calculated.

In addition to this analysis, extraction of the feed samples with the neutral-detergent solution of Van Soest (1967) enabled separation of the cellular material from the cell-wall constituents. Crude protein (AOAC, 1965) and ash (AOAC, 1965) determinations on the residual cell-wall constituents enabled the estimation of soluble carbohydrate content (including starch) by difference (i.e. Cellular Contents + Cell Wall Bound Protein and Ash - Ether Extract - Crude Protein - Ash = Soluble Carbohydrate).

This procedure resulted in a comprehensive analysis of the cellular or 'soluble' fraction of the feeds, and acid-detergent fibre determinations (Van Soest, 1963), in conjunction with the crude fibre determinations of the Proximate Analysis (AOAC, 1965), facilitated a breakdown of the cell-wall constituents (or insoluble fraction) into hemi-cellulose, and cellulose and lignin (Fonnesbeck, 1968).

The crude protein fraction of the feeds was also further broken down into true protein and NPN by means of an alcohol extraction (Bailey, pers. comm.), and Kjeldahl total nitrogen (AOAC, 1965) on the residue.

Because of their particular relevance to maize silage and non-protein nitrogen feeding (see section 1.4.4), sulphur (gravimetric determination, Scott, 1925), calcium (atomic absorption spectroscopy) and phosphorus (colorimetrically, Fiske and Gubbarow, 1925) determinations were also made on the feeds.

2.4.3 Digestibility of Rations

Following the third week of the standardisation period, three animals were selected at random from each of the three treatments and fitted with faecal collection harnesses (see Plate 2.2). Dry matter digestibilities were determined by total collection of faeces over a period of 14 days. The same nine animals were used during the comparison period, and four days were allowed in both cases for the animals to become adapted to the harnesses before the 14-day digestibility trials were started. Feed intake was measured as described in section 2.4.4.

Faeces were collected once daily at 8.30 a.m., weighed to an accuracy of $\pm$ 0.0025 kg, mixed carefully, and a 10% aliquot taken for individual bulked samples stored at -10°C. These composite samples were later again mixed thoroughly and sub-sampled for duplicate dry matter determinations in a forced draft oven at 90°C.

Further sub-samples were freeze dried, ground through a one mm sieve (Wiley Mill) and stored in glass jars for chemical analysis. Crude protein (Kjeldahl, AOAC, 1965), acid-detergent fibre (Van Soest, 1963), organic matter (AOAC, 1965), and gross energy (Adiabatic Bomb Calorimeter) determinations were made on the faecal samples to enable calculation of apparent digestibilities for each of these components.

2.4.4 Voluntary Intake

To avoid extensive deterioration in the feed bins over twenty-four hours, the maize silage was fed twice daily in equal portions. Similarly, to help avoid digestive upsets, the barley meal was also fed twice daily. However, the hay was fed only once a day, at the morning feeding, its deterioration being considered insignificant. Likewise, on the basis of the slow rumen degradation of biuret (see section 1.4.1.3), it was also fed only once a day, at the afternoon feeding.

All animals were individually fed at 8.30 a.m. and 3.30 p.m.,

PLATE 2.2 *Steers harnessed for total collection of urine and faeces. The urine collectors were designed during the study, and details of their construction can be found in Section 2.4.5.*

the feeds being weighed on spring balances, accurate to ± 0.015 kg. Both the maize silage and the hay were offered ad lib. (10% excess), necessitating the collection and weighing of individual feed refusals at the morning feeding. These were weighed on the same balances and to the same accuracy as the feeds.

For determination of DM intakes, daily samples (70-200 g depending on material) were taken from both feeds and individual refusals, and dried for 24 hours in a forced drought oven at 90°C.

DOM, DE, ME, crude protein and digestible crude protein intakes were also calculated from the results of the chemical analysis (2.4.2) and digestibility data (2.4.3).

2.4.5 Nitrogen (N) Balance

Two N balance determinations were made, one in the standardisation period and the other in the comparison period. The former was carried out not only to provide information necessary for an analysis of covariance, but also to perfect the procedure for the comparison period.

As N balance work had not previously been done with steers at this University, a technique for total collection of urine had to be developed. Observation indicated that the steers never urinated while lying down. This meant that urine collection funnels could be designed that crumpled when the animals lay on them, and provided they returned to the correct shape when the animals stood up, no urine should be lost. After trial of a number of designs and materials, it was found that butyl rubber funnels, 30 cm in diameter and 20 cm deep, held in shape at the top by vacuum strip seal, and fastened to rubber gas tubing at the bottom, collected the urine very satisfactorily.

The funnels were mounted under the steers with nylon cord fastened to wide elastic bands over the animals' backs to allow for movement. In this way they were held lightly but firmly against the underside of the steers' bellies, regardless of the positions or stances adopted by the animals. It was found necessary, however, to shave their bellies prior to the commencement of the collection periods, so as to avoid both contamination of the urine and blocking of the urine collectors with loose hair.

The design, construction and fitting of these funnels is shown in plates 2.3, 2.4 and 2.5.

PLATE 2.3 Urine collection funnel mounted in place during a collection period.

PLATE 2.4 Butyl rubber, urine collection funnel partially dismantled. Note the vacuum strip seal alkathene frame and the totally collapsible rubber funnel.

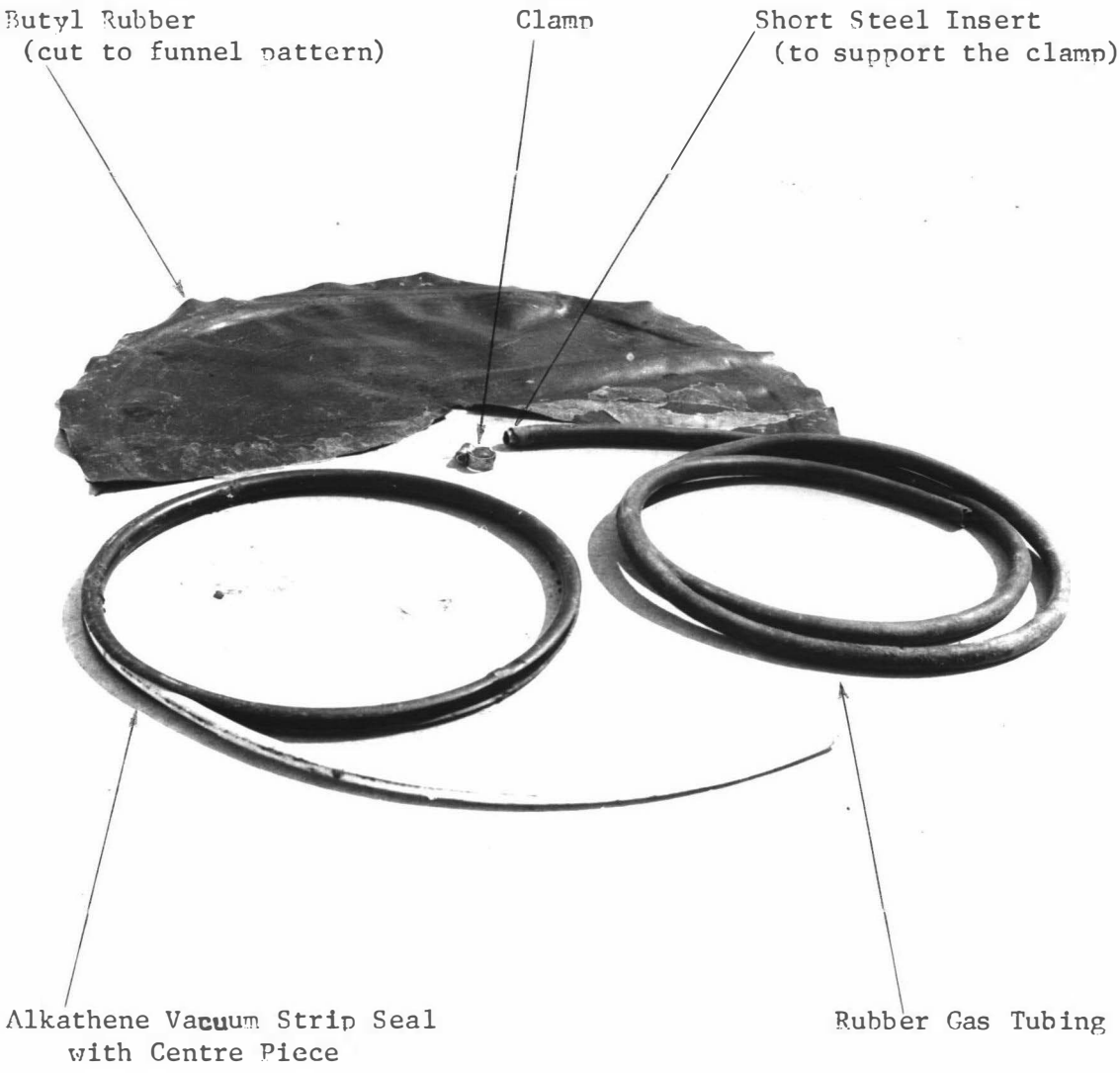

PLATE 2.5 Urine collection funnel completely dismantled

The N balance studies were carried out concurrently with the digestibility trials, the steers being harnessed for both urine and faeces collections four days prior to the commencement of the 14-day collection periods.

The faeces were collected, weighed, sampled for frozen storage of individual composites, and sub-sampled for DM determinations as already outlined in 2.4.3. The crude protein content data obtained in section 2.4.3 was also used to calculate faecal N output.

Urine was collected in 19 litre buckets placed behind the steers, in a collection pit. The collection buckets contained concentrated (50% $^{v}/_{v}$) hydrochloric acid, sufficient to lower the pH of the urine to two (see section 1.5.2.2), thus avoiding serious loss of nitrogen.

The urine was collected concurrently with the faeces every morning at 8.30 a.m. Acidity was checked prior to weighing, and if required, more acid was added. Weights were recorded to an accuracy of $\pm$ 0.0025 kg and 10% aliquots were taken, after thorough mixing, for individual bulked composite samples stored in lidded buckets at 5°C.

The individual animal composite urine samples were later mixed thoroughly and sub-sampled for determination of both specific gravity (by weight) and total N content (Kjeldahl Method, AOAC, 1965).

From the feed intake (section 2.4.4) and chemical analysis data (section 2.4.2), total N intakes during the collection period were calculated, and by subtracting from these the total faecal and urinary N produced during the same period, the N balance status of the animals under the different treatments was determined.

2.4.6 Liveweight Gain

The steers were weighed at 8.00 a.m. on two consecutive days each week, usually Mondays and Tuesdays. The animals were not starved prior to weighing, the recording of liveweights at exactly the same point in the feeding cycle each day being considered to combat gut fill variation between animals equally effectively (see section 1.5.2.1).

An Avery weigh-bridge accurate to $\pm$ 0.25 kg was used for measuring the liveweights of the steers and was checked periodically for accuracy.

The liveweight of any steer in any one week was taken as the mean of the two weighings for that week, and overall liveweight gain

was calculated by averaging changes in liveweight over two three-week periods.

No liveweight measurements were made whilst the steers were harnessed for urinary and faecal collections.

2.5 STATISTICAL PROCEDURES

Analysis of variance and covariance models in conjunction with Duncan's Multiple Range Test for testing the significance of differences between means formed the basis of the statistical analysis of the data. It was intended, where possible, to use covariance models not only to test with greater precision for differences between group means, but also to examine relationships between variates. However, a general lack of relationship between the standardisation and comparison period data (see section 4.1) made the extensive use of covariance analysis (ancova) unnecessary.

As a preliminary to the data analyses using the models described in this section, homoscedasticity was verified by the F-max. test rather than the more conventional Bartlett's Test. The former was considered to be less sensitive to the less important departures from normality than the latter (Sokal and Rohlf, 1969).

2.5.1 Physical Analysis of Rations, and 2.5.2 Chemical Analysis of Feeds

These data required no statistical analysis.

2.5.3 Digestibility of Rations

Significantly different ($P < 0.01$) regression coefficients within treatments (see Appendix IX) meant that analysis of the DM digestibility data by standard ancova, using the SP data as the concomitant variable, was not possible. The treatments were considered to be fixed effects (Searle and Fawcett, 1970; Searle, 1971) and a type I analysis of variance (anova) (Eisenhart, 1947) was used to analyse the data according to Model (1).

$$y_{ij} = \mu + t_i + e_{ij} \qquad \text{-----(1)}$$

where μ = a general mean

t_i = the effect of the ith treatment, i = 1 ... 3

and e_{ij} = the residual error, which is assumed to have zero mean, constant variance and no correlation with other errors.

Because a fixed effects model was assumed, the computation of F statistics was based on the expectations of mean squares shown in generalised form for the more complex three-way model, number (2) (see table 2.2). To minimise the risk of erroneous conclusions from anova analysis, the SP data were tested for homogeneity among treatment group means also using Model (1).

Model (1) was again used in analysing both the fibre and crude protein digestibility data, although only one example of the analyses, that of DM digestibility, is presented in Appendix IX.

In the analysis of the DOM value data, precisely the same set of circumstances prevailed as for the DM digestibility data, precluding the use of standard ancova analysis. Consequently, Model (1) was used in the analyses of the DOM, DE, ME and digestible crude protein content data.

2.5.4 Voluntary Intake

Despite a significant ($P < 0.05$) regression relationship between comparison period DM intakes and those of the standardisation period, covariance analysis was ineffective in reducing the residual variance or error mean squares. As an analysis of variance showed no significant differences between the SP treatment means, a three-way, type I anova was employed in analysing the comparison period DM intake data according to Model (2) (see Appendix X).

$$y_{ijk} = \mu + t_i + p_j + b_k + (tp)_{ij} + (tb)_{ik} + (pb)_{jk} + e_{ijk} \quad ---(2)$$

where μ = a general mean

t_i = the effect of the ith treatment, i = 1 ... 3

p_j = the effect of the jth period, j = 1 ... 6

b_k = the effect of the kth block, k = 1 ... 5

$(tp)_{ij}$ = the effect of the first order interaction between treatment and period

$(tb)_{ik}$ = the effect of the first order intearction between treatment and block

$(pb)_{jk}$ = the effect of the first order interaction between period and block

e_{ijk} = the residual error about which the same assumptions are made as shown for Model (1) It should be noted that the effect of the second order interaction $(tpb)_{ijk}$ cannot be separated out in this particular model, because there is no replication in sub-group ijk.

In order to calculate valid F values for these effects it was

necessary to consider their expected mean squares in an analysis of variance (Sokal and Rohlf, 1969). A generalised terminology is used in order to avoid repetition of the following table (Table 2.2) where other similar type I, fixed effects models are used.

TABLE 2.2: Type I anova for Model (2) with expected mean squares

Source of Variation	df	Expected Mean Squares
Main Effects		
T	a*-1	$\sigma_e^2 + \frac{bc}{(a-1)} \sum_i^a t_i^2$
P	b-1	$\sigma_e^2 + \frac{ac}{(b-1)} \sum_j^b p_j^2$
B	c-1	$\sigma_e^2 + \frac{ab}{(c-1)} \sum_k^c b_k^2$
First Order Interaction		
T x P	(a-1)(b-1)	$\sigma_e^2 + \frac{c}{(a-1)(b-1)} \sum_{ij}^{ab} (tp)_{ij}^2$
T x B	(a-1)(c-1)	$\sigma_e^2 + \frac{b}{(a-1)(c-1)} \sum_{ik}^{ac} (tb)_{ik}^2$
P x B	(b-1)(c-1)	$\sigma_e^2 + \frac{a}{(b-1)(c-1)} \sum_{jk}^{bc} (pb)_{jk}^2$
Error (including second order interaction	(a-1)(b-1)(c-1)	$\sigma_e^2 + \frac{1}{(a-1)(b-1)(c-1)} \sum_{ijk}^{abc} (tpb)_{ijk}^2$

* The notation a, b and c refers to the number of each of the main effects in the analysis.

As can be seen from the expected mean squares in Table 2.2, to enable strictly valid computation of F values (i.e. significance testing) it is necessary to assume that the added mean square due to the second-order interaction ($\frac{1}{(a-1)(b-1)(c-1)} \sum_{ijk}^{abc} (tpb)_{ijk}^2$) is equal to zero. If however, a second order interaction does exist, as is likely with this DM intake data, the bias is not too serious provided the interaction is not large. The F value is merely deflated and the probability of a type I error, in fact, becomes less than that stated.

On completing the DM intake analysis using Model (2), a highly significant T x P interaction effect was found. To obtain more information on this important interaction, a two-way, fixed effects serial ancova (Appendix XI) was carried out according to Model (3).

$$y_{ijk} = \mu + t_i + p_j + (tp)_{ij} + \beta_{j,j-1}x_{ijk} + e_{ijk} \quad \text{---- (3)}$$

where μ = a general mean

t_i = the effect of the ith treatment, i = 1 ... 3

p_j = the effect of the jth period, j = 1 ... 6

$(tp)_{ij}$ = the effect of the treatment by period interaction

$\beta_{j,j-1}$ = the coefficient of the regression of period j on period j-1 DM intakes

x_{ijk} = $(X_{ijk} - \bar{X})$ where X_{ijk} is DM intake in period j-1

e_{ijk} = the residual error about which the same assumptions are made as shown for Model (1)

The confounding influence of adaptation responses by the steers to the CP maize silage rations led to the ommission of certain periods from the analysis of the intake data. The same type I anova, according to Model (2), with period effect modified, was employed. Only one example of this modified analysis, that of DM intake expressed as a percentage of liveweight, is presented in Appendix XII. However, all other intake measures not included in Appendix XII, crude protein, digestible crude protein, true protein, non-protein nitrogen, DOM, DE and ME were also analysed using Model (2), modified for period effect by removal of period one.

2.5.5 Nitrogen (N) Balance

A non-significant ($P > 0.05$) regression relationship between CP and SP N balance data prevented the use of ancova analysis. Consequently a fixed effects, two-way anova was used according to Model (4) the SP data also being tested as a safeguard against drawing erroneous conclusions (see Appendix XIII).

$$y_{ij} = \mu + t_i + b_j + e_{ij} \quad \text{---- (4)}$$

where μ = a general mean

t_i = the effect of the ith treatment, i = 1 ... 3

b_j = the effect of the jth block, j = 1 ... 3

e_{ij} = the residual error, about which the same assumptions are made as shown for Model(1). It should be noted that

the interaction effect $(tb)_{ij}$ cannot be separated out in this model because there is no replication in sub-group ij.

Tests of significance were based on the expectation of mean squares as shown in generalised form in Table 2.2, with the same assumption about the confounding of the interaction effect with the residual error.

Model (4) was also used in the analysis of both the N intake and urinary N excretion data, while the faecal N excretion data was tested using Model (1). Only the one example of these analyses, that of N retention, is presented in Appendix XIII.

2.5.6 Liveweight Gain

Once again a lack of relationship between CP and SP data prevented the use of ancova analysis. Instead, a three-way, fixed effects anova model similar to Model (2), as shown in 2.5.4, was used (see Appendix XIV). The only modification was in the period effect, where (j = 1, 2) replaced (j = 1 ... 6). The same assumptions about the magnitude of the second order interaction were made and the same procedure followed in the computation of F values.

A highly significant T x P interaction (see Appendix XIV) resulted from this analysis of the liveweight gain data. While information was not available for the further analysis of period two gains (see section 2.4.6), intermediate liveweights for period one enabled investigation of the cause of the T x P interaction in that period. This was done by serial covariance analysis of weekly mean liveweights. A one-way, fixed effects ancova based on Model (5) was used (see Appendix XV).

$$y_{ij} = \mu + t_i + \beta x_{ij} + e_{ij} \qquad \text{-----(5)}$$

where μ = a general mean

t_i = the effect of the ith treatment i = 1 ... 3

β = the coefficient of the regression of liveweight on the preceding week's liveweight

$x_{ij} = (X_{ij} - \bar{X})$, where X_{ij} is liveweight for any week less one, in period one

e_{ij} = the residual error about which the same assumptions are made as shown for Model (1).

The serial ancova strongly indicated DM intake to be having a confounding influence on measured liveweight gain, and correction for intake was considered desirable. To this end, liveweight gain within

each three-week period was regressed on DM intake per 100 kg LW for that period and the adjusted means analysed by one-way, fixed effects ancova according to Model (6) (see Appendix XVI).

$$y_{ij} = \mu + t_i + \beta x_{ij} + e_{ij} \qquad \text{-----(6)}$$

where μ = a general mean

t_i = the effect of the ith treatment, i = 1 ... 3

β = the coefficient of the regression of liveweight gain within each period, on DM intake per 100 kg liveweight for that period

$x_{ij} = (X_{ij} - \bar{X})$, where X_{ij} is DM intake per 100 kg liveweight, within a period

e_{ij} = the residual error, about which the same assumptions are made as shown for Model (1).

CHAPTER THREE

RESULTS

Data presentation in this chapter is brief, its extent being determined only by compatability with the purpose of accurate representation of results. Repeated reference is made, throughout, to the appendices where supporting information and pertinent data analyses can be found. Unless otherwise stated, data variability is expressed in the form of standard error of the mean. To facilitate ease of cross reference, the chapter is designed to follow the layout of section 2.4 of Methods and Materials, Experimental Procedures.

3.1 PHYSICAL ANALYSIS OF RATIONS

The component feed contents of the dry matter of the three Comparison Period (CP) rations are presented in Table 3.1.

TABLE 3.1: Component feed contents of the dry matter of the CP rations

Treatment	Percentage Composition (DM basis)	
HB	Hay	56 ± 4
	Barley Meal	44 ± 4
MS	Maize Silage	99.0 ± 0.05
	Mineral Supplement	1.0 ± 0.05
MSB	Maize Silage	97.5 ± 0.1
	Mineral Supplement	0.9 ± 0.03
	Biuret	1.6 ± 0.06

Table 3.2 contains the results from the further physical analysis of the maize silage.

TABLE 3.2: Physical composition of the maize silage

Source of Material	Component	Percentage Composition (DM basis)	
At Harvest	Grain	45.0 ± 1.0	Ear = 57.0
	Husk	12.0 ± 0.4	
	Leaf	11.5 ± 0.5	Leaf = 17.0
	Leaf Sheath	5.5 ± 1.0	
	Stem	26.0 ± 1.0	
After Ensiling	Grain	32.0 ± 5.0	

3.2 CHEMICAL ANALYSIS OF FEEDS

The proximate analysis (AOAC, 1965) of the CP rations is shown in Table 3.3.

TABLE 3.3: Proximate analysis of CP rations (DM basis)

Chemical Fractions	Ration Components %	HB %	Ration Components %	MS %	Ration Components %	MSB %
Dry Matter	Hay 34±5 Barley 89±1	85	Maize Silage 31±2 Mineral Supplement 97.6	32	Maize Silage 31±2 Mineral Supplement 97.6 Biuret 91.2	33
Crude Fibre	Hay 29.0 Barley 5.3	18.6	Maize Silage 19.5	19.5	Maize Silage 19.5	19.5
Crude Protein	Hay 10.0 Barley 12.5	11.1	Maize Silage 9.7 Mineral Supplement 10.3	9.7	Maize Silage 9.7 Mineral Supplement 10.3 Biuret 250.0	13.6
Ash	Hay 7.9 Barley 2.9	5.7	Maize Silage 6.6 Mineral Supplement 83.0	7.4	Maize Silage 6.6 Mineral Supplement 83.0 Biuret 0.1	7.2
Ether Extract	Hay 4.5 Barley 1.0	3.0	Maize Silage 3.9	3.9	Maize Silage 3.9	3.8
Nitrogen Free Extract	Hay 48.6 Barley 78.3	61.6	Maize Silage 60.3	59.5	Maize Silage 60.3	55.9

This analysis was performed largely to enable comparisons to be made with silages used by other workers. It is considered by the writer to be not entirely satisfactory (see 4.2.2), and further analyses of the carbohydrate fraction were carried out (see 2.4.2). Results are presented in table 3.4.

TABLE 3.4: Analysis of the carbohydrate fractions of the CP rations by the acid detergent fibre and neutral detergent fibre techniques (Van Soest, 1963, 1967) (% of total DM)

Chemical Fractions	Ration Components %	HB %	Ration Components %	MS %	Ration Components %	MSB %
Acid Detergent Fibre (Lignin + Cellulose)	Hay 36.2 Barley 7.4	23.5	Maize Silage 26.3	26.0	Maize Silage 26.3	25.6
Neutral Detergent Fibre (Cell Wall Consituents - lignin, cellulose, hemicellulose & bound protein and ash)	Hay 61.1 Barley 20.5	43.2	Maize Silage 37.1	36.7	Maize Silage 37.1	36.2
Readily fermentable carbohydrate (incl.starch)	Hay 19.5 Barley 67.3	40.8	Maize Silage 44.2	43.8	Maize Silage 44.2	43.1

The further analysis of the crude protein fraction of the rations is presented in table 3.5, while the calcium, phosphorus and sulphur contents are shown in table 3.6.

TABLE 3.5: Composition of the crude protein fraction of the CP rations (% of total DM)

Chemical Fractions	Ration Components %	HB %	Ration Components %	MS %	Ration Components %	MSB %
True Protein	Hay 7.7 Barley 11.0	9.2	Maize Silage 3.1	3.1	Maize Silage 3.1 Biuret 0.0	3.0
NPN - Protein (N x 6.25)	Hay 2.3 Barley 1.5	1.9	Maize Silage 6.6	6.5	Maize Silage 6.6 Biuret 250.0	10.4

TABLE 3.6: Calcium, phosphorus and sulphur contents of the CP rations

Mineral	Ration Components %	HB %	Ration Components %	MS %	Ration Components %	MSB %
Ca	Hay 1.230 Barley 0.056	0.71	Maize Silage 0.460 Mineral Suppl.12.341	0.53	Maize Silage 0.460 Mineral Suppl.12.341	0.56
P	Hay 0.280 Barley 0.340	0.31	Maize Silage 0.270 Mineral Suppl. 4.928	0.32	Maize Silage 0.270 Mineral Suppl. 4.928	0.31
S	Hay 0.22 Barley 0.14	0.18	Maize Silage 0.13 Mineral Suppl. 0.47	0.13	Maize Silage 0.13 Mineral Suppl. 0.47	0.13
N/S	Hay 7/1 Barley 14/1	10/1	Maize Silage 12/1	12/1	Maize Silage 12/1	17/1

3.3 DIGESTIBILITY OF RATIONS

The DM, fibre (Van Soest, 1963) and crude protein (AOAC, 1965) apparent digestibilities of the three CP rations are presented in Table 3.7.

TABLE 3.7: Apparent digestibilities of the DM, fibre and crude protein fractions of the CP rations

Ration	DM Digestibility (%)	Fibre (Cellulose + Lignin) Digestibility (%)	Crude Protein Digestibility (%)
HB	67	48	62
MS	62 ± 1	50 ± 1.5	57 ± 1
MSB	62	47	64

The statistical significance of the differences between treatment means for each of the above parameters is shown in Table 3.8. An example of their analysis by anova, using model (1), is presented in Appendix IX.

TABLE 3.8: Statistical significance of the differences between treatment means for DM, fibre and crude protein digestibilities

DM Digestibility	Fibre Digestibility	Crude Protein Digestibility
HB > MS**	No Significant Differences	HB > MS**
MS = MSB ($P > 0.05$)		MS < MSB**
MSB < HB**		MSB = HB ($P > 0.05$)

** 1% level of significance.

The digestible organic matter (DOM) and digestible energy (DE) contents of the CP rations, as measured, are contained in Table 3.9, along with estimated metabolisable energy (ME) contents (Blaxter et al, 1966; Bryant, 1971). In Table 3.10 are presented the digestible crude protein contents.

TABLE 3.9: DOM, DE and ME contents of the CP rations, including the apparent digestibility of GE

Ration	DOM (% of DM)	DE (% of GE)	DE (Kcal/kg DM)	ME (Kcal/kg DM)
HB	56	67	2980	2430
MS	59 ± 1	61 ± 1	2670 ± 40	2210 ± 30
MSB	59	61	2640	2170

There were no significant differences between the MS and MSB rations in terms of DOM content, percentage digestibility of GE, and DE and ME contents. In each case however, the HB ration was significantly higher ($P < 0.01$).

TABLE 3.10: Digestible crude protein contents of CP rations

Treatment	Dig. Crude Protein Content (% of DM)
HB	6.9
MS	5.5 ± 0.1
MSB	8.7

All digestible crude protein content treatment means were highly significantly different from each other ($P < 0.01$).

3.4 VOLUNTARY INTAKE

Treatment mean daily dry matter intakes averaged over each week of the experiment are shown graphically in Figure 3.1. Overall mean daily dry matter intakes for the three groups of steers during the comparison period are presented in Table 3.11.

TABLE 3.11: Mean daily dry matter intakes of treatments during the comparison period (kg/day)

Treatment	All Six Periods	Period One Omitted	Periods One & Two Omitted	Periods One, Two & Three Omitted
HB	6.04	6.08	5.91	5.74
MS	5.58 ± 0.08	5.91 ± 0.08	5.96 ± 0.06	6.05 ± 0.06
MSB	5.17	5.56	5.75	5.98

A series of figures relating to differing time periods are presented, since at the start of the comparison period the two maize silage groups were suddenly introduced to their experimental rations, and a period of adaption to the new diet could be expected.

Consideration is given to the statistical significance of the differences between treatments in Table 3.12. An example of the analysis of these data, using Model (2) is presented in Appendix X.

TABLE 3.12: Statistical significance of differences between the treatment mean daily dry matter intakes shown in Table 3.11

All six periods	Period One Omitted	Periods One & Two Omitted	Periods One, Two & Three Omitted
HB $\geq$ MSB**	HB $\geq$ MSB**	MS $\geq$ MSB*	MS $>$ HB**
HB $>$ MS**	HB = MS ($P \geq 0.05$)	MS = HB ($P > 0.05$)	MS = MSB ($P > 0.05$)
MS $\geq$ MSB**	MS $>$ MSB**	HB = MSB ($P \geq 0.05$)	MSB $>$ HB*

* 5% level of significance

The time trend in the animals' response (see Appendix X) showed DM intakes during period one to be significantly lower ($P < 0.01$) than during the five subsequent periods, which did not differ significantly among themselves. Moreover, a serial covariance analysis using Model (3)·· (see 2.5.4), showed the significant treatment by period interaction of Model (2), apparent in periods one, two and three (Table X.v, Appendix X), to be at least partially generated by the large drop in intakes of the two silage groups in period one (see Figure 3.1 and Appendix XI).

With these analyses indicating adaptation responses to the maize silage rations, most evident in the first period, to be so powerfully influencing results, it was considered that most meaningful estimates of DM intake for comparative purposes would be those excluding period one. Such figures are reproduced in Table 3.13, along with equivalent data expressing intake as a percentage of liveweight.

TABLE 3.13: Mean daily DM intakes of CP rations with period one omitted

Treatment	Ration DM Intake kg/day		Component Feeds DM Intake kg/day	DM Intake (% Liveweight)	
HB	6.08±0.08	HB > MSB**	Hay 3.40 Barley Meal 2.68	2.68±0.03	HB > MSB**
MS	5.91±0.08	MS = HB ($P > 0.05$)	Maize Silage 5.85 Mineral Suppl. 0.06	2.72±0.03	MS = HB ($P > 0.05$)
MSB	5.56±0.08	MSB < MS**	Maize Silage 5.41 Mineral Suppl. 0.05 Biuret 0.09	2.56±0.03	MSB < MS**

In the analysis of the DM intake data for the entire comparison period, using Model (2) (see Appendix X), the first order interactions contributed significantly ($P < 0.01$) to the total mean squares. Consideration of the DM intake response to treatment must therefore

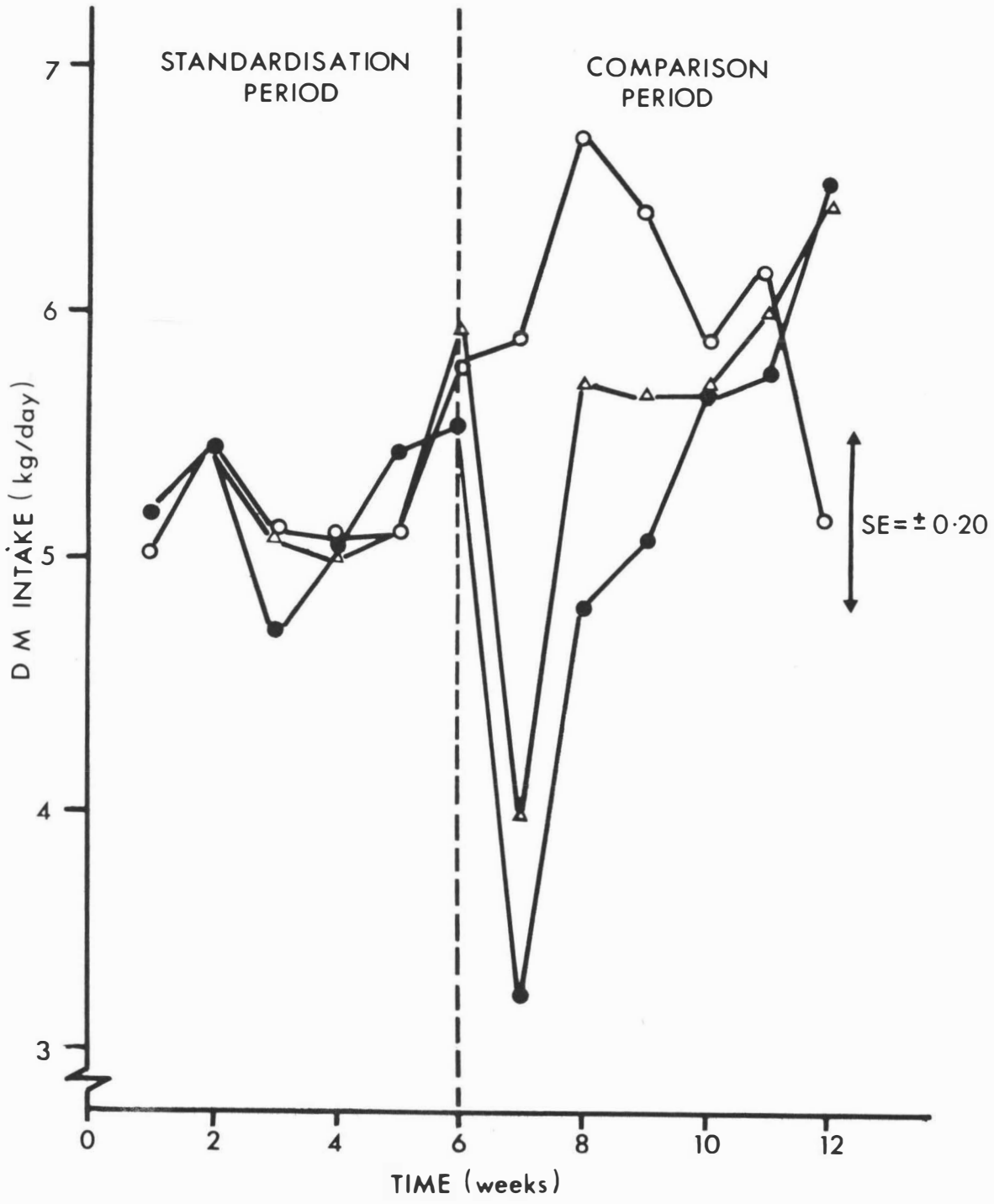

FIGURE 3.1: Treatment mean daily DM intakes averaged over each week, showing the treatment by period interaction

○ HB △ MS ● MSB

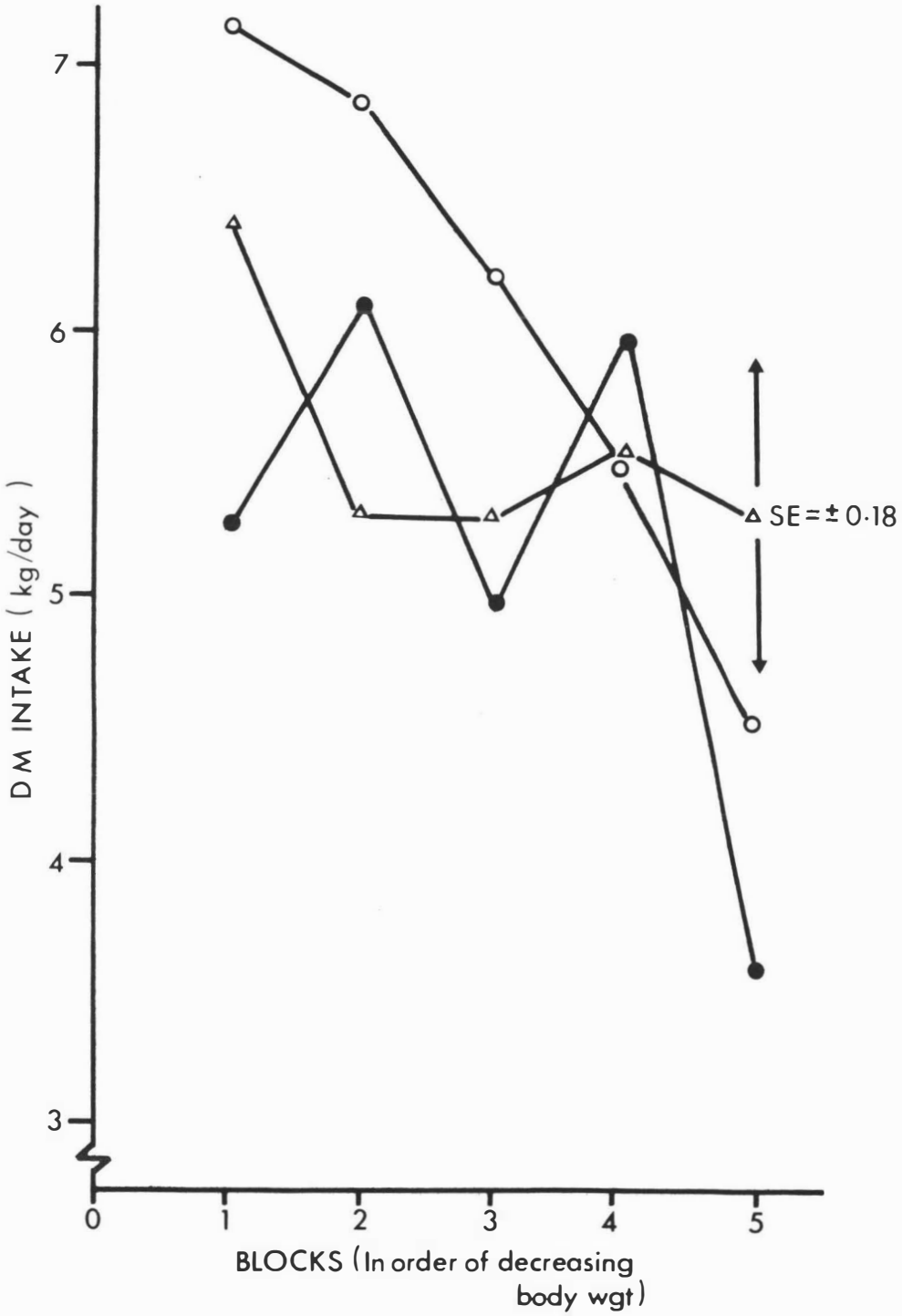

FIGURE 3.2: Treatment mean daily DM intakes during the comparison period, plotted against blocks to indicate the cause of the treatment by block interaction

○ HB △ MS ● MSB

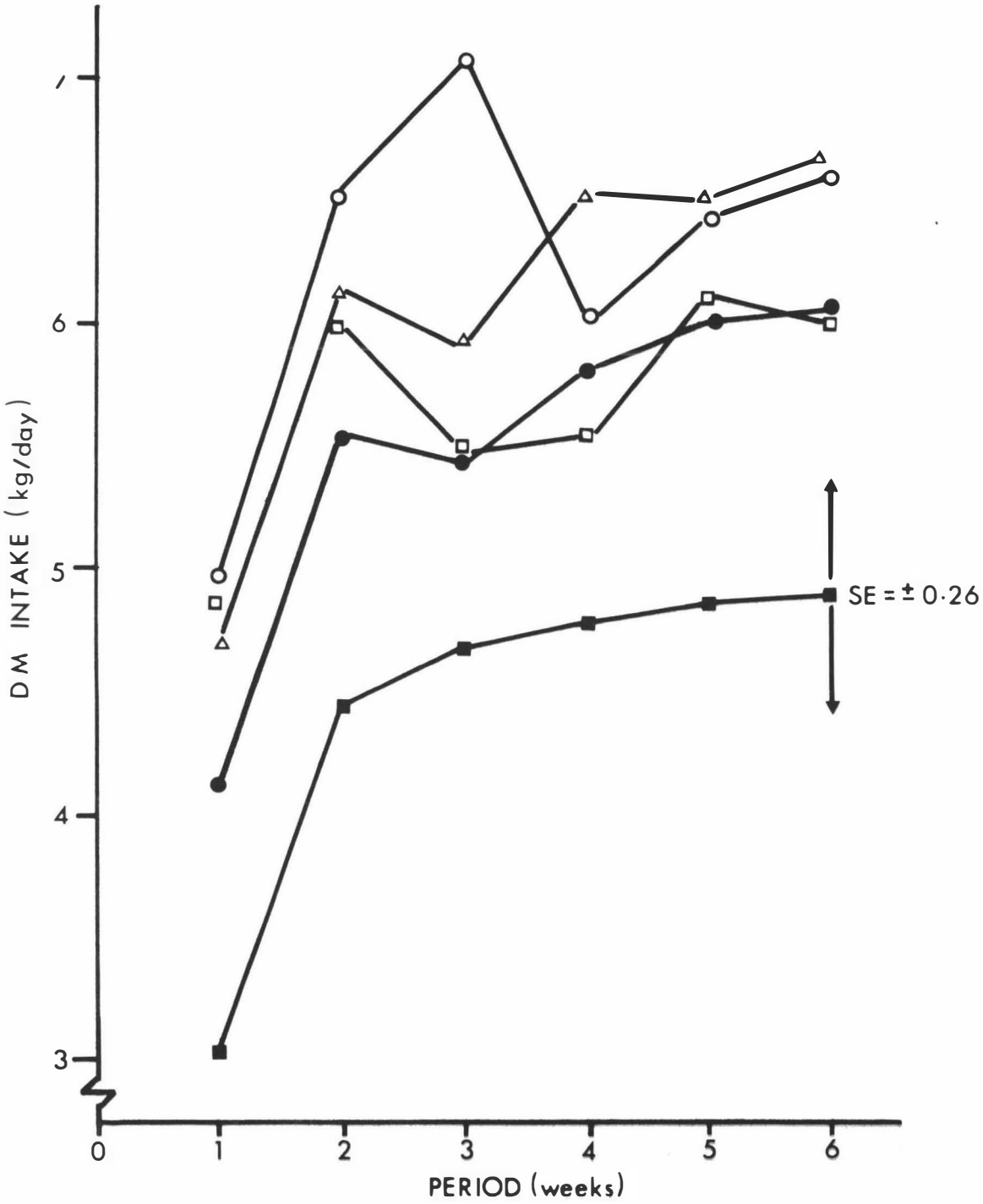

FIGURE 3.3: Comparison period mean daily DM intakes of blocks plotted against time, showing the absence of a period by block interaction.

○ Block 1 △ Block 2 ● Block 3 □ Block 4 ■ Block 5

include these interactions. They are presented graphically in Figures 3.1, 3.2, and 3.3, and are discussed in section 4.2.4.

The DOM and DE intakes, together with calculated ME intakes, are presented in Table 3.14, while N intakes, in various forms, are contained in Table 3.15.

TABLE 3.14: Mean daily DOM, DE and ME intakes of treatments, based on the DM intake data of Table 3.13 (i.e. Period one omitted)

Treatment	DOM Intake kg/day	DE Intake Megacalories/day	ME Intake Megacalories/day
HB	4.01	18.1	14.8
MS	3.49 ± 0.05	15.8 ± 0.2	13.1 ± 0.2
MSB	3.28	14.7	12.1

All intake parameter means in Tables 3.14 and 3.15 were analysed using Model (2) (2.5.4) modified by exclusion of period one. An example of this type of anova (DM intake as a percentage of liveweight) is shown in Appendix XII. The treatment means for DOM, DE and ME intakes all differed from each other highly significantly ($P < 0.01$).

TABLE 3.15: Mean daily crude protein, digestible crude protein, true protein and non-protein nitrogen intakes of treatments, based on the DM intake data of Table 3.13

Treatment	Crude Protein Intake kg/day	Digestible Crude Protein Intake kg/day	True Protein Intake kg/day	Non-Protein Nitrogen Intake kg/day
HB	0.67	0.418	0.559	0.018
MS	0.57 ± 0.01	0.327 ± 0.006	0 185 ± 0.005	0.059 ± 0.001
MSB	0.76	0.484	0.169	0.095

The treatment means for the intake parameters contained in Table 3.15, except true protein intake, were all highly significantly different from each other ($P < 0.01$). The true protein intake for the HB ration was significantly greater than that for either the MS or MSB

rations ($P < 0.01$), while the difference between the two maize silage rations was significant only at the 5% level.

3.5 NITROGEN (N) BALANCE

Mean daily N intakes, and urinary and faecal N outputs, recorded during the comparison period, are presented in Table 3.16.

TABLE 3.16: Mean daily N intake, urinary N output, faecal N output and N balance for each treatment during the comparison period

Treatment Group	N Intake g/day	Faecal N Output g/day	Urinary N Output g/day	N Balance g/day
HB	107	40	40	27
MS	96 ± 5	42 ± 3	30 ± 2	24 ± 3
MSB	141	51	65	25

Table 3.17 contains a summary of the statistical significance of the differences between the three treatments for each of the parameters shown in Table 3.16. These data were all analysed using Model (4), except faecal N, for which Model (1) was employed (see 2.5.5). An example of these analyses, that of the N retention data, is presented in Appendix XIII.

TABLE 3.17: Statistical significance of differences between treatment mean daily N intake, faecal N output, urinary N output and N balance during the comparison period

Daily N Intake	Faecal N Output	Urinary N Output	N Balance
MSB > MS**	MSB > HB ($P < 0.10$)	MSB > MS**	No Significant
MSB > HB**	MSB > MS ($P < 0.10$)	MSB > HB**	Differences
HB = MS ($P > 0.05$)	MS = HB ($P > 0.10$)	HB > MS*	

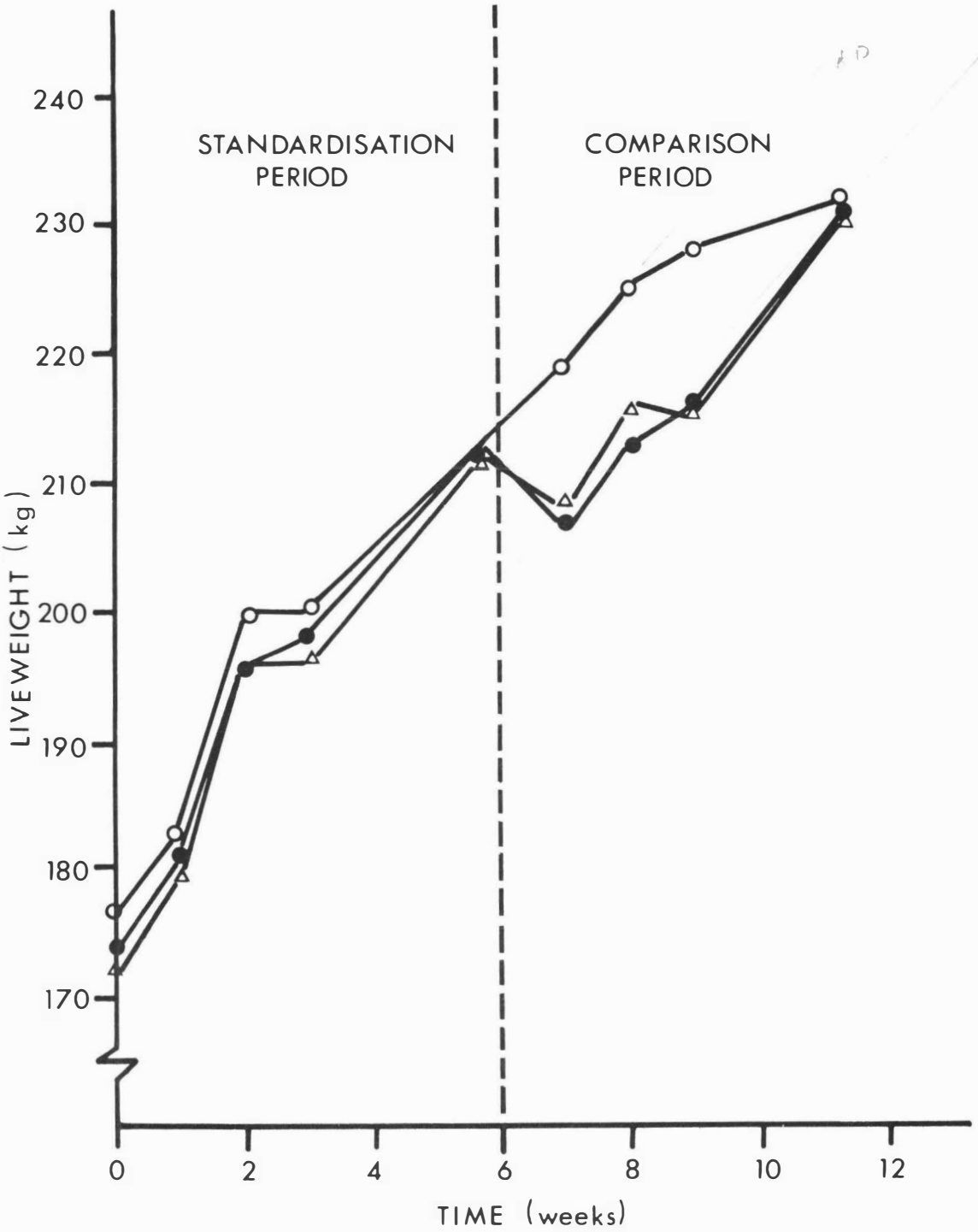

Figure 3.4: Weekly mean liveweights for each treatment in both the standardisation and comparison periods.

○ HB △ MS ● MSB

analysis of the liveweight gain data by Model (2) can be found in Appendix XIV.

TABLE 3.18: Treatment mean daily liveweight gains during the comparison period

Treatment	Mean Daily Liveweight Gains over Six-week Comparison Period (kg/day)
HB	0.44
MS	0.52 ± 0.06
MSB	0.51

While there were no significant differences between the three treatment means, rates of gain were on average significantly higher ($P \leq 0.01$) in period two than in period one. However, because of the highly significant treatment by period (T x P) interaction ($P < 0.01$), the period effect has limited meaning. The T x P interaction (see Appendix XIV) reveals that while in period one (first three weeks of the comparison period) the mean rate of gain of the HB treatment was significantly higher than either the MS or MSB treatment ($P < 0.01$), during period two, precisely the reverse situation existed. For the MS and MSB treatments, the increase in rate of gain from period one to period two was significant at the 1% level, while the decrease in rate of gain for the HB treatment during the same time was significant at the 5% level.

Unfortunately, the absence of intermediate liveweights in the period two data prevented further statistical consideration of the apparent depression in rate of gain of the HB treatment during that period. It is suggested, however, that it was possibly a result of the highly significant reduction in intake ($P < 0.01$) (see Figure 3.1 and Appendix X) of the HB group in the sixth, compared with the fifth week of the comparison period. This was likely the result of an unavoidable change in the nature of the hay fed the HB group during the last week of the comparison period.

The availability of intermediate liveweights in period one made possible the further analysis of the significant T x P sub-group differences during that period. Serial ancova analysis of mean liveweights for each of the three weeks during period one, using Model (5)

indicated the between treatment differences in period one to have in fact been generated in the first week of the three week period. An example of these serial ancova analyses is presented in Appendix XV. Reference to Appendices X and XI further reveals that it was in this same first week of the comparison period that the significant T x P interaction in DM intake over weeks one, two and three was largely generated. It would seem clear that the 30 to 40% decrease in mean DM intake during week one of the comparison period (see Figure 3.1) as the MS and MSB treatments adapted to completely new diets, had a considerable effect on apparent rate of liveweight gain.

On the basis of this evidence it was felt that more meaningful interpretation of the treatment effects could be obtained by adjustment of the liveweight gain data through regression on DM intake. To overcome the influence of animal size on maintenance intake requirements, the DM intake data were expressed per 100 kg liveweight. The adjusted data were analysed by ancova using Model (6) (see Appendix XVI), and the treatment means are presented in Table 3.19.

TABLE 3.19: Mean liveweight gains of CP treatments adjusted by regression on DM intake per 100 kg LW

Treatment	Adjusted Mean Daily Liveweight Gain kg/day		Significance of Differences
HB	0.30	± 0.08	HB < MSB*
MS	0.48		HB = MS (P > 0.10)
MSB	0.70		MSB > MS (P < 0.10)

The implications of this analysis are discussed in section 4.2.6.

CHAPTER FOUR

DISCUSSION OF RESULTS

In the main, this chapter is confined to the discussion of results as presented in Chapter Three. Where, however, ideas emerge from consideration of the results, and these have been tested, new material may be presented.

The chapter falls into two sections, the first of which considers the success of the experiment as designed. Discussion of the actual results forms the second section and follows precisely the layout of Chapter Three.

4.1 A POSTERIORI CONSIDERATION OF THE EXPERIMENTAL DESIGN

On the evidence of Kincaid *et al* (1945), Ashton *et al* (1955b) and Bailey *et al* (1958), it was suggested in Section 1.5.1 that under stringent managerial conditions with covariance design, a coefficient of variation of 10% in measured liveweight gain might be expected. With the five animals per treatment and three treatments, this would have permitted detection of between treatment differences of 0.15 kg at the 5% level of significance with a probability of 65%.

However, the coefficient of variation for liveweight gain in the present study was found to be 41%, and covariance analysis was of no direct use whatsoever. Only the more sophisticated anova procedures followed would have enabled significant differences of 0.20 kg/day to have been detected at the 5% level, had they existed. The question immediately arises as to the reasons for both the ineffectiveness of the covariance design and the high variability of the liveweight gain data.

Beyond doubt, DM intake exerted a powerful influence on liveweight gain during the comparison period (see Figures 3.1, 3.4 and XVI.1) despite the standardised weighing and regular feeding practices adopted. In light of this fact, the writer, in reconsidering the procedures followed during the experiment, would strongly criticise the decision to switch the MS and MSB treatments from the hay and barley ration of the standardisation period directly to the maize silage of the comparison period. This sudden change effectively obliterated the desirable stabilising influence of the standardisation period and resulted in the interpretative problems of adaptation responses in the CP data.

The other rather unfortunate managerial circumstance, which would tend to inflate the variability of weight gain, was the slight change in the nature of the hay of the HB treatment in the sixth week of the comparison period. This was both unavoidable and undesirable, and together with the adaptation response to the maize silage feeding, is considered responsible, at least in part, for the high coefficient of variation of the liveweight gain data.

Of greater concern, however, was the ineffectiveness of the covariance design in reducing the coefficient of variation of adjusted treatment means. The CP gain data failed to bare any significant relationship to that of the standardisation period (Section 2.5.6).

This seemed to be in complete contrast to the evidence presented in Section 1.5.1. Further perusal of the literature, however, reveals an important omission from the considerations discussed in Section 1.5. That is, that while the advantages of covariance design have been variable (Green et al, 1952), they have been consistently absent from short term growth studies lasting up to 10 weeks (Patchell, 1956; Bailey et al, 1958). The reasons for this are not obscure and should have been realised in planning the present experiment.

It was shown by Knapp and Clark (1947) that in three successive 84-day periods, the liveweight gain correlation was considerably greater between the second and third periods than either the first and second, or the first and third periods. Genetic influences accounted for 10,54 and 84% of the variation in gains for each of the three periods respectively. This is consistent with the reports of both Ashton et al (1955c) and Bailey et al (1958), showing reduced coefficients of variation for liveweight gain with time.

The success of the covariance design is clearly dependent on the proportion of the error variance which is due to the between animal variability in inherent growth characteristics and genetic potential. In short term growth studies of six weeks, such as the present, it is clear that these genetic influences account for a surprisingly small proportion (less than 10%) of the variability in liveweight gain (Knapp & Clark,1947; Bailey et al, 1958). In view of this evidence, the non-significance of the regression of CP weight gain on SP weight gain is not surprising and similar circumstances would explain the poor relationship in the N retention data.

In terms of DM intake, Stone et al (1960) showed consistent intake ranking of cattle on a variety of forages, and a regression relationship between the two periods of observation in this present study might have been expected. While a significant regression was recorded (see 2.5.4) it was of no value in reducing the error variance in ancova analysis.

The digestibility data also provided significant CP/SP relationships, but within-treatment regression coefficients were significantly different between treatment groups (see Figure IX.1). The reasons for this are obscure. If meaningful, the regressions show that the steers with the highest DM digestibility on the HB ration in the standardisation period, held the same ranking in the comparison period

on the HB and MSB rations, but ranked in exactly the opposite order on the MS ration. The chemical characteristics of the three rations (see Section 3.2) were not sufficiently different to support a sound argument for adaptation in explaining this finding. It is therefore considered an artifact.

In contrast to the inefffectualness of the covariance design in analysis of the measured parameters, the randomised block feature of the design proved most successful in reducing the unaccountable variation especially with the intake data. The procedure also provided three treatment groups with almost identical liveweights at the start of the comparison period, a consideration greatly assisting interpretation of results. Randomised blocking is suggested as being essential in this type of growth study.

Were the experiment to be redesigned under the same limiting circumstances of animal numbers and length of time available for a comparison period, it would not be altered greatly. The standardisation period is considered important in its stabilising effect on animals' rates of gain. The change to the comparison period rations, however, would be made gradually so as to avoid the confounding influence of large intake fluctuations on liveweight gain. Such a procedure should reduce the coefficient of variation for weight gain below the 41% recorded in the present study, and result in a more sensitive experiment.

Should more animals and a greater time period have been available it is important, on the basis of the present findings, to ascertain the relative advantages of increased animal numbers and increased length of comparison period. In this situation, interest no longer centres on the treatments, blocks, and periods as specific, fixed factors. Rather, they can be considered merely as examples of a range of possible selections from a population of each. Under these circumstances the liveweight gain analysis using model (2), changes from a type I to a type II anova (Searle,1971), enabling estimation of variance components (see Appendix XVII). While Searle and Fawcett (1970) have elucidated the errors association with a finite rather than the infinite population implied by the type II model, adjustments were not made as they were considered not to be of importance to the present situation.

In this way, it was shown that in an experiment such as the

present, a greater reduction in error variance can be effected by an increase in the number of periods (length of experiment) rather than increasing the number of animals. This conclusion is in accord with the findings of Bailey et al (1958) based on a large number of short term growth studies with cattle. They report greater experimental precision through doubling the comparison period from three to six weeks than through doubling replication from five to ten animals per treatment. For the present study, doubling the number of animals per treatment from five to ten would have in fact effected a lesser reduction in the variance of a treatment mean than lengthening the experiment by one period (i.e. three weeks).

4.2 INTERPRETATION OF RESULTS

4.2.1 Physical Analysis of Rations

The data presented in Table 3.1 would indicate that the ration feeding procedures were satisfactory. To attain the target liveweight gain of 0.75 kg/day, 42% of the DM consumed by the HB treatment had to be barley meal (see Appendix III). The 44% achieved closely approximates this objective. Moreover, it was estimated that the biuret supplementation would raise the crude protein content of the maize silage to between 13 and 14% (DM basis) (see Section 2.2.3). As can be seen from Table 3.3, the quantity of biuret consumed did exactly this.

Comparison of Tables 3.2 and 1.1 reveals that the physical composition of the New Zealand grown maize silage used in the present study, aligns closely with that of silage produced in America from maize harvested at the similar dent-glaze stage of maturity (30-35% DM content). The outstanding feature of this data is the high grain content, 45% of total DM. To further demonstrate its significance, gross energy determinations (adiabatic bomb calorimeter) were made on samples in which the grain had been separated from the rest. Gross energy digestibilities were assumed (Morrison, 1957) and the estimated proportion of the total digestible energy in the grain fraction of the silage at harvest is shown in Table 4.1. It can be seen that the grain constituted approximately 60% of the DE of the silage at harvest.

The importance of not harvesting too early is demonstrated by the only similar work carried out in New Zealand recently, that of Waghorn (1973), where maize harvested only a little earlier at the

TABLE 4.1: Importance of the grain fraction in maize silage

Characteristic	Grain %	Rest %
DM	45	55
Gross Energy	46	54
Digestible Energy*	59	41

* Assumed digestibility of GE - 85% for grain
- 50% for rest.

dough-dent stage of maturity (27% DM) contained only 36% grain (DM basis). The loss in both DM yield and digestible energy content is immediately apparent from both Figure 1.1 and Table 4.1.

One apparent anomaly in the data presented in Table 3.2 is the reduction in measured grain content from levels estimated prior to harvest, to those found following harvest and ensiling. The magnitude of the drop from 45 to 32% grain content is similar to that in the data of Waghorn (1973). It would seem improbable that the missing grain is being fermented during ensilage, as both Barnett (1954) and Johnson et al (1966b) suggest that very little hydrolysis of starch occurs during this process. While the unsuitability of harvesting equipment available for the present work resulted in the loss of considerable grain both in the field and in transit to the silage stacks, the most plausible explanation seems to be that the fine chopping of the material may render part of the grain fraction unrecognisable in the hand separation technique used in this analysis. Consequently, the measured grain content in the maize silage following ensiling is likely to underestimate the influence of the grain on its nutritive value.

4.2.2 Chemical Analysis of Feeds

Comparison of Tables 1.2 and 3.3 reveals a striking chemical similarity between New Zealand produced maize silage and that produced in America. The only differences appear to be in the crude protein and crude fibre contents. The higher crude protein content of the silage being studied (9.7 versus 8.6% of DM) is likely a result of the high level of N fertilizer used to produce the maize crop (see Appendix IV). The influence of N fertilizer levels was clearly established in Section 1.3.1 and is not further discussed. In contrast the slightly lower crude fibre content of the present silage (19.5 versus 23.0) is possibly a

reflection of its higher DM content (31 versus 28.5) and associated higher grain content.

While the crude protein content (Table 3.3) varied between CP rations, 9.7, 11.1 and 13.6% for MS, HB and MSB rations respectively, there was a close similarity between crude fibre contents and, with the exception of MSB, the NFE fractions. The higher crude protein content of the MSB ration was naturally reflected in its lower NFE content.

The question arises, however, as to the meaning of these compositional similarities or dissimilarities. Norman (1935) and Van Soest (1966) have shown quite clearly that while the crude fibre measurement gives some indication of the amount of roughage in a ration, it does not measure the same material in all rations. Although the crude fibre fraction contains only cellulose and lignin, it contains varying amounts of each depending on the feed; the loss of lignin from the fraction often being extensive (Norman, 1935). Consequently, it was considered that more meaningful comparisons between rations could be made on the basis of acid-detergent fibre (ADF) measurements (Van Soest, 1963) which include all of both the lignin and cellulose fractions. Moreover, just as the composition of the crude fibre fraction varies between feeds, so it is likely to vary between feed and the faeces produced from that feed (Norman, 1935), tending to invalidate crude fibre digestibility as a measure of feed value. For this reason, the more reliable ADF measurement was used to determine fibre digestibility in the present study.

Despite its superiority over crude fibre, ADF is probably not the fibre measure most closely correlated with nutritive value (Van Soest, 1971). It has been pointed out by Van Soest (1966) that certain fractions of the hemicellulose, not inluded in the ADF, such as the xylans, can be less digestible than cellulose. Van Soest and Marcus (1964) and Van Soest and Wine (1967) have therefore proposed a neutral-detergent fibre (NDF) measurment which separates the cellular material from the cell wall constituents. The writer considers this the most satisfactory fibre measure, as it distinguishes between the almost completely digestible cellular contents (98% true digestibility), and the cell wall fraction of variable digestibility (Van Soest, 1967). Unfortunately the extreme filtration problems encountered with this technique precluded its use in comparing the digestibilities of the

cell wall constituents of the rations used in the present study. Its measurement in the rations themselves, however, did enable the estimation of readily fermentable carbohydrate content, a fraction of more meaningful value than the conventional NFE. Not only is it devoid of hemicellulose (Van Soest and Marcus, 1964), but it is also free of the variable lignin content of the NFE (Norman, 1935).

In contrast to the crude fibre, the ADF measurements (Table 3.4) reveal differences between the rations. The lower level of ADF in the HB ration probably reflects an extremely low lignin content of the barley meal. Despite this, the high hemicellulose content of the hay (Table 3.4, subtracting ADF from NDF and adjusting for cell wall-bound ash and protein) meant that both the maize silage rations contained a little more readily fermentable carbohydrate than the hay and barley.

The absence of major differences between the carbohydrate fractions of the rations as fed is perhaps not surprising when it is considered that all three were combinations of roughage and concentrate in not vastly differing proportions.

The crude protein fractions, however, revealed large between-ration differences on their further analysis (see Table 3.5). While 83% of the crude protein of the HB ration was true protein, equivalent figures for the MS and MSB rations were 32% and 22% respectively. The ethanol extraction technique, however, used to remove the NPN may be subject to a systematic error caused by its failure to extract the nucleic acid N (Bailey, pers. comm.). The low true protein values for the maize silage rations are therefore unlikely to be under-estimates.

While it is known that silages may contain much NPN (Barnett, 1954; Macpherson and Violante, 1966a, 1966b; Voss, 1956), Johnson *et al* (1967) (see Table 1.3) found 56% of the crude protein in maize silage to be true protein. The reason for the relatively low level in the maize silage being studied is not clear. A discrepancy due to the different methodology of Johnson *et al* (1967) (tungstic acid precipitation of true protein) is undoubtedly possible, although it is likely that both methods exclude amino acids and small peptides from the true protein fraction. However, as is mentioned in Appendix VI, air entry into the silage stacks was unavoidable, rendering the preservation of the silage less than excellent. Under such conditions

extensive proteolysis may take place (Macpherson and Violante, 1966a).

The significance of both the protein and carbohydrate analyses will be discussed in relation to ration digestibility in Section 4.2.3 and N utilisation in Section 4.2.5.

The phosphorus content of the maize silage being studied was very similar to that reported in the literature (comparing Tables 3.6 and 1.3; 0.27 versus 0.23% of DM). The NRC (1970) suggests a requirement of 0.28% for rapidly growing 200 kg steers. Consequently, the addition of the mineral supplement to raise the P content to 0.32% avoided any possibility of a deficiency.

In contrast, however, the calcium content was considerably above generally accepted values at 0.46% compared with 0.29% of DM (Table 1.3), and in excess of the NCR (1970) requirement of 0.36%. While the literature is adamant that maize silage is marginal in its Ca and P contents, the present evidence would suggest that except for rapidly growing calves, mineral supplementation may be unnecessary. The present findings do not, however, refute the marginal nature of the mineral content of maize silage.

It was suggested in Section 1.4.4 that sulphur was required in N metabolism. An N/S ratio of 10/1 was considered to be required by ruminants (Allaway and Thompson, 1966), and typical maize silage was found to have a ratio of 13/1. The silage used in the present study resembled this figure closely at 12/1, the addition of biuret widening the N/S ratio to 17/1. The sulphur contained in the mineral supplement was insufficient to alter the overall ration content and the possibility exists that sulphur may have been limiting N utilisation in the MSB treatment.

4.2.3 Digestibility of Rations

The apparent DM digestibilities of the two maize silage treatments presented in Table 3.7, seem considerably lower than those reported in the literature (see Table 1.5). The six percentage unit depression from the typical value of 68%, for American produced maize silage of similar dry matter content, was first thought due to the appearance of seemingly large quantities of whole maize grain in the faeces. However, measurement of the extent of passage of undigested grain through the digestive tract yielded a figure of only 6 to 7% of the grain DM. This was slightly less than comparable figures recorded in the literature (8.5%) for

maize silage made at the glaze stage of maturity (Becker and Gallup, 1929) 0.2 to 8.3% being reported for silage made at the dough-dent stage of maturity (Huffman and Duncan, 1959).

The undigested grain theory therefore seemed an improbable explanation for the apparent depression of DM digestibility recorded in the present study. The possibility existed, however, that estimation of undigested grain was both erroneous and low, as the grain content of the silage actually consumed by the steers was assumed to be the same as that in the silage offered the steers. To check the validity of this assumption, a further five day faecal collection trial was run with two cows. While it appeared that refusals may have contained relatively more grain than the silage offered, causing over-estimation of grain intake, hand separations revealed no difference.

There is strong evidence that apparent DM digestibility may be influenced by level of feeding, and that the magnitude of the influence may differ from feed to feed (Watson *et al*, 1939; Van Soest, 1971). In the present study the maize silage was fed to appetite (2.7% of LW), while for much of the work reported in Table 1.5, lower levels of intake were used (Noller *et al*, 1963; Colovos *et al*, 1970).

Watson *et al* (1939), Colovos *et al* (1970) and Wilson (1973) have reported variable declines in DM digestibility of maize silage by cattle, with increasing levels of intake. The former workers recorded a depression of nine percentage units from 70.4%, as DM intake was increased from 1.45% of body weight to appetite. Such a response to high levels of intake could well account for the lower apparent digestibilities recorded in the present study. The question arises, however, as to why the apparent digestibility of the HB ration appeared not to be similarly depressed with the *ad lib* feeding. It would seem that the apparent digestibility of hay DM is less sensitive to level of feeding, Watson *et al* (1939), in keeping with the findings of Watson *et al* (1935), reporting small depressions only with extremely high intakes. It is therefore possible that lower apparent DM digestibility at high levels of intake may be a characteristic of maize silage.

It is quite evident from Table 3.7 that the difference in DM digestibility between the hay and barley ration and the maize silage was not a funtion of the apparent digestibilities of their respective

fibre fractions (ADF-lignin and cellulose). These were essentially the same. Neither was the effect entirely due to the lower apparent digestibility of the crude protein of the maize silage in relation to the hay and barley (Table 3.7), as the presence of supplemental NPN, sufficient to raise the apparent digestibility of the crude protein by seven percentage units, failed to alter the overall DM digestibility of the MSB ration. Similarly, while no measurements were made on hemicellulose digestibility, its percentage content in the maize silage (9.3% of DM by difference) would have been too low to have substantially affected overall DM digestibility, unless it was almost totally indigestible. The only remaining fraction, the cellular contents (56.8, 63.3, 63.8% of DM for HB, MS and MSB rations respectively), are considered almost totally digestible in a variety of rations (Van Soest, 1967). Consequently, it would seem that potentially the three experimental rations should have been of similar digestibility, tending to exclude a possible argument that the present maize was of inferior digestibility through problems encountered during ensiling (see Section 2.2.2 and Appendix V).

While level of intake may have contributed to the low DM digestibilities recorded for maize silage in the present study, the possibility of measurement bias, not accounted for in the standard errors presented with the results, cannot be overlooked. That most readily apparent, resulting from the oven determination of silage DM content, would be an under-estimation of DM intake (Coppock and Stone, 1965), which would in turn depress apparent DM digestibility. However, Bryant (1970) recorded only small (0.3 percentage units) but significant differences between over-drying (100°C for 36 hours) and toluene distillation of maize silage; differences sufficient to raise the apparent DM digestibility figure by no more than 0.5 percentage units. Further mention of this DM determination problem will be made with the consideration of voluntary intake in Section 4.2.4.

As could be expected, the DOM content of the DM, and the digestibility of the GE (Table 3.9) of the rations, followed closely the pattern set by the apparent DM digestibility. The estimated metabolizable energy (ME) contents however, revealed lessened, though still significant differences between the MS and HB rations; the percent metabolizability of the DE of maize silage being estimated

to be higher than for the HB ration (Blaxter et al, 1966; Bryant, 1971). Consequently the energetic nutritive value of the HB and MS rations could be expected to be more similar than is reflected by their respective DOM contents of 66 and 59%. The supplemental NPN in the MSB ration was considered likely to increase the urinary energy loss, lowering the DE metabolizability to a level below the MS ration.

Finally, the digestible crude protein content of the maize silage (see Table 3.10 - 5.5%) was very similar to the 5.7% used in calculating possible levels of production in Section 1.2. The higher digestible crude protein content of the MSB ration would indicate that the supplemental biuret was disappearing in some way or other from the digestive tract. This point will be further discussed with the N balance results in Section 4.2.5.

4.2.4 Voluntary Intake

Measurement of voluntary DM intake of maize silage proved difficult in the present study. The procedure was dependent on accurate determination of feed and refusal DM contents. These were found to vary in the oven-drying technique used, with both the size of sample taken and length of time in the oven.

Bryant et al (1970) had recorded statistically significant, but practically unimportant depressions of estimated DM content (0.8%) by oven-drying compared with toluene distillation. Moreover, a standard error of $\pm$ 0.06, indicated little problem with the oven-drying technique, and consequently no particular precautions were taken prior to commencement of the maize silage feeding.

However, on realising the variability in measured DM percentage with oven-drying, depending on procedure followed, a small experiment was conducted with samples of varying size (300, 200 and 100 g) dried for either 24 or 48 hours. The analysis of the results is presented in Appendix XVIII. It can be seen that samples of size 300 g had a significantly higher measured DM content than either 100 g ($P < 0.05$), or 200 g samples ($P < 0.10$). Furthermore, DM percentages calculated after 24 hours drying were significantly higher than those after 48 hours drying ($P < 0.10$). Analysis of the 24/48 hour differences (see Appendix XVIII) showed the further moisture loss after 24 hours to be significantly greater ($P < 0.01$) for the 300 g samples than for either the 100 or 200 g samples. The size of the differences were clearly of practical importance, the mean difference between the 100 and 300 g samples being 3.9 percentage units. The difference between the 24 and 48 hour oven-

drying for 300 g samples was 5.9 percentage units, sufficient to alter estimated DM intake by 17% from say 8.2 down to 6.8 kg DM/day.

The problem emerging from this secondary experiment was which degree of drying represented free water loss, and after what time period were the volatile fractions of the DM removed. It was considered that a 24 hour drying time was less likely to result in DM loss than 48 hours drying, and was also more desirable from a logistics point of view. While larger samples for DM determinations represented better sampling of the silage fed, 300 g samples still seemed to contain free moisture after 24 hours. It was also likely that secondary fermentation continued for a greater length of time in the larger samples, adding yet another source of error to the problem. Consequently it was decided to standardise the procedure and use a 200 g sample oven-dried for 24 hours at 90°C.

To check on the loss of DM involved with this procedure, a series of maize silage samples were later halved, one half being freeze-dried and the other oven-dried, using the same 200 g, 24 hour procedure for the oven-drying. It was considered that freeze-drying would result in very little DM loss and give an accurate measurement of free moisture content. The freeze-drying in fact resulted in a consistent seven percent (1.3 percentage units) higher estimation of DM content than oven-drying. Such a difference would have been sufficient to have raised estimated DM intake by 4.4%, and DM digestibility by 1.6 percentage units from 62% to 63.6%. Clearly, the under-estimation of DM digestibility caused by the oven-drying could have been greater than was estimated in Section 4.2.3 on the basis of Bryant's (1970) oven-drying results. It is suggested that this possible bias be kept in mind during the subsequent discussion of the intake results.

The analysis of the comparison period DM intake data (Appendix X) revealed significant ($P < 0.01$) treatment by period (T x P) and treatment by block (T x B) interactions and a non-significant period by block (P x B) interaction. It is intended to establish the implications of these interactions prior to consideration of the main treatment effect.

The T x B interaction is shown graphically in Figure 3.2. It can be seen that while for the HB treatment, DM intakes bore a well defined relationship to block number (i.e. steer liveweight at the start of the experiment), the same did not occur with the MS and MSB

treatments. This would indicate that DM intake on the maize silage rations was being influenced by factors other than liveweight. It is suggested that the interaction was caused by the varying lengths of time required by different steers to adapt to the maize silage feeding, the non-significant P x B interaction indicating the time trend of the adaption response not to be affected by steer size (block number - see Figure 3.3). There is no evidence however, that the significant T x B interaction would bias the main treatment effect in any way.

The significance of the T x P interaction, shown graphically in Figure 3.1, indicates that in certain periods the DM intakes of the treatment groups could not be accounted for by the mean effect of the treatment. Therefore, depending on the cause and severity of this interaction, the treatment DM intake means may have limited meaning. While Table X.v shows the interaction effect to be present in periods one, two, three and six, serial ancova analysis (Appendix XI) revealed the interaction during these periods to have been initially generated largely in periods one and six. It is highly likely that the change from the SP to the CP rations for the MS and MSB groups was responsible for the former, while a slight change in the nature of the hay of the HB group was thought to have caused the latter. Clearly, the 38% depression in the DM intakes of the MS and MSB treatments in period one would have had a greater influence on the main treatment effect than the 17% decrease of the HB treatment in period six. It was therefore considered that the main treatment effects on DM intake would be more meaningful with the period one data excluded (see Section 3.4). Mean DM intakes obtained in this way were felt to more closely approximate levels of intake which might be expected on feeding maize silage of a similar nature to other steers.

While the omission of the period one data raised the mean DM intake of the MS treatment to a level similar to the HB treatment, the MSB treatment remained significantly lower than either of the others ($P < 0.01$), even when expressed as a percentage of liveweight (see Table 3.13).

With the small number of animals used in this study the risk is always greater, despite statistical significance, that differences between means may be merely artifacts, rather than effects ascribable to the treatments applied. The present difference between the MSB

and the other two groups is such a case. The only difference between the MS and the MSB treatments was the addition of biuret to the latter. While the lower intake of the MSB treatment could therefore be justifiably ascribed to the presence of biuret, its being neither toxic, nor bitter tasting (Armstrong and Trinder, 1966), would make this seem less than likely.

Consideration of Figure 3.1 reveals that the mean DM intake of the MSB treatment had the greater decrease ($P < 0.05$) with the ration change at the start of the comparison period, returning to the level of the MS treatment only after the first three periods (see Table 3.11). Consequently the lower intake for the MSB treatment in Table 3.13 is largely a reflection of the second and third periods, thereafter there being no difference. That the greater initial intake drop could have been caused by the biuret, with subsequent adaption of the N metabolic system raising intake, is a possibility. It was certainly not, however, caused by palatability differences, the biuret fed with the rock salt - bone flour, mineral supplement being consumed most readily.

The possibility existed that the fairly slow apparent adaptation of the MSB group to the CP diet (see Figure 3.1) was a result of the change of diet depressing initial intake and decreasing body weight, which in turn prevented a return to higher intake levels. However, it is argued that had this occurred, the expression DM intake as a proportion of liveweight (Table 3.13) would have eliminated, or at least reduced substantially, the difference between the MSB and the other two treatments. Such was not the case.

Evidence has been produced to show that adaptation responses caused by diet changes were responsible for the significant interactions found in the analysis of the DM intake data. These adaptation responses were also largely responsible for the treatment differences in intake and it is concluded that both similar and satisfactory (NRC, 1970) levels of DM intake could be expected with cattle on the three experimental rations (see Table 3.11). However, it is also suggested that maize silage of lower DM content (less than 30% DM) may be associated with levels of intake below that of a hay and barley ration, Waghorn (1973) recording ad lib. intakes of 2.35% of liveweight for steers on 27% DM maize silage. This would tend to confirm the conclusion drawn from the evidence presented in Section 1.3.4.

All energy and protein intake figures were based on the DM intake data of Table 3.13 (i.e. first period omitted). The energy intakes, DOM, DE and ME (Table 3.14) appear to be overwhelmingly influenced by the mean level of both DM intake and DM digestibility for each treatment. Mean differences were all highly significant, the lower estimated urinary DE loss for maize silage compared with the hay and barley (Blaxter et al, 1966; Bryant, 1971), resulting in the smallest differences being in ME intake (MS 89% of HB ration compared with 87% for DOM intake). Even the lowest mean ME intake , that of the MSB steers, was sufficient, albeit only just, to meet the NRC (1970) requirements for a 200 kg steer growing at 0.75 kg/day.

The energetic ceiling imposed by the poor DM or GE digestibilities of the maize silage in the present study is of concern. Even with nitrogen supplementation, the maize silage would appear able to promote gains in 200 kg steers of only 0.75 kg/day. While such rates of gain are satisfactory, higher digestibilities of maize silage GE than recorded here (61% of GE), with ad lib. levels of feeding, would be desirable. Such higher gross energy digestibilities for similar silage have been reported by Bryant (1971) (70% digestibilty of GE) but have continued to elude studies at this University (Smith, 1971; Waghorn, 1973; Wilson, 1973). The possibility, expressed earlier in this section, that DM digestibility had been under-estimated through the use of oven DM determinations, would obviously have a two fold effect on DOM, DE and ME intakes, one mediated via the higher DM intake and the other via the higher DM digestibility. This could be sufficient to raise MS and MSB, ME intakes 7% from 13.1 and 12.1 to 14.0 and 13.0 megacalories/day respectively. This is unfortunately a serious possible bias, in all four studies at this University.

Total crude protein intakes (Table 3.15) seemed to be in excess of NCR (1970) requirements for rapidly growing (0.75 kg/day), 200 kg steers, except for the MS ration which was marginally adequate. However, in terms of digestible crude protein requirements (Morrison, 1957; NRC, 1970) the MS ration fell short by 12%, both the HB and MSB rations containing excess quantities.

A striking feature of the N intake data was the high level of NPN consumed by the MSB treatment (0.45 kg/1000 kg liveweight). It was, however, established in Section 1.4.1.2 of the review that while

such a level of NPN feeding would have been reaching the animal safety maximum for urea, its being largely biuret posed no health hazard whatsoever.

4.2.5 Nitrogen (N) Balance

In considering the N balance results, the fate of the supplemental biuret fed the MSB group is perhaps of greatest interest. Comparing the MS and MSB figures in Table 3.16, it can be calculated that approximately 80% of the supplemental biuret disappeared from the digestive tract. Whether however, this amount was actually broken down prior to absorption by the animal is unclear (Hatfield et al, 1959; Karr et al, 1965a). If substantial amounts had been hydrolysed and resynthesised into microbial protein, it might have been expected that the faecal output of true protein for the MSB group expressed as a percentage of true protein intake, would have been higher than the similar figures for the MS treatment. Faecal true protein output was determined using the ethanol extraction procedure of Bailey (pers. comm.), and when expressed as a percentage of true protein intake, was analysed for between treatment differences by anova according to Model (1) shown in Section 2.5.3 (see Appendix XIX).

As would be expected, the proportion of true protein intake in the faeces was significantly greater ($P < 0.01$) for both the MS and MSB treatments than for the HB treatment. This could be considered a reflection of both the low level of true protein in the MS and MSB rations and an actively synthesising rumen microbial population. However, the analysis showed the greater proportion of the dietary true protein in the faecal output of the MSB (10% greater) compared with the MS treatment to be non-significant. Despite its non-significance, if the difference was real it would be of a magnitude of nutritional importance. The finding does indicate the possibility of some incorporation of biuret N into microbial protein, although it would seem that the bulk of the biuret was absorbed unaltered and excreted in the urine. No significant affect on N retention was observed (see Tables 3.16 and 3.17) and it is possible that the comparison period was not long enough to allow for adaptation to the biuret.

It was suggested in Section 1.4.1.2 of the review that maize silage may contain insufficient readily fermentable carbohydrate for efficient utilisation of NPN, especially urea. Conrad and Hibbs (1968) had

demonstrated the need for one kg readily fermentable carbohydrate (CHO) for the utilisation of 100 g of dietary urea, and on the evidence of Johnson et al (1966b) showing maize silage to contain only 9% soluble CHO (DM basis), Connock (1969) offered the above suggestion. It was pointed out by the writer in Section 1.4.1.2 that Connock (1969) had overlooked an important difference between the Johnson et al (1966b) soluble CHO content and the Conrad and Hibbs (1968) readily fermentable CHO content; a difference resulting from the exclusion of starch from the former determination. Through the measurement of the readily fermentable CHO content of the maize silage in the present study, further elucidation of this question is possible.

The maize silage used in the present study was shown to contain 44% readily fermentable CHO (DM basis) (see Table 3.4). Using the Johnson et al (1966b) figure for maximum soluble carbohydrate content of maize silage (9%), it can be calculated that approximately 80% of the readily fermentable CHO in the present maize silage was starch.

For the situation discussed in the review (1.4.1.2) in which 5 kg urea were added per 1000 kg whole plant maize silage (33% DM), with a 90% recovery of the urea, the maize silage would contain 0.0135 g urea/g DM and for every 100 g urea, 3260 g readily fermentable CHO; a quantity well in excess of the requirement suggested by Conrad and Hibbs (1968). Such a level of urea addition would be sufficient to increase the crude protein content of the maize silage by 4% (DM basis), a similar increase to that resulting from the biuret addition in the present study. While the pathways of utilisation of biuret remain obscure, it is quite clearly not hydrolysed in the rumen with the rapidity of urea (Oltjen et al, 1969) and readily fermentable CHO requirements are likely to be less stringent. It is therefore suggested that levels of readily fermentable CHO were quite adequate for efficient utilisation of the NPN in the present study, and would be likely to have been adequate had urea been used in place of the biuret.

The mean daily nitrogen retentions for the three treatments were similar (see Table 3.16). All the experimental animals being of similar age and under essentially similar conditions,it could be expected that N retention might correlate with growth or liveweight gain.

The HB treatment data were excluded from the derivation of an N retention/ liveweight gain relationship as the DM intake drop towards

the end of the comparison period (see Figure 3.1) with associated changes in gut fill, obliterated any association between N retention and apparent liveweight gain. For the remaining two groups a correlation of 0.72 ($P < 0.10$) was found and regressing daily N retention on liveweight gain produced a significant ($P < 0.10$) linear regression coefficient of 11.8 (see Figure 4.1). However, an animal on zero gain without body compositional shifts must also have zero N retention, and consequently the above regression line must pass through the origin. The only way a line fitting the data can meet this requirement is for it to be a curve. While the data are considered by the writer to be inadequate for the mathematical description of such a curve, a line of apparently best fit is drawn in Figure 4.1, using the regression line through the plotted points as part of it. From this curve it can be seen that a steer retaining N at the rate of 25 g/day would have been gaining at the rate of 1 kg/day. The similar figures of Lofgreen (1964) would add credence to the magnitude of this relationship. In an experiment lasting 133 days, Lofgreen (1964) reported steers retaining 26 g N/day to be gaining in body weight at the rate of 1.01 kg/day when fed ad lib. With restricted feeding and similar steers gaining at 29% of the rate of those fed ad lib., 35% as much nitrogen was retained, a result tending to support the curvilinear relationship suggested above.

Although N balance studies tend to have a number of inherent inaccuracies (see Section 1.5.2.2) the evidence of Lofgreen (1964) would indicate a reasonable accuracy of measurement in the present study, at least for the MS and MSB treatments.

4.2.6 Liveweight Gain

Mean daily liveweight gains for the three treatments during the comparison period were remarkably similar (see Table 3.18). However, the highly significant treatment by period interaction ($P < 0.01$, see Appendix XIV) made any conclusions based on the overall mean liveweight gains of limited meaning. Evidence was presented in Section 3.6, showing DM intake to be largely responsible for this interaction, and it was considered that more meaningful comparisons could be made between treatments by correcting for intake.

It is claimed that the DM intake fluctuations were not so much

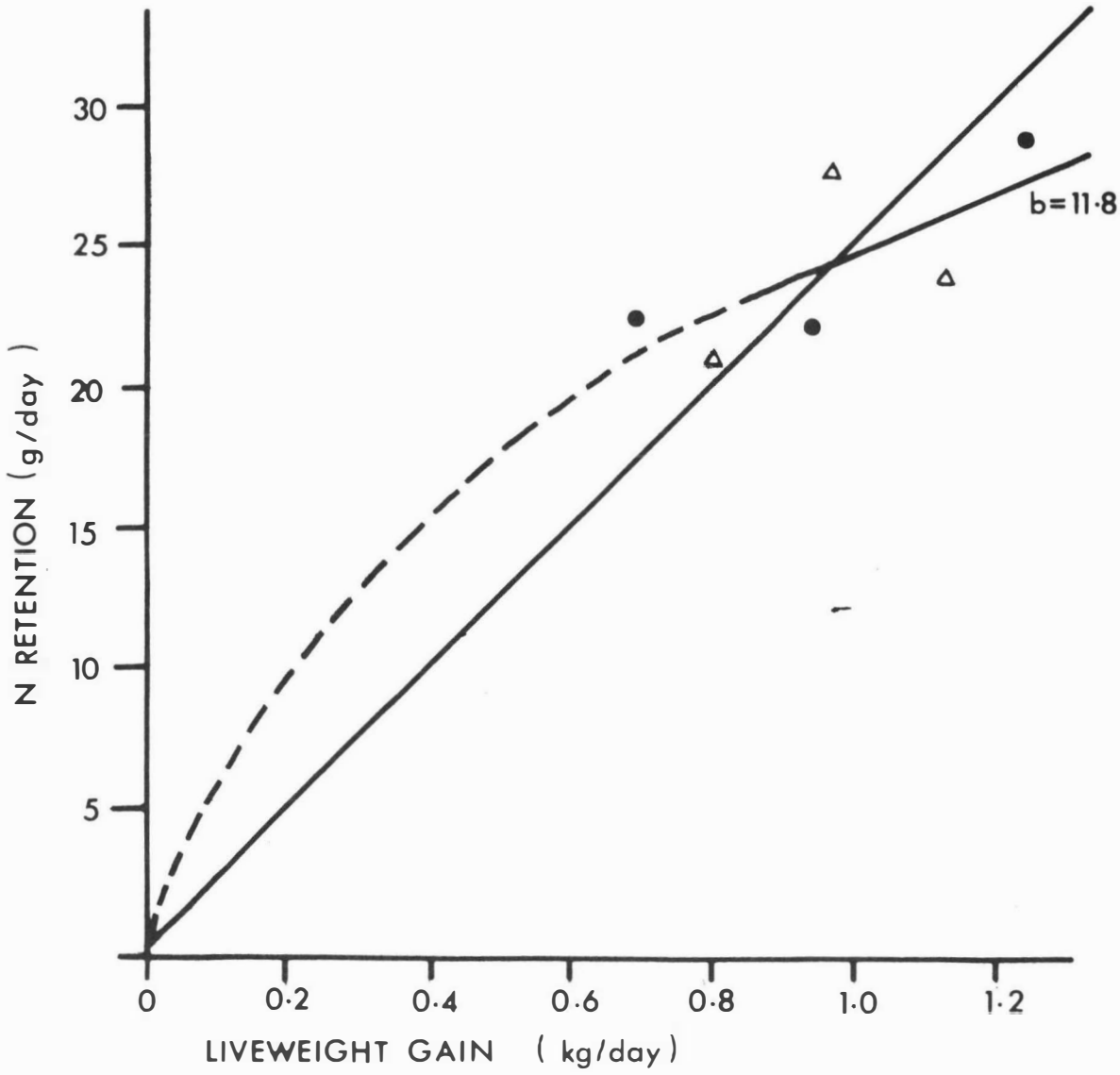

Figure 4.1: The relationship between N retention and liveweight gain of the MS (△) and MSB (●) treatments during the comparison period

properties of the rations, but the circumstances under which the rations were fed. If the intakes from the first two weeks of the comparison period, when the MS and MSB treatments were adapting to ration changes, are ignored, the mean intakes of the three groups over weeks three to six inclusive were very similar (see Tables 3.11 and 3.12). Consequently consistently depressed intake levels were not a characteristic of any of the rations fed, and adjustment of the liveweight gain data for intake could therefore not systematically elevate any one treatment, but rather would correct for circumstantial intake depressions, such as followed ration changes.

In order to overcome the problem of animal size influencing maintenance requirements, liveweight gain within each period was regressed on DM intake per 100 kg liveweight for the period. The adjusted means were presented in Table 3.19, and ranked in the order HB, MS $<$ MSB ($P < 0.05$ and $P < 0.10$ respectively).

To automatically conclude as to the superiority of the MSB ration would be dangerous. Consideration of Figure 3.1 reveals a steady increase in the DM intake of the MSB treatment from weeks two to six inclusive. It could be argued that the increasing levels of intake would elevate measured liveweight gain through gradually increasing levels of gut fill, without necessarily causing the appropriate regression adjustment. However, the N retention data, the accuracy of which was well established in Section 4.2.5, would suggest that the measured liveweight gains over periods four, five and six for both the MS and MSB treatments did in fact represent true body gain, and were not elevated by increasing gut fill. Despite this evidence, a definite conclusion is not made in favour of the higher nutritive value of the MSB treatment. Rather, it is pointed out that under the conditions of the experiment, the MSB ration was unequivocally the most efficient in promoting liveweight gain.

While it could be argued that this superior efficiency was a function of the lower intakes of MSB group from weeks one to three inclusive (see Table X.v), or a function of the increasing level of intakes from weeks two to six inclusive, N retention evidence has been presented to refute to a degree the latter. Moreover, the former was certainly not mediated via more favourable energy digestibilities of the maize silage treatment (see Table 3.9) and on the evidence of

of Almquist et al (1971), lower intakes are unlikely to increase the efficiency of utilisation of DE.

The possible superiority over, or at least equality of the maize silage rations to the hay and barley is difficult to reconcile with the lower measured digestibilities and metabolisable energy intakes of the former rations (see Sections 3.3, 3.4, 4.2.3 and 4.2.4). Notwithstanding the reasonably convincing evidence presented in this section, favouring the reliability of the present efficiency of weight gain findings, it remains possible that the superiority of the MSB over the other treatments is purely an artifact resulting from the short time period and gut fill biases not adequately accounted for. This qualification should be borne in mind through the following discussion.

It is suggested that the situation may be similar to that found in comparing the growth performance of sheep on Manawa short-rotation and Ruanui perennial ryegrass pastures. While of a similar DM digestibility, short-rotation has supported considerably greater liveweight gains than perennial ryegrass when consumed by sheep to similar levels of intake (Butler et al, 1968). Differences in the efficiency of utilisation of DE were found (Ulyatt, 1971) and were largely attributed to the efficiency advantages associated with a greater proportion of post-ruminal digestion occurring in animals consuming the short-rotation ryegrass (Ulyatt, 1969; Ulyatt and MacRae, 1971).

It was shown in Section 4.2.5 that maize silage contains considerable amounts of unfermented starch, possibly up to 35% of the DM. A steer eating 5 kg DM/day could therefore be consuming 1700 g starch/day. With such high levels of starch intake, it has been convincingly shown that considerable proportions may pass unfermented through the rumen (MacRae and Armstrong, 1966; Topps et al, 1968; Nicholson and Sutton, 1969), the proportion seeming greater with maize than with barley feeding (Tucker et al, 1966; MacRae and Armstrong, 1969; Armstrong and Beever, 1969). Tucker et al (1966) reported quantities as high as 31% of dietary starch to have reached the abomasum when sheep were fed ground maize rations containing 46% starch. Whether the same phenomenon would occur with the starch in maize silage is open to question. It is however a possibility with this fine chopped high grain material.

The efficiency superiority of the MSB over the MS treatment may have been a result of the higher crude protein content of the latter. The MS ration at 9.7% crude protein was just below the 10-11% suggested by Goodrich et al (1961), ARC (1965) and Armstrong (1968) as being the level up to which nitrogen responses can be expected. While utilisation of the supplemental biuret was shown by the N retention data to be poor, that amount utilised was possibly sufficient to effect this small advantage.

CHAPTER FIVE

CONCLUSIONS

Interpretation of the results of this experiment is made complex by an unfortunate combination of circumstances. As the ultimate criterion of nutritive value in the study presented, the liveweight gain data is problematical. All three treatments commenced and concluded the comparison period at similar liveweights. The paths followed in reaching these similar final weights however, differed markedly.

It would be rather simple to conclude, on the basis of the similar overall rates of gain, that the experiment failed to demonstrate a nutritive value advantage in favour of any one ration over the others. However, such would be a shallow interpretation of the data considering the other interpretative evidence available.

Despite the significant superiority of the liveweight gain response of the MSB treatment following intake adjustment , it is not concluded that this ration is likely to promote faster rates of gain in growing cattle than the others examined in this study. Such a conclusion can only result from extrapolation of the findings, based on certain intake assumptions expressed in Section 4.2.6. It is offered as a suggestion rather than a conclusion.

It is concluded, however, that under the prevailing circumstances of the experiment described, the MSB ration was most efficient in promoting liveweight gain in young growing cattle. The possibility that this was a result of systematic intake changes elevating measured liveweight gain cannot be excluded, although the N retention data would tend to refute it. If the apparent superiority of the MSB over the HB ration is truly a nutritional effect, its cause is obscure. The lower digestible and estimated metabolisable energy contents of the maize silage rations would suggest a lower nutritive value than the hay and barley ration.

It is therefore postulated that the equivalent if not superior performance of the growing steers on the maize silage rations can only be attributed to enhanced efficiency of utilisation of metabolisable energy, possibly mediated via increased post-ruminal digestion.

Similarly, if the apparent efficiency advantage of the MSB ration over the unsupplemented MS ration is a result of nutritional superiority, it in turn can only be attributed to the addition of biuret to the former ration.

The results tentatively support the evidence discussed in Section 1.4.1, that positive growth rates of cattle can be expected on maize silage without supplemental protein, although superior performance may result from N supplementation.

It is acknowledged that such a finding is inconclusive and fails to answer fully the question posed on the adequacy of the protein content of maize silage for growth in young cattle. It is therefore suggested that the need exists for a further, more specific study of this aspect. A longer period of maize silage feeding, and the inclusion of an extra treatment containing a natural protein supplement would be considered necessary.

APPENDICES

The importance of the information contained in this section of the thesis was stressed in the introduction to Chapter Two, Methods and Materials. Its contents have not been included in the script as it was considered they would digress the direction of thought maintained throughout the five preceding chapters. The study is one of the nutritive value of a feed, and effort has been made not to cloud this issue.

In the following appendices, a number of conventions have been adopted. Their meaning is hereby recorded.

A line down the right hand side of a list of values indicates that they do not differ significantly ($P > 0.05$),

e.g. 2.6 |
2.4 |

Double asterisks (**) indicate that either an F value is significant at the 1% level, or that two means are statistically significantly different with the probability of a type one error less than 1% (i.e. $P < 0.01$).

A single asterisk (*) refers to the 5% level of significance or $P < 0.05$.

In all ancovas, x^2, y^2 and xy refer to the corrected sums of squares for X, Y and cross products respectively. Moreover, S'S refers to sums of squares adjusted for regression.

Through necessity of space, only summaries of selected analyses are presented in this section. However, all the major models employed in the analysis of data are represented.

APPENDIX I

Time Sequence of Events

Date		Event
15.11.69	-	Maize planted.
14. 4.70	-	Maize crop ensiled.
1.12.70	-	Steers introduced to feeding barn. Various headstall and urinary collection equipment tested and faecal collection harnesses tried for size.
1-21.12.70	-	Steers gradually adapted to diet and environment of feeding barn.
7-24.12.70	-	Ringworm treated with iodine - aerosol.
11.12.70	-	First drench - Nilverm 89 mls (3 fl. oz.). First spray for lice - Neocidal.
21.12.70	-	Commencement of Standardisation Period.
26.12.70	-	Second drench - Nilverm 89 mls (3 fl. oz.).
27.12.70	-	Second spray for lice - Neocidal.
10. 1.71	-	Selected steers harnessed in preparation for urine and faeces collection.
11. 1.71	-	Faecal collection shutes fitted.
13. 1.71	-	Urine collection funnels fitted.
14. 1.71	-	Standardisation total collection period commenced.
28. 1.71	-	Standardisation total collection period completed - 14 days.
1. 2.71	-	Commencement of Comparison Period and feeding of maize silage.
22. 2.71	-	Same steers harnessed in preparation for urine and faeces collections.
26. 2.71	-	Commencement of Comparison total collection period.
11. 3.71	-	Comparison total collection period completed - 14 days.
12. 3.71	-	Experiment concluded.

APPENDIX II

Sensitivity of the Experiment as Designed (see section 1.5) for Liveweight Gain Comparisons

The sample size required for a given precision can be calculated using the formula

$$n \geq \left(\frac{\sigma}{\delta}\right)^2 \left\{t_{\alpha[v]} + t_{2(1-P)[v]}\right\}^2$$

where n = number of replications

σ = true standard deviation

δ = the smallest true difference that it is desired to detect. (N.B. It is necessary to know only the ratio of σ to δ not their actual values).

v = degrees of freedom of the sample standard deviation with 'a' groups and 'n' replications per group.

α = significance level

P = desired probability that a difference will be found to be significant

$t_{\alpha[v]}$ and $t_{2(1-P)[v]}$ = values from a two tailed t-table with v degrees of freedom and corresponding to probabilities of α and 2(1-P) respectively (Sokal and Rohlf, 1969).

In the present study the number of replicates 'n' was fixed at five animals per treatment (3 treatments), and a coefficient of variation of 10% was considered to be the best likely to be attained.

By rephrasing the equation it was possible to calculate the probability (P) of detecting a significant difference of a given magnitude between the treatment groups.

$$t_{\alpha[v]} + t_{2(1-P)[v]} = \sqrt{\frac{n}{2\left(\frac{\sigma}{\delta}\right)^2}}$$

$$\therefore t_{2(1-P)[v]} = \sqrt{\frac{n}{2\left(\frac{\sigma}{\delta}\right)^2}} - t_{\alpha[v]}$$

The value $t_{2(1-P)[v]}$ was first calculated, and the probability P associated with this value of 't' was taken from a table of the critical values of Students' t-distribution. The probabilities of detecting two differences was calculated.

A Probability of detecting a between group difference of 0.15 kg/day at the 5% level of significance:

$$t_{2(1-P)[12]} = \sqrt{\frac{5}{2(\frac{10}{20})^2}} - 2.179$$

$$= 0.983$$

$$\therefore P = 65\%$$

B Probability of detecting a between group difference of 0.20 kg/day at the 5% level of significance:

$$t_{2(1-P)[12]} = \sqrt{\frac{5}{2(\frac{10}{26.667})^2}} - 2.179$$

$$= 2.039$$

$$\therefore P = 93\%$$

C Probability of detecting a between group difference of 0.20 kg/day at the 1% level of significance:

$$t_{2(1-P)[12]} = \sqrt{\frac{5}{2(\frac{10}{26.667})^2}} - 3.055$$

$$= 1.163$$

$$\therefore P = 72\%$$

APPENDIX III

Level of Barley Meal Feeding

At the start of the standardisation period the mean liveweight of the fifteen experimental steers was 175 kg. With a projected rate of gain of 0.75 kg/day, it was estimated that this mean liveweight would approximate 206 kg by the commencement of the comparison period, midway through the experiment. Requirements for growing steers of this liveweight, taken from the NRC feeding standards (NRC, 1970) are presented in Table III.i.

TABLE III.i: Nutrient requirements of steers gaining at 0.75 kg/day.

Liveweight (kg)	Dry Matter Intake (kg/day)	Total Protein (kg/day)	Digestible Protein (kg/day)	TDN (kg/day)
200	5.0	0.56	0.36	3.5

It was felt that a 5.0 kg dry matter intake (2.5% of liveweight) was below that which stalled animals fed ad lib. would attain (Davey, pers. comm.). A more probable level of intake was considered to be 2.75% LW or 5.5 kg DM.

Nutritive value figures for both hay and barley were necessary to calculate the level of meal feeding required to achieve the 3.5 kg/day TDN intake, and these are presented in Table III.ii.

TABLE III.ii: Assumed nutritive values for meadow hay and barley meal.

Feed	Dry Matter %	TDN Value* (DM Basis)
Hay	88	50
Barley Meal	89	83

* Based on both NRC (1970) and Unpublished Massey University data.

On the basis of this information, the level of barley meal feeding was computed:

Let barley meal dry matter intake = x kg/day

and hay dry matter intake = y kg/day

Given a total dry matter intake of 6.0 kg/day

and a required TDN intake of 3.5 kg/day

$x + y = 5.5$ .. .. (1)

and $0.83 x + 0.54 y = 3.50$ .. .. (2)

$\therefore y = 5.5 - x$

and $0.83 x + 0.50 (5.5 - x) = 3.50$

$\therefore x = 2.3$ kg DM/day (i.e. 42.0% of DM intake)

$\therefore$ Barley meal required for steers to obtain projected growth rate = 2.60 kg/day.

It was assumed that:

Metabolic body weight = $LW^{0.75}$

= 53.18

$\therefore$ 2.6 kg barley meal to be fed an animal with a metabolic body weight of 53.18 kg - i.e. 0.049 kg meal/kg metabolic weight.

APPENDIX IV

Maize Silage Production Details

Graded Wisconsin 575 maize seed, a three way hybrid maturing at 115 days, was planted in a cultivated 0.36 hectare paddock on November 15th, 1969. The area had previously been in pasture. A four-row Burch precision planter was used, the seed being sown in 76 cm (30 inch) rows and 16.5 cm (6.5 inch) spacings to give a sowing density of 80,000 seeds per hectare (32,000 seeds/acre). An effective germination rate of 80% resulted in a measured plant population of 64,000 $\pm$ 8,000 plants/hectare (26,000 $\pm$ 3,000 plants/acre).

The crop was planted in a Manawatu fine sandy loam soil fertilized with Amophos 10:18:8 NPK (900 kg/ha), muriate of potash (250 kg/ha) and urea (170 kg/ha). A further 170 kg/ha of urea was added as a side dressing prior to flowering. The levels of fertilizer applied were determined by both soil tests and the need for a large quantity of good quality silage from the small area of land (see section 1.3.1).

Complete weed control was effected by the use of Propachlor (4.5 kg/ha) pre-sowing and Atrazine (2.5 kg a.i./ha) post emergent.

Despite the 1969/70 summer drought, the crop was not irrigated, and was harvested on April 14th, 1970, at the dent stage of maturity with a dry matter content of 33 $\pm$ 3%. A single row New Holland 717 Maize Chopper was used.

APPENDIX V

Maize Silage Yield Data

The following data were obtained by sampling the maize crop four days prior to machine harvesting. Three samples were taken from different parts of the crop, each sample space being 7.6 metres in length and two rows (1.5 m) in width.

TABLE V.i: Maize plant characteristics

Characteristics	Mean
Height of plants (m)	2.3 ± 0.1
Number of tillers per plant	1.04 ± 0.04
Number of cobs per plant	1.5 ± 0.1

TABLE V.ii: Dry matter content of plant components

Component	DM%
Leaf	41.0 ± 2.0
Sheath	39.0 ± 3.0
Stem	18.5 ± 1.0
Ear	60.0 ± 1.0

TABLE V.iii: Crop and component yields

Component	Mean DM Yield (kg/ha)
Crop	20,000 ± 2000 (18,000 ± 2000 lbs/acre)
Leaf	3300 ± 600
Stem	5200 ± 500
Grain	8900 ± 1100
Rest	2400 ± 300

APPENDIX VI

Maize Silage Storage

The finely chopped maize silage was vacuum packed in two elongated stacks, designed for compatability with usage rate (see section 2.2.2). Single layer, 0.005 gauge, polythene ground sheets, 2.4 m in width, were used under the silage, and were fastened to the 0.005 gauge, 3.7 m wide, polythene top sheets with vacuum strip seal. Following air evacuation, both stacks were covered with a second similar sheet for complete air exclusion, the first sheet generally being slightly damaged by the evacuation process. A 10 cm layer of earth over both stacks, to maintain compaction and protect the polythene, completed the coverings.

The only major problem encountered was quickly apparent. The double layer polythene top sheet afforded the silage no protection from rodents, rats and mice mutilating the polythene and allowing air entry. Where possible, the damage to the covers was repaired, but air exclusion was far from complete.

While apparent silage wastage was measured at only 20-30% in cross sections of the stacks, the overall loss of dry matter as measured by total drymatter taken out of the stacks was 40%. Clearly air entry, facilitated by rodent damage, had led to respiratory dry matter losses far in excess of that apparent from residual wastes.

It is recommended that if maize silage stacks must be positioned in rodent infested areas, effort must be made to safeguard this highly palatable material, in order to prevent substantial dry matter losses.

The entry of air into the stacks did enable observations to be made on the shape of stack required for satisfactory maize silage storage. As can be seen in Plate VI.i, the major area of silage loss was the sides of the stacks. Losses at the top of the stack under the top sheet were negligible. It is therefore suggested that for satisfactory storage of small quantities of maize silage in stacks, these should be shallow with sloping tops and no sides, or low vertical sides, so as to avoid both poor compaction and bridging by the covers.

For the production of really good quality maize silage there is no substitute for bunkers or tower silos.

PLATE VI.i: Silage wastage. A cross-section of one of the silage stacks showing the areas of greatest loss. Material above the white line had to be discarded. The negligible wastage at the top of the stack is as distinctive as the considerable losses on the sides.

APPENDIX VII

Calculation of Biuret Requirements for MSB Treatment

Nitrogen content of biuret	= 36%
Expected crude protein content of maize silage	= 8.7% of DM
Required crude protein content	= 13.4% of DM
Deficit	= 4.7 kg Cr.Prot./100 kg DM
	= 0.757 kg N/100 kg DM
∴ Biuret required	= 2.103 kg/100 kg DM
	= 0.021 kg biuret/kg DM Fed

APPENDIX VIII

The Blocking of the Experimental Animals and their Random Allocation to Treatment Groups

TABLE VIII.i: Classification of steers by blocks based on liveweight following the second week of the standardisation period

Block Number	1	2	3	4	5
Steer Number	4 7 10	6 11 14	1 3 13	5 9 17	8 15 16
Liveweight range at time of blocking	234-225 kg	218-206 kg	205-191 kg	187-180 kg	178-153 kg

TABLE VIII.ii: Random allocation within blocks of steers to treatment groups

Group	HB	MB	MSB
Steer Number (Liveweight in kg at time of allocation in parenthesis)	10 (234) 6 (218) 3 (200) 9 (187) 15 (163)	7 (225) 11 (208) 13 (191) 17 (180) 8 (178)	4 (234) 14 (206) 1 (205) 5 (180) 16 (153)

APPENDIX IX

Analysis of DM Digestibility Data

TABLE IX.i: Calculation of within-treatment regressions of CP on SP dry matter digestibility data.

Treatment	df	x^2	xy	y^2	b	s's	df
1	2	27.860	7.600	2.087	0.273	0.014	1
2	2	7.980	-6.620	5.687	-0.830	0.195	1
3	2	12.827	4.200	1.520	0.327	0.145	1
Pooled regression	6	48.667	5.180	9.294	-0.077	0.354	3

The within-group regression lines are shown in Figure IX.i.

TABLE IX.ii: Analysis of differences between within-treatment regression coefficients

Source of Variation	df	ss	ms	F
Deviations from Average Regression	5	8.743		
Deviations from Individual Regresssions	3	0.354	0.118	
Between Regression Coefficients	2	8.389	4.195	35.551**

TABLE IX.iii: Analysis of comparison period DM digestibility data by anova using model (1)

Source of Variation	df	ss	ms	F
Total	8	76.229		
T	2	66.935	33.468	21.606**
Error	6	9.294	1.549	

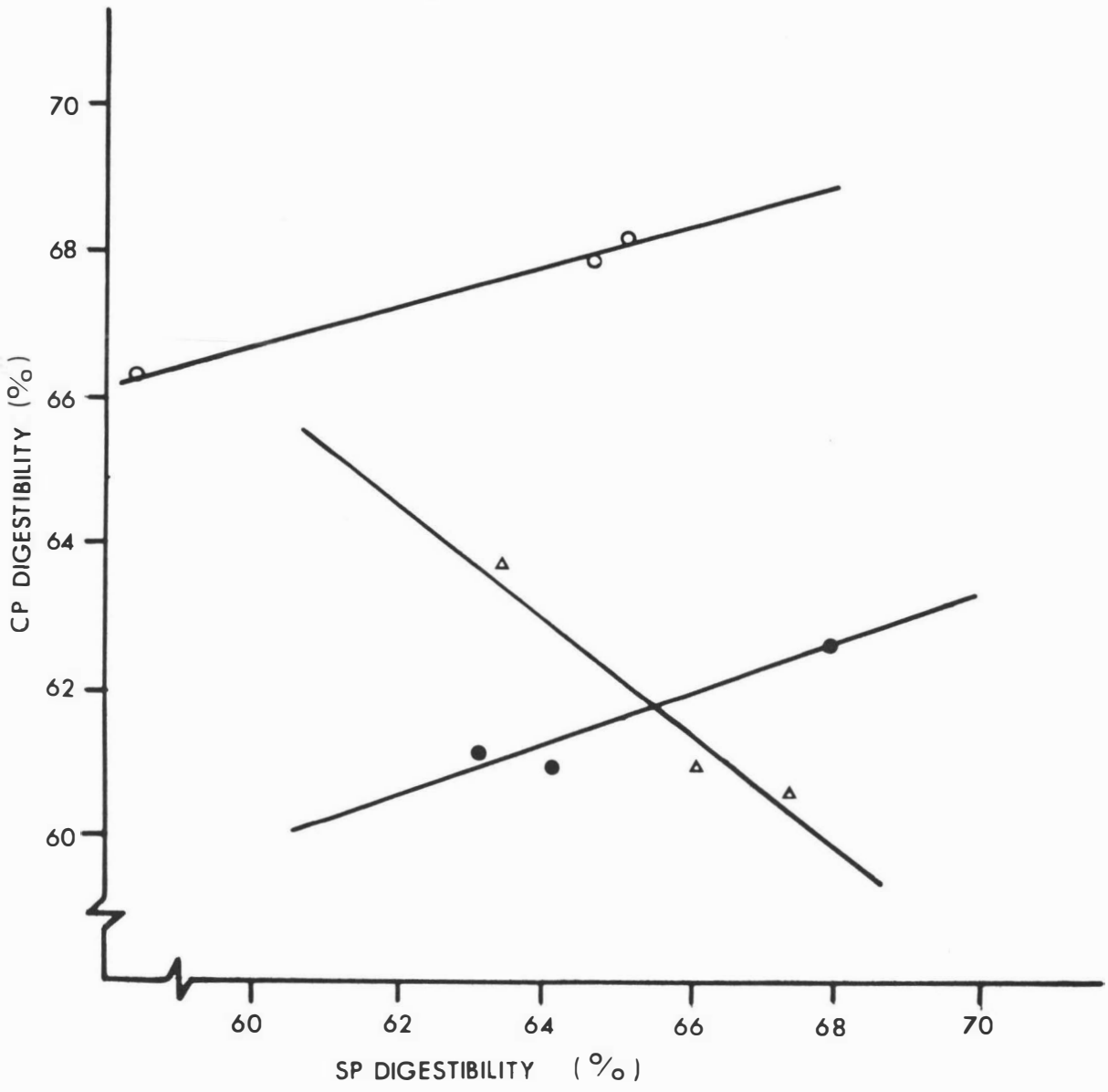

FIGURE IX.i: Within treatment regressions of CP DM digestibility on on SP DM digestibility.

○ HB △ MS ● MSB

TABLE IX.iv: Duncan's Multiple Range test of significance between treatment means

p	3	2	Level of Significance
Rp x $S_{\bar{T}}$	2.58 3.97	2.49 3.77	5% 1%
HB = 67% MS = 62% MSB = 62%		HB > MS** HB > MSB**	

TABLE IX.v: Anova analysis of standarisation period DM digestibility data, using model (1) to check the validity of conclusions drawn from Table IX.iv.

Source of Variation	df	ss	ms	F
Total	8	62.842		
T	2	14.175	7.088	0.864 NS
Error	6	48.667	8.111	

APPENDIX V

Analysis of DM Intake Data

TABLE X.i: Anova analysis of comparison period DM intake data (kg/day) using model (2) (entire six week period duration)

Source of Variation	df	ss	ms	F
Total	89	141.812		
T	2	11.456	5.728	28.356**
P	5	29.913	5.983	29.619**
B	4	36.497	9.124	45.163**
T x P	10	28.465	2.847	14.094**
T x B	8	22.552	2.819	13.955**
P x B	20	4.865	0.243	1.203 NS
Error	40	8.064	0.202	

TABLE X.ii: DMR test of significance between treatment means

p	3	2	Level of Significance
$R_p x S_{\bar{T}}$	0.25	0.24	5%
	0.36	0.32	1%
HB = 6.04		HB > MSB**	
MS = 5.58		HB > MS**	
MSB = 5.17		MS > MSB**	

TABLE X.iii: DMR test of significance between period means

p	6	5	4	3	2	Level of Significance
$R_p x S_{\bar{P}}$	0.37	0.36	0.35	0.34	0.33	5%
	0.48	0.48	0.47	0.46	0.44	1%
P_1 = 4.34						
P_3 = 5.72						
P_4 = 5.74						
P_2 = 5.75						
P_5 = 5.99						
P_6 = 6.04						
$P_1 < P_3, P_4, P_2, P_5, P_6$**						

TABLE X.iv: DMR test of significance between block means

p	5	4	3	2	Level of Significance
$R_p x S_{\overline{B}}$	0.33	0.33	0.32	0.30	5%
	0.44	0.43	0.42	0.40	1%
$B_1 = 6.28$ $B_2 = 6.09$ $B_4 = 5.67$ $B_3 = 5.49$ $B_5 = 4.46$			$B_1 > B_5, B_3, B_4$** $B_2 > B_5, B_3, B_4$**		$B_4 > B_5$** $B_3 > B_5$**

TABLE X.v: DMR test of significance btween treatment by period sub-group means, showing the source of the T x P interaction

p		6	5	4	3	2	
$R_p x S_{\overline{TP}}$	5%	0.64	0.63	0.62	0.60	0.57	
	1%	0.85	0.83	0.82	0.80	0.76	
Period		1	2	3	4	5	6
TP Sub-Group Means		HB 5.86	HB 6.73	HB 6.44	HB 5.87	HB 6.21	HB 5.13
		MS 3.92	MS 5.72	MS 5.67	MS 5.68	MS 6.01	MS 6.46
		MSB 3.22	MSB 4.80	MSB 5.06	MSB 5.65	MSB 5.74	MSB 6.54

APPENDIX XI

Serial Analysis of DM Intake Data

TABLE XI.i: Serial analysis of comparison period DM intake data (kg/day) by ancova using model (3)

Source of Variation	df	x^2	xy	y^2	s's	df	ms	F
Total	89	144.917	76.010	141.812				
T	2	20.006	15.123	11.456				
P	5	27.102	-2.026	29.913				
T x P	10	61.207	18.030	28.465	43.035	10	4.304	18.008**
Error	72	36.602	44.883	71.978	16.941	71	0.239	

TABLE XI.ii: DMR test of significance between T x P sub-group means adjusted serially using the equation $\hat{y}_{ij} = \bar{y}_{ij} - b(\bar{x}_{ij} - \bar{\bar{x}})$ where $\bar{\bar{x}} = 5.549$ and $b = 1.226$

p		6	5	4	3	2	
$RpxS_{\overline{TP}}$	5%	0.70	0.69	0.68	0.65	0.62	
	1%	0.92	0.90	0.88	0.85	0.83	
Period		1	2	3	4	5	6
Adjusted TP Sub-group Means		HB 5.58	MS 7.72	MSB 5.97	MSB 6.22	MS 5.84	MSB 6.31
		MS 3.42	MSB 7.66	MS 5.46	MS 5.54	HB 5.82	MS 5.90
		MSB 3.24	HB 6.33	HB 4.98	HB 4.79	MSB 5.62	HB 4.32

APPENDIX XII

Analysis of DM Intake as a Percentage of Liveweight

TABLE XII.i: Anova analysis of DM intake data expressed as a percentage of liveweight, by model (2) modified by omission of period one.

Source of Variation	df	ss	ms	F
Total	74	7.270		
T	2	0.361	0.181	6.464**
P	4	0.125	0.031	1.107 NS
B	4	0.395	0.099	3.536*
T x P	8	2.909	0.364	13.000**
T x B	8	1.916	0.240	8.571**
P x B	16	0.678	0.042	1.500 NS
Error	32	0.886	0.028	

TABLE XII.ii: DMR test of significance between treatment means

p	3	2	Level of significance
$RpxS_{\overline{T}}$	0.09 0.12	0.09 0.12	5% 1%
HB = 2.68 MS = 2.72 MSB = 2.56		MS > MSB** HB > MSB**	

TABLE XII.iii: DMR test of significance between block means

p	5	4	3	2	Level of Significance
$RpxS_{\overline{B}}$	0.14 0.19	0.14 0.19	0.14 0.18	0.13 0.18	5% 1%
B_1 = 2.55 B_3 = 2.63 B_5 = 2.65 B_2 = 2.67 B_4 = 2.77				$B_4 > B_1$** $B_4 > B_3$*	

APPENDIX XIII

Analysis of N Retention Data

TABLE XIII.i: Anova analysis of N retention data (g/day) during the comparison period using model (4)

Source of Variation	df	ss	ms	F
Total	8	223.92		
T	2	12.81	6.41	0.29 NS
B	2	122.07	61.04	2.74 NS
Error	4	89.04	22.26	

TABLE XIII.ii: Anova analysis of N retention data (g/day) during the standardisation period using model (4)

Source of Variation	df	ss	ms	F
Total	8	146.10		
T	2	32.97	16.49	1.14 NS
B	2	55.11	27.56	1.90 NS
Error	4	58.02	14.51	

APPENDIX XIV

Analysis of Comparison Period Liveweight Gain Data

TABLE XIV.i: Analysis of CP mean daily liveweight changes by anova using model (2), modified for period effect.

Source of Variation	df	ss	ms	F
Total	29	4.853		
T	2	0.038	0.019	0.463 NS
P	1	1.221	1.221	29.780**
B	4	0.211	0.053	1.293 NS
T x P	2	2.205	1.103	26.902**
T x B	8	0.614	0.077	1.878 NS
P x B	4	0.235	0.059	1.439 NS
Error	8	0.329	0.041	

TABLE XIV.ii: Treatment by period subgroup means showing the source of the T x P interaction (significance of differences by DMR test)

Treatment	Period 1		Period 2	
HB ($P_1 > P_2$*)	0.62	HB > MS**	0.26	HB < MS**
MS ($P_2 > P_1$**)	0.10	MS=MSB (P>0.05)	0.93	MS=MSB (P>0.05)
MSB ($P_2 > P_1$**)	0.14	MSB < HB**	0.88	MSB>HB**

APPENDIX XV

Serial Analysis of CP Period One Intermediate Liveweights

TABLE XV.i: Serial ancova analysis of CP period one, week one liveweights (kg) using model (5)

Source of Variation	df	x^2	xy	y^2	s's	df	ms	F
Total	14	11754.9	11693.5	12333.7	701.3	13		
T	2	1.7	25.5	433.7	384.7	2	192.4	6.68*
Error	12	11753.2	11668.0	11900.0	316.6	11	28.8	

TABLE XV.ii: Adjustment of treatment mean liveweights for week one of CP period one, according to liveweight in preceding week, using the equation $\hat{\bar{y}}_i = \bar{y}_i - b(\bar{x}_i - \bar{\bar{x}})$ where $b = 1.00$ and $\bar{\bar{x}} = 213$ kg

Treatment	$\bar{y}_i$	$\bar{x}_i - \bar{\bar{x}}$	$b(\bar{x}_i - \bar{\bar{x}})$	$\hat{\bar{y}}_i$
HB	218	0	0	218
MS	208	-1	-1	209
MSB	206	0	0	206

TABLE XV.iii: DMR test of significance between adjusted treatment means

p	3	2	Level of Significance
$R_p x S_{\hat{\bar{y}}}$	7.8	7.5	5%
HB = 218 MS = 209 MSB = 206		HB > MSB* HB > MS* MS = MSB ($P > 0.05$)	

TABLE XV.iv: Significance of reduction in error mean squares due to regression

Source of Variation	df	ss	ms	F
Error for unadjusted means	12	11900.0		
Reduction due to regression	1	11583.4	11583.4	402.2**
Error for adjusted means	11	316.6	28.8	

TABLE XV.v: Calculation of within treatment regression coefficients for testing validity of the analysis summarized in Table XV.i

Treatment	df	x^2	xy	y^2	b	ss	df
HB	4	5142.8	5169.6	5197.2	1.0	0.7	3
MS	4	1829.2	1716.0	1692.0	0.9	82.2	3
MSB	4	4781.2	4782.4	5010.8	1.0	227.2	3
Pooled Regression	12	11753.2	11668.0	11900.0	1.0	310.1	9

TABLE XV.vi: Analysis of differences between within treatment regression coefficients

Source of Variation	df	ss	ms	F
Deviations from average regression	11	316.6		
Deviations from individual regressions	9	310.1	34.5	0.1 NS
Between regression coefficients	2	6.5	3.3	

APPENDIX XVI

Analysis of CP Liveweight Gain Data Adjusted for DM Intake per 100 kg Liveweight

TABLE XVI.i: Ancova analysis of CP liveweight gain data adjusted for DM intake per 100 kg liveweight using model (6)

Source of Variation	df	x^2	xy	y^2	s's	df	ms	F
Total	29	3.425	2.872	4.853	2.445	28		
T	2	0.533	-0.097	0.038	0.678	2	0.339	4.985*
Error	27	2.892	2.969	4.815	1.767	26	0.068	

TABLE XVI.ii: Adjustment of CP treatment mean liveweight gains for DM intake per 100 kg LW using the equation $\hat{y}_i = \bar{y}_i - b(\bar{x}_i - \bar{\bar{x}})$ where b = 1.03 and $\bar{\bar{x}} = 2.54$

Treatment	$\bar{y}_i$	$(\bar{x}_i - \bar{\bar{x}})$	$b(\bar{x}_i - \bar{\bar{x}})$	$\hat{y}_i$
HB	0.44	0.14	0.14	0.30
MS	0.52	0.04	0.04	0.48
MSB	0.51	-0.18	-0.19	0.70

TABLE XVI.iii: DMR test of significance for differences between adjusted treatment means

p	3	2	Level of Significance
$R_p x S_{\hat{y}}$	0.24	0.19	10%
	0.24	0.23	5%
HB = 0.30		HB < MSB*	
MS = 0.48		HB = MS (P > 0.10)	
MSB = 0.70		MSB > MS (P < 0.10)	

TABLE XVI.iv: Significance of reduction in error mean squares due to regression

Source of Variation	df	ss	ms	F
Error for unadjusted means	27	4.815		
Reduction due to regression	1	3.048	3.048	44.8 ($P < 0.001$
Error for adjusted means	26	1.767	0.068	

TABLE XVI.v: Calculation of within treatment regression coefficients for testing validity of the analysis summarized in Table XVI.i (see Figure XVI.i)

Treatment	df	x^2	xy	y^2	b	s's	df
HB	9	0.531	0.504	0.699	0.95	0.221	8
MS	9	0.531	0.815	1.889	1.53	0.638	8
MSB	9	1.830	1.649	2.227	0.90	0.723	8
Pooled Regression	27	2.892	2.968	4.815	1.03	1.582	24

TABLE XVI.vi: Analysis of differences between within treatment regression coefficients

Source of Variation	df	ss	ms	F
Deviations from average regression	26	1.767		
Deviations from individual regressions	24	1.582	0.066	1.409 NS
Between regression coefficients	2	0.185	0.093	

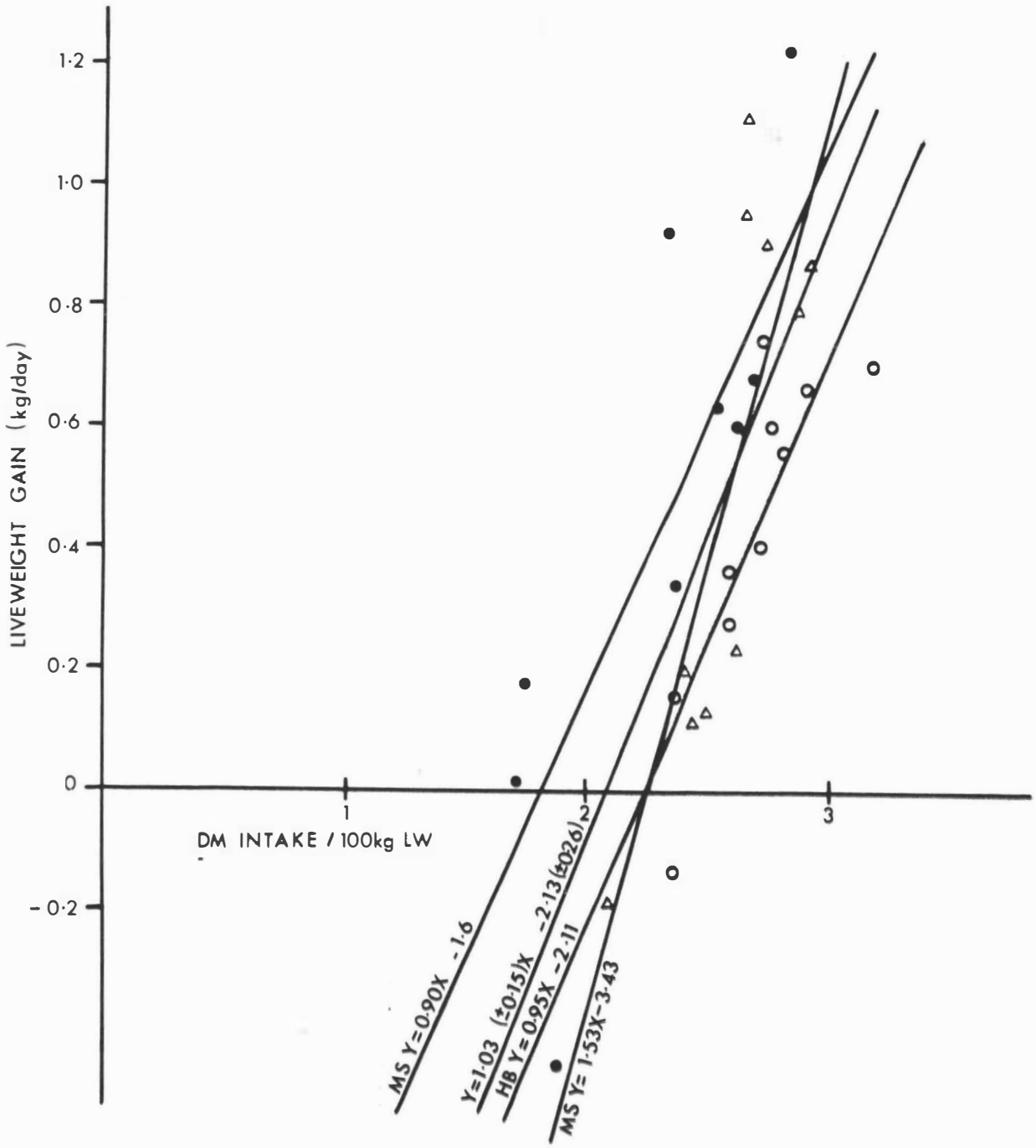

FIGURE XVI.i: Regression of CP liveweight gain during each period on DM intake per 100 kg liveweight for that period.

○ HB △ MS ● MSB

APPENDIX XVII

Estimation of Variance Components for Liveweight Gain Data

TABLE XVII.i. Expected and recorded mean squares for liveweight gain anova, using model (2) converted from a fixed effects to a random model.

Source of Variation	df	EMS	ms
T	2	$\sigma_e^2 + \sigma_{TPB}^2 + p\sigma_{TB}^2 + b\sigma_{TP}^2 + pb\sigma_T^2$	0.011
P	1	$\sigma_e^2 + \sigma_{TPB}^2 + t\sigma_{PB}^2 + b\sigma_{TP}^2 + tb\sigma_P^2$	1.221
B	4	$\sigma_e^2 + \sigma_{TPB}^2 + p\sigma_{TB}^2 + t\sigma_{PB}^2 + tp\sigma_B^2$	0.053
TXP	2	$\sigma_e^2 + \sigma_{TPB}^2 + b\sigma_{TP}^2$	1.103
TXB	8	$\sigma_e^2 + \sigma_{TPB}^2 + p\sigma_{TB}^2$	0.077
PXB	4	$\sigma_e^2 + \sigma_{TPB}^2 + t\sigma_{PB}^2$	0.059
Error	8	$\sigma_e^2 + \sigma_{TPB}^2$	0.041

NB: t = 3; p = 2; b = 5

Computation of the variance component due to the main treatment effect T

$$s_T^2 = \frac{1}{10}(ms_T - ms_{TXP} - ms_{TXB} + ms_{Error})$$

$$= -0.112$$

$$s_{TP}^2 = \frac{1}{5}(ms_{TXP} - ms_{Error})$$

$$= 0.212$$

$$s_{TB}^2 = \tfrac{1}{2}(ms_{TXB} - ms_{Error})$$

$$= 0.018$$

s_E^2 is assumed to contain both s_e^2 and s_{TPB}^2

$$\therefore s_E^2 = 0.041$$

Estimated variance of treatment mean T after increasing the number of steers per treatment (blocks) by one

$$S_T^2 = 0.041 + 0.036 + 1.272 - 1.344$$
$$= 0.005$$

Reduction in variance of T = 0.036

Estimated variance of treatment mean T after increasing period number by one.

$$S_T^2 = 0.041 + 0.054 + 1.060 - 1.680$$
$$= -0.525$$

Reduction in variance of T = 0.566

Estimated variance of treatment mean T after doubling steer number per treatment

$$S_T^2 = 0.041 + 0.036 + 2.120 - 2.240$$
$$= -0.043$$

Reduction in variance of T = 0.084.

APPENDIX XVIII

Analysis of Oven DM Determination Data for Maize Silage

TABLE XVIII.i: Analysis by two-way, type I anova of maize silage DM determination data - effect of sample size (S = 300, 200 or 100 g) and length of oven-drying time (L = 24 or 48 hours)

Source	df	ss	ms	F
Total	63	1278.0		
S	2	134.5	67.3	3.80*
L	1	53.3	53.3	3.01 (10%)
S x L	2	62.1	31.1	1.76 NS
Error	58	1028.1	17.7	

TABLE XVIII.ii: DMR test of significance between S and L means

p	3	2	Level of Significance
$RpxS_{\overline{S}}$	3.13	2.97	5%
$RpxS_{\overline{S}}$	3.11	2.48	10%
$RpxS_{\overline{L}}$		1.75	10%
Sample Size (S)	300 > 100*		
	300 > 200 ($P < 0.10$)		
Oven Time (L)	24 > 48 ($P < 0.10$)		

TABLE XVIII.iii: Analysis by one-way, type I anova of data showing the effect of sample size on differences between 24 and 48 hours oven-drying

Source	df	ss	ms	F
Total	20	219.770		
S	2	128.430	64.215	12.656**
Error	18	91.340	5.074	

TABLE XVIII.iv: DMR test of significance between sample size means

p	3	2	Level of Significance
$R_{p}xS_{\bar{S}}$	2.64 3.59	2.51 3.42	5% 1%
300 > 100, 200**			

APPENDIX XIX

Analysis of Biuret Utilisation Data

TABLE XIX.i: Anova analysis of CP faecal true protein output expressed as a percentage of true protein intake, using model (1)

Source of Variation	df	ss	ms	F
Total	8	8961		
T	2	8529	4265	59.24**
Error	6	432	72	

TABLE XIX.ii: DMR test for significant differences between treatment means

p	3	2	Level of Significance
$S_{\bar{T}} x R_p$	17.4	13.5	10%
	27.0	25.7	1%
HB = 31%		MSB > HB**	
MS = 91%		MSB = MS (P > 0.10)	
MSB = 100%		MS > HB**	

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