THE UTILIZATION OF LACTOSE

BY THE

GROWING PIG

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INTRODUCTION

The results of a recent Nationwide survey (Davenport 1966) showed that 93% of all pig units were still dependent on supplies of liquid dairy by-products - whey and skim milk - for their major source of pig food.

Calculations made from figures for cheese and casein production (N.Z. Govt. Statistics, 1967) show that in 1966, approximately 500 million gallons of whey alone, were produced. Of this, a comparatively small amount is utilized by the dairy industry to produce alternative by-products. Condensed or dried whey production involves the costly removal of large volumes of water. This necessarily results in a high price to the consumer, and consequently a low consumer demand. The quantities of lactose produced are unlikely to increase appreciably as there is only a limited demand for this sugar, and it is still too early to tell whether current research into alternative uses for the whey, such as the production of food yeast (Chapman, 1966), will make significant inroads into the very large whey surpluses.

It is clear that the conversion of these surpluses into pigmeat is still the most profitable single outlet for a large amount of the whey produced. On the basis of calculations similar to those made by Owtram, (1961) full utilization of the whey produced in 1966 if fed alone, could yield up to 19,000 tons of pigmeat. Needless to say, under the more normal feeding systems in which 1 lb meal daily, is also fed, production levels even higher could be envisaged.

Lactose comprises approximately 70% of the total solids of whey, and a little over 50% of the total solids of skim milk. The ability of the pig to utilize this sugar is therefore a crucial factor in the present and possible future production of pigmeat in this country. Despite its importance, very little published work is available concerning the effects of feeding high dietary levels of lactose to growing pigs. In view of this lack of information, investigations were carried out to provide some

indication of the extent to which the growing pig can utilize diets containing high levels of lactose.

PART I

PHYSIOLOGICAL AND ANATOMICAL ASPECTS INVOLVED IN THE UTILIZATION OF LACTOSE BY THE GROWING PIG

CHAPTER 1

REVIEW OF LITERATURE

1.1 LACTOSE UTILIZATION BY THE GROWING PIG

High levels of dietary lactose may result either from the direct incorporation of the sugar into the diet, or from the use of a variety of dairy by-products available for pig feeding. Evidence suggests, that when these high intake levels are reached, the ability of the growing pig to make efficient use of this particular source of energy, is exceeded, resulting in poor growth, decreased feed intake, diarrhoea and other physiological effects (Fischer and Sutton, 1949; Duncan, 1955).

Due to the possible associative effects of the various constituents of dairy by-products the discussion will be subdivided, according to the origin of the dietary lactose, into:

(a) Diets containing lactose powder.

(b) Diets containing dried whey powder.

(c) Diets containing condensed whey.

(d) The use of fresh whey in growing pig diets.

(e) Summary

(a) Diets containing lactose powder

Limited work suggests that, as pigs grow older, the percentage level of lactose in the diet must be reduced if efficient utilization of the diet is to continue. This does not suggest that, as the pig ages, less lactose can be hydrolysed, because absolute intake of lactose will rise as the daily ration, containing a fixed percentage of lactose, increases. More correctly, it suggests that the pigs' hydrolytic activity either increases (but at only a slow rate) or remains relatively constant as the animal increases in age. This, therefore, enables the older animal to successfully consume greater amounts of lactose than were possible early in life.

A diet containing 40 % lactose, was reported by McCrea and Tribe,(1956) to produce satisfactory growth when fed to baby pigs for four weeks. However, in earlier work (Whittier, Cary and Ellis, 1935) growing pigs (initial liveweight 80 lb) fed a diet containing the same level of lactose, (40 %) grew more slowly and less efficiently than a control group receiving no lactose. Work at Illinois (Becker, Ullrey and Terrill, 1954) showed that baby pigs fed synthetic milk diets from 7-35 days of age, readily utilized a diet containing over 56 % lactose. Furthermore, significantly better performance was produced on this diet than was produced by other pigs on diets containing the same percentage of glucose, corn starch, or dextrin. When similar experiments were carried out with growing pigs (Becker and Terrill, 1954) it was clear that the same lactose levels could not be achieved with any measure of success. Two experiments were conducted, the first with pigs aged 9 weeks, the second with 16-week old pigs. In the first experiment, glucose, lactose, sucrose, dextrin and corn starch, comprising 50 % of the diet were full fed for a period of 39 days. Satisfactory rate, and efficiency of liveweight gains were reported for all diets except that containing lactose, this diet producing depressed feed intake, slow growth, and a moderate diarrhoea. In the second experiment glucose was replaced by lactose to yield dietary levels of 0, 6.25, 12.5, 25 and 50 % lactose. Here again the 50 % level depressed feed intake and growth rate, and produced a moderate diarrhoea. However, at the 25 % level growth rates comparable with the lower lactose levels and the control diet were achieved, suggesting that at some level intermediate between 25 % and 50 % lactose, the amount of lactose ingested exceeded the pigs ability to utilize this sugar.

(b) Diets containing dried whey powder

Within the limitations mentioned earlier, it is possible to gain further information from a number of investigations in which dried whey has been fed to growing pigs.

Schmidt, Kleisch and Schmalenbach, (1939) reported that up to 1.7 lb/day, of dried whey could be given satisfactorily to fattening pigs when it replaced 75% of a predominantly cereal meal diet. A similar level was also arrived at by Bünger (1940) who, earlier (Bünger, Fissmer, Harre, Schmidt, Boehm and Reising, 1939) reported that levels above 1.1 lb per day tended to depress the appetite of animals. At Illinois, feeding trials were conducted by Becker, Terrill, Jensen and Hanson, (1957) to study the carbohydrate replacement value of high levels of dried whey fed to swine of various ages. In the first experiment baby pigs initially 14 days of age, were fed diets containing up to 60 % dried whey. No harmful effects were reported. In the second experiment six levels of dried whey ranging from zero to 60 % were fed to pigs weighing approximately 85 lb at the start of the experiment. Each diet was full-fed to six individually penned pigs and half of each treatment group received an antibiotic supplement. The results after 35 days on the treatment diets showed that the 60 % level, had had a marked depressing effect on both food consumption and the rate of liveweight gain. A smaller effect was also apparent with pigs on the 40 % level of whey with antibiotic. A marked diarrhoea was reported for many of the pigs on the 60 % level, and some diarrhoea was also noted at the 40 % level of dried whey feeding.

Considerably higher intake levels of dried whey were achieved by Dunkin (1963 a). Three different, fixed levels of meal were supplemented with lactic casein whey powder to make the total daily ration up to a restricted scale of feeding for growing pigs. The resultant whey treatment diets (treatments 1, 2 and 3) contained 75.7 - 87.9%; 51.5 - 75.8 %; or 2.9 - 51.6 % whey powder respectively for liveweights ranging between 44 lb and 124 lb. No growth depressions and negligible scouring were reported in any treatment containing whey, including treatment 1 where whey powder formed approximately 83 % of the total food consumed. The results are

somewhat unusual in that from 48-110 lb liveweight, even the pigs receiving only ½ lb meatmeal plus lactic casein whey powder to scale, grew significantly faster than the control pigs receiving the same amount of meatmeal together with barley meal to scale. This suggested that weight for weight, under the existing conditions whey was superior to barley meal.

(c) Diets Containing Condensed Whey

Early reports by Scott and Graham, (1929) and Edin and Nordfeldt, (1941) suggested that condensed forms of whey could be used to advantage. Although handling difficulties (Dunkin, 1958) detract from its value, it has the advantage that a large amount (about 60 %) of the water has been removed and so is considerably less bulky than liquid whey. This makes it more suitable for feeding to pigs of all ages, and greatly improves it's keeping quality.

Dunkin, (1958) carried out a trial using condensed whey diluted 1 : 1 with water, in which three diets containing condensed whey and different fixed levels of meal were compared with an all meal diet. A restricted scale of feeding was used which resulted in up to 88 % of the total dietary dry matter consisting of whey. Results showed that when whey percent intake rose from 29 % at 45 lb, to 52 % at 125 lb, growth rate and food conversion efficiency were similar to that achieved on an all-meal control diet. When the whey intake at 45 lb liveweight was either 52 % or 76 %, and rose to 76 % or 88 % respectively at 125 lb liveweight, significant depressions were recorded in both rate and efficiency of liveweight gains.

(d) The Use of Fresh Whey in Growing Pig Diets

Workers at Reading (U.K.), (Braude, Clarke, Mitchell, Cray, Franke and Sedgwick 1957; Braude, Mitchell, Cray, Franke and Sedgwick, 1958; 1959 a, b; Mitchell and Sedgwick, 1963) have conducted several experiments with growing pigs fed unrestricted quantities of whey. A similar meal mixture containing barley meal, white fish meal and weatings or millers offals, was fed in all experiments, 3 lb per pig per day, reduced to 2 lb at 13 weeks of age, being the usual level of supplementation. As a consequence, the results published are fairly comparable, particularly in so far as all experiments were conducted at the same commercial unit and pigs were selected from a comparable pig population.

All data showed that an intake of about 40 lb of whey was reached at 120 lb liveweight, this representing an approximate intake of 2 lb whey D.M. and so constituted 50 % of the daily ration. Much later, at liveweights around 200 lb, only slightly greater average intakes were recorded, levels of 45 - 50 lb whey being common. These levels represented 56 % of the total daily ration. Similar calculations for whey intake at 70 lb liveweight show the percentage to be as low as 20 % of the total daily ration.

It is evident that the rapid increase in whey intake (20 % - 50 % from 70 lb - 120 lb) continued until approximately half the total daily D.M. ration consisted of whey. After this point, little increase in whey was apparent. This 'levelling out' in whey intake at about 150 lb liveweight, and its associated reduction in rate of growth were noted by Braude et al (1958) who commented that similar effects were not noted with pigs in an earlier investigation (Braude, Clarke, Mitchell, Cray, Franke and Sedgwick 1958) using all meal rations fed under similar conditions.

The suggestion was made that the pigs were unable to consume sufficient whey, but no reasons were given for this voluntary feed

restriction. Possible factors contributing to this will be dealt with following the summary.

(e) Summary

In view of the different sources of lactose used in the publications reviewed, the data has been summarised in terms of the absolute (lb) level of lactose ingested daily, in Table 1.1. The table clearly illustrates the difficulties involved in interpreting and drawing conclusions from the published data. It is evident that higher levels of lactose are successfully fed (irrespective of the source) in New Zealand and the United Kingdom compared with the United States of America. This is probably due to the selection and development in the U.S.A. of strains and breeds best suited to ad lib grain feeding conditions. The higher levels achieved under New Zealand, compared with United Kingdom conditions, is possibly a reflection of the greater utilization of dairy by-products and lower use of grain in this country, resulting in unconscious selection of animals better suited to this type of feed.

LACTOSE LEVELS REPORTED TO CAUSE DIARRHOEA &/OR GROWTH DEPRESSION (cont'd)

REFERENCE		L/WT (LB)	LACTOSE SOURCE	APPROX. % IN DIET	APPROX. DAILY LACTOSE INTAKE (LB)	SYSTEM OF FEEDING	COMMENTS
Braude et al (1957)		180-->200	Fresh whey	48.2-47.5	1.30-1.27	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age. Growth rate and feed intake depression after 150 lb liveweight.
Braude et al (1958)		180	" "	51.8	1.51	" "	
Dunkin (1958)		50- 110	Condensed Whey	3-52 52-76 76-88	-1.45 0.75-2.10 1.10-2.40	Restricted to scale	Refusals small, but highly significant depression in growth rate of the two highest lactose levels.
Dunkin (1959)		50- 120	Fresh whey	42.9-76.0	.53-2.24	Restricted to scale	Produced high incidence of Diarrhoea. Not possible to assess effect on G.R. or food intake as all Tr.Groups on similar intake level.
Braude et al (1959 a)		50- 170	" "	14.3-52.6	.25-1.55	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age. Depression in G.R. clearly apparent at 120 lb (43.2 %, 1.06 lb) and got progressively worse.
Dunkin (1961)	I	50- 110	" "	47.4-70.0	.63-1.65	Restricted to scale	Consistent refusals and eratic growth. High incidence Diarrhoea. Differences in G.R. due to caloric content of meal supplements.
Dunkin (1963 b)		50- 140	Dried whey	50	1.25-2.50	Restricted to scale	Poorer growth with neutralized whey only - higher total ash.

LACTOSE LEVELS REPORTED AS BEING MAXIMAL

REFERENCE	L/WT (LB)	LACTOSE SOURCE	APPROX. % IN DIET	APPROX. DAILY LACTOSE INTAKE (LB)	SYSTEM OF FEEDING	COMMENTS
Schmidt et al (1939)	60-?	Dried Whey	?-75	1.19	?	Assumes Lactose 70% of whey D.M.
Bünger et al (1939)		" "		0.77		" " " " "
Bünger (1940)		" "		1.19		" " " " "
Becker et al (1957)	85-140	" "	40	1.12	Full Fed	Slight Depression in presence of Antibiotics

LACTOSE LEVELS REPORTED TO CAUSE DIARRHOEA &/OR GROWTH DEPRESSION

REFERENCE	L/WT (LB)	LACTOSE SOURCE	APPROX. % IN DIET	APPROX. DAILY LACTOSE INTAKE (LB)	SYSTEM OF FEEDING	COMMENTS
Krider et al (1949)	I 30- 75	Dried whey	4.0	.05	Self Fed	Considerable Diarrhoea but did not affect growth. High ash 12.9 % of D.M. May have had some effect on Diarrhoea.
			8.0	.12	" "	
	II 50- 90	Dried whey	4.0	.09	Self Fed	Persistent Diarrhoea. Whey ash 12.9% D.M.
		" "	2.6	.08	" "	Negligible Diarrhoea. Whey ash 20.6% D.M.
		" "	3.2	.04	" "	Negligible Diarrhoea. Whey ash 14.8% D.M.
		Lactose	2.4	.06	" "	Persistent Diarrhoea
Becker & Terrill (1954)	I 45- 80	Lactose	50	1.15	Full Fed	Av. over 39 days, depressed food intake, growth rate, marked diarrhoea.
	II 125-160	"	50	1.56	" "	
Becker et al (1957)	II 85-125	Dried whey	60	1.19	" "	Av. over 35 days. Depressed food intake. Growth rate, marked Diarrhoea.
	IV 40- 80	" "	20	0.60	Ad Lib	Av. over 34 days, marked depression in daily Gain.
			30	0.90	" "	Negligible Diarrhoea. Differences in mineral level implicated.

TABLE 1.1

SUMMARY OF LACTOSE, AND WHEY, FEEDING DATA

LACTOSE LEVELS REPORTED AS YIELDING SATISFACTORY RESULTS

REFERENCE	L/WT (LB)	LACTOSE SOURCE	APPROX. % IN DIET	APPROX. DAILY LACTOSE INTAKE (LB)	SYSTEM OF FEEDING	COMMENTS
Becker et al (1954)	6- 22	Lactose	56	0.5	Ad Lib	Intake Av. over 28 days
Becker & Terrill (1954)	125-175	"	25	1.00	Full fed	Intake Av. over 39 days
Becker et al (1957)	8- 22	Dried whey	60	0.35	Ad Lib	Intake Av. over 28 days
	85-145	" "	20	0.60	Full fed	Intake Av. over 35 days
	34- 86	" "	30	1.00	Full fed	Intake Av. over 42 days
Braude et al (1957)	60-120	Fresh whey	18.5-46.1	0.46 -1.20	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age
Braude et al (1958)	70-120	" "	18.3-46.7	0.46 -1.23	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age - Protein source white fish meal
Braude et al (1959)	50-120	Fresh whey	15 -54.5	.25 -1.68	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age
Dunkin Trial II (1961)	50-110	" "	39 -73.8	.70 -2.00	Restricted to Scale	Refusals and scouring very low suggesting levels not above tolerance. Differences in G.R. due to caloric content of meal supplement.
Mitchell & Sedgwick (1963)	50-120	" "	10.0-42.0	0.21 -1.02	Whey Ad Lib	Meal 3 lb dropping to 2 lb at 13 weeks of age
Dunkin (1963 a) I	50-140	Dried whey	3 -52	-1.45	Restricted to Scale	Refusals and scouring negligible G.R. depression 110-140 (I), 50-120 (II) on two highest intake levels.
II	50-120	" "	52 -76	1.75 -2.10		
			76 -88	1.10 -2.40		
Dunkin (1963 b)	50-140	" "	25	.7 -1.25	Restricted to Scale	Satisfactory at high and low ash
			50	1.25 -2.50		Satisfactory only at lower ash level.

1.2 ADDITIONAL FACTORS AFFECTING THE PERFORMANCE OF GROWING PIGS FED WHEY

Evidence suggests that other factors characteristic of whey, in particular the high soluble salt concentration and the excessive bulk, may also affect pig performance.

(a) Soluble Salt Concentration

Daniel and Harvey, (1947) first demonstrated the deleterious effects of whey salts by feeding dialysed and untreated whey to rats. The untreated whey diet caused a lowered appetite, diarrhoea, and impaired food utilization. Comparable symptoms were produced with the dialysed whey diet only after the salts, which had been removed, were returned to the diet. In an unreplicated group feeding experiment with pigs (Wegelin, 1952) in which normal or desalted whey powder was fed at two levels to growing pigs, poorer growth rates and inferior feed consumption and utilization was reported for the two groups on the untreated whey.

However, differences were small and no tests of significance were performed.

Wegelin concluded that:

> "The retardation of growth by feeding high quantities of normal whey powder is not due to the whey proteins but it has to be ascribed to the accompanying salts and/or lactose."

At Illinois, Becker et al (1957) suggested that mineral composition might have made an important contribution to the different effects noted with a semi-purified and a practical diet. Both contained 30 % dried whey but severe growth rate depression was only apparent in the practical diet which differed considerably, in mineral composition, from the semi-purified diet. More recently, the intake level of mineral salts was further implicated in results published by Dunkin (1963 b). A neutralized form of lactic casein whey powder was used which had a much lower level of acidity than the normal powder. However, as a consequence of the

neutralizing process using caustic soda, a large (50 %) increase in the total ash level of the powder resulted. When the two forms of whey powder were fed as 50 % of the daily ration, a significant growth depression was reported in the group receiving the neutralized whey powder.

(b) Bulk

The effect of liquid whey bulk on pig performance was demonstrated in experiments carried out with concentrated whey (Dunkin, 1957 unpubl.).

The whey was fed at two dilutions 1:1 and 1:9, the second ratio resulting in a dry matter concentration similar to that of fresh whey. Each dilution was fed either on a restricted scale of feeding, or ad lib, thereby giving two levels of dilution and two feeding levels. Results obtained from the two ad lib groups showed that the pigs on the 1:1 dilution consumed significantly ($P < 0.05$) more food (18 - 20 %) and grew at a faster rate (8 - 10 %) than those on the 1:9 dilution. In a second investigation (Dunkin, 1958 unpubl.) two restricted scales of feeding were used, one being 113 % higher than the other. The same dilutions were again used. Growth rate and food conversion efficiency of the group on the lower feeding scale but receiving the more concentrated diet were both superior to those of the group receiving the higher level of feeding but in a more dilute form.

1.3 LACTOSE EFFECT ON CARCASS QUALITY

Only one publication is available in which carcass evaluation has been carried out on lactose fed pigs. In a small experiment involving 12 pigs (Whittier et al, 1935) four pigs were fed a diet containing 40 % lactose. Carcass appraisal revealed only slight differences in water and fat content compared with the control pigs, much fatter carcasses resulting from the use of a diet containing 40 % sucrose.

Evaluation of carcass quality has been carried out in a limited number of the whey feeding investigations reported earlier. In general the results show that whey fed pigs may be slightly less fat than all-meal fed pigs, but these results could have also been produced by the slower growth rates reported in the whey fed groups after approximately 150 lb liveweight. The differences were mainly associated with a reduced back-fat thickness and increased length, but no apparent differences in eye muscle area were reported (Braude et al 1957; 1959 a). In all experiments involving different levels of meal supplementation where all treatments received whey ad lib, no significant differences have been reported. (Braude et al 1958; 1959 b; Mitchell and Sedgwick, 1963). In these, a much more uniform growth between treatments was evident, again suggesting that the carcass differences reported in other investigations were a reflection of different rates of growth.

In summary it is clear that no satisfactory results are available from which conclusions can be drawn as to the effect of lactose on carcass quality. Results presented by Whittier et al (1935) are clearly inadequate, and the results produced from whey feeding investigations are subject to the influences of whey protein, whey salts, and greater water intake, as well as the effect of growth depression.

1.4 ADAPTATION TO HIGH LEVELS OF LACTOSE

Two definitions of "Adaptation" can be found in the literature. Lawrence, Fischer, Sutton and Weiser, (1956) defined it as:

> "The remission of lactose - induced diarrhea in rats maintained on a lactose diet, or failure of a test animal to develop diarrhea on a lactose diet which elicits this symptom in control rats,"
>
> and stated that:
>
> "Other changes in the animal such as enlargement of the caecum, are not included in the term adaptation as used here."

A more widely accepted definition (Knox, Auerbach, and Liu, 1956), Herzenberg and Herzenberg, 1959) was proposed by Lightbody and Kleinman (1939):

> "In general, changes in enzyme systems caused by changes in type or quantity of food ingested may be expected to result in two types of adaptations, those that may be considered emergency measures, and those requiring slow changes in physiological processes ..... Those of the second group may be considered adaptations in the quantities of the enzymes required to accomplish a given purpose."

It is therefore apparent that "the remission of lactose-induced diarrhoea" described by Lawrence et al, (1956) could be regarded as a physiological consequence of the enzymic adaptation to lactose as defined by Lightbody and Kleinman (1939).

As reported earlier, reduced growth rate, depressed food intake and diarrhoea, are the three main effects noted when pigs are fed diets containing high levels of lactose. Limited available evidence suggests that diarrhoea plays a very small part in the reduced growth rate. Krider et al (1949) reported considerable diarrhoea in pigs fed small amounts of dried whey, but concluded that rate or economy of live-weight gains were not affected. More recently a marked treatment difference in incidence of diarrhoea was reported by Dunkin, (1959) but no differences in liveweight gain were apparent.

It is much more likely that reductions in growth rate result from the depressed feed intake. However, knowledge of what causes this intake depression, or of what processes enable some animals to recover their appetite after a period of reduced intake is very limited. Nevertheless, the fact that some pigs do appear to regain their appetite after refusing food for a short period, suggests that this would be a more valuable index of "adaptation" than a reduction or cessation of diarrhoea.

1.5 LACTOSE INDUCED CAECUM DEVELOPMENT, AND BACTERIAL LACTASE ACTIVITY

The conflicting information regarding lactose induction of mucosal lactase activity in the small intestine, will be dealt with more fully in the second review. Although the above speculation is still unresolved, there is considerable evidence to show that lactose can induce greater bacterial lactase activity in the caecum of the rat. Enlarged caecae resulting from the intake of considerable amounts of the sugar have been reported by Ershoff and Deuel Jr, (1944), Fischer, Sutton, Lawrence, Weiser, and Stahly, (1949), Lawrence et al (1956), Fischer, (1957 b), Fournier and Digaud, (1959), Fournier and Guillam, (1960) and Tomarelli, Hartz and Bernhart, (1960).

Where measurements of lactase activity were made (Fischer et al 1949, Lawrence, Weiser, Stahly, Fischer and Sutton, 1949) an increased bacterial lactase activity in the caecal contents was reported to be associated with the caecal enlargement.

That this source of lactase activity plays an important part in the utilization of lactose was indicated recently by Dahlqvist and Thomson (1964). When 800 milligrams of lactose was administered to rats, sample analysis of gut contents revealed that a considerable proportion escaped absorption in the small intestine. However, no lactose was found in the stools. When lactase activity was assessed (mg. lactose hydrolysed per hour) it was found that the caecal contents contained at least ten times the hydrolytic activity found in the colon and rectum. This clearly implicated the bacterial lactase in the caecum as being largely responsible for the hydrolysis of the lactose entering the large intestine.

Although very little monosaccharide absorption occurs in the caecum and colon (1.1 Part II) several reports show that these organs are important sites of volatile fatty-acid (v.f.a.) and lactic acid absorption (Barcroft, McAnally, and Phillipson 1944; Elsden, Hitchcock, Marshall and

Phillipson 1946; Friend, Cunningham and Nicholson, 1962; 1963; Friend, Nicholson and Cunningham 1964). It is therefore postulated that the monosaccharides resulting from lactose cleavage, are converted to v.f.a.'s and lactic acid, thereby enabling their absorption into the bloodstream.

Furthermore, the conversion of lactose to lactic acid could explain the presence of the latter in diarrhoeal faeces (3.5/1 Part II). The inhibitory action of high levels of lactose in the caecum and colon, on the normal water reabsorption process, could also result in an associated decrease in lactic acid absorption, and so greater quantities would be lost from the body, in the faeces.

Practically all the available literature on lactose induced caecum development, relates to work with rats, and at present it is not possible to decide whether similar caecum development, or increased bacterial lactase activity occurs in pigs fed lactose. A very limited amount of work has been carried out to determine the importance of the caecum to the pig's digestive processes. Nasr. (1950) fed either raw or boiled potato starch to two separate groups of pigs and concluded that the caecum played an important part in the animals' digestion of the raw starch offered. On the other hand, Lloyd, Dale and Crampton (1958) using unoperated, and caecectomized pigs fed both experimental and practical diets, concluded that the caecum did not contribute appreciably to the general breakdown of the food substances.

Two factors may have been involved in producing the more recent results. The dissaccharide components of the diet used, (wheat, oats, cereal grass, corn starch) would be readily digested in the small intestine. Consequently very little carbohydrate would reach the large intestine and affect caecum development or bacterial enzyme activity. This suggestion is supported by the fact that Lawrence et al (1956) used raw potato starch to produce caecal distension in rats. The authors concluded that

caecal enlargement in the rats fed the raw potato starch was due to the inability of the alimentary tract to 'handle' this less digestible form of carbohydrate.

In addition, the removal of the caecum could have resulted in colonic hypertrophy (see Lawrence et al 1956) thereby providing an alternative site for the high concentrations of bacteria normally found in the caecum.

If then it can be assumed that the caecum only contributes to the overall hydrolytic activity when unhydrolysed di- and poly-saccharides reach the large intestine, there is the distinct possibility that lactose would be substantially hydrolysed by bacterial lactase activity in the caecum, when fed at levels which exceed the capacity of the mucosal lactase. This was demonstrated in rats (Dahlqvist and Thomson 1964) but no reports of work on this aspect of lactose feeding to pigs are available.

CHAPTER 2

FIRST PRELIMINARY INVESTIGATION

2.1 INTRODUCTION

The investigation was designed with three objectives in mind: to assess the adequacy of a proposed Basal Mixture fed to treatment 2 pigs from 45-80 lbs liveweight, to get some indication of the maximum lactose percentage levels which could be reached under the existing conditions, and to see if age at which lactose substitution commenced, affected the pigs' subsequent performance.

Two treatments, commencing at 45 lb for treatment 1, and at 80 lb for treatment 2, involved progressive lactose substitution of the wheat starch of the Basal Mixture. Each treatment contained two individually fed pigs.

2.2 EXPERIMENTAL

2.2/1 SELECTION OF ANIMALS AND EXPERIMENTAL DESIGN

The investigation involved four pigs from a Large White x (Berkshire x Large White) litter weaned at three weeks of age. Two castrate males and two females, of similar liveweight were selected at 8 weeks and one pig of each sex was randomly allocated to each of the two treatments.

2.2/2 PRE-EXPERIMENTAL FEEDS AND FEEDING PROCEDURES

Prior to selection the pigs had been fed a pelleted 'carry-on' meal mixture containing 25% dried buttermilk powder. Following selection, this mixture was gradually replaced by a cereal-meatmeal mixture fed at the daily rate of $1\frac{1}{2}$ lb per pig. This was supplemented by $4\frac{1}{2}$ lb of fresh whey to ensure a continued intake of small amounts of lactose, formerly provided by the buttermilk powder.

After allowing the pigs ten days in which to become accustomed to the individual penning facilities in a room in the Animal Physiology Unit (A.P.U.), the whey feeding was discontinued and a full cereal-meatmeal

ration was fed according to individual liveweights. This ration was maintained until 45 lb liveweight when the basal, or the lactose substituted diets were introduced. The daily ration was divided into two approximately equal feeds which were given at 7.30 a.m. and 4.30 p.m. The composition of the two pre-experimental meal mixtures may be found in the appendix. (App. 2.2/1, 2.2/2)

2.2/3 HOUSING

The room housing the pigs in the A.P.U. was totally enclosed and had provision for mechanical ventilation and thermostatically controlled minimum air temperature. Due to an error in the installation of the thermostat in the air intake duct, the temperature control equipment was never functioning and temperature fluctuations were quite large. However, as a result of fairly high climatic temperatures, coupled with the very effective insulation, the ambient temperature rarely fell below 66°F or rose above 78°F, a range of 12°F.

2.2/4 TREATMENT DIETS AND THEIR INTRODUCTION

A Basal Mixture common to both treatments was formulated, and consisted of:

Wheat Starch	50 %
Pollard	30 %
Fortified Meatmeal	20 %
Premix *	0.25 %

* Premix contained (per pound):

20 gm. 'Rovimix' A, B_2 D_3 - 40,000 I.U. Vitamin A per gm.
- 10,000 I.U. Vitamin D_3 per gm.
- 40 mg. Vitamin B_2 per gm.

4.8 gm. Pantothenic Acid

0.4 gm. Folic Acid

40 gm. 'Rovimix' Vit E_{25} - 250 I.U. Vitamin E per gm.

0.6 lb D.L. Methionine

0.2 lb 'Tasminal' Trace Element Supplement

For both treatments, total daily food allowances were rationed according to Evans' 'B' Scale of meal feeding (Evans 1966), as shown in Table 2.2/1.

TABLE 2.2/1 FEEDING SCALE *

Liveweight (lb)	Meal (lb)	Liveweight (lb)	Meal (lb)
20	1.10	80	3.50
30	1.60	100	4.10
40	2.10	120	4.60
50	2.50	140	5.10
60	2.90	160	5.60

* Intermediate values obtained by interpolation.

The Basal Mixture, in which lactose substituted for 10 % of the wheat starch, was fed to treatment 1 pigs from 45 lb liveweight, and to treatment 2 pigs from 80 lb liveweight. The unsubstituted Basal Mixture being fed to treatment 2 pigs from 45-80 lb liveweight as a control diet.

Following every 10 lb increase in liveweight of pigs on the lactose substituted diets, a further 10 % increase in lactose was made until food refusals commenced. The lactose level was then lowered by 10 %, and the pigs remained on the lowered level for three weeks, a period arbitrarily chosen as being of sufficient length to enable adaptation to the lactose, to occur. After three weeks, a further increase of 5 % lactose was attempted.

2.2/5 MANAGEMENT

(a) Feeding Routine

The pigs were weighed once a week before the morning feed, and adjustments to the daily ration allowances were made according to these weights. When feed refusals were becoming common, feed troughs were removed at 8.00 p.m. the previous night to ensure that weighings were made on a 'constant stomach fill'.

The day's food allowances were weighed into plastic buckets to which water was added at the rate of 3 parts water to 1 part meal on a weight basis. This was mixed the afternoon before the day of feeding, to ensure sufficient time for the lactose (in the α hydrate form) to attain the α/β equilibrium form which is less likely to cause digestive upsets (Primnig and Turkus 1943).

No bedding and no additional drinking water were provided.

Refusals were collected in the morning before feeding. Each refusal was weighed, stirred, and a 500 gram sample was taken and dried at 100°C for 48 hours, preliminary observations indicating that these conditions were adequate, and that duplicate samples were unnecessary. Meal dry matter and water refusals could then be calculated separately.

(b) Dung Consistency

Daily dung observations were made in the morning before cleaning out the pens. Dung consistency was arbitrarily classified by visual appraisal into five classes. These were:

Hard - (H)

Normal - (N)

Soft - (S) - retaining its characteristic form but softer than the normal stool.

Porridge-(P) - having a consistency not unlike thick porridge.

Watery -(W) - Amorphous and with very little 'depth'

(c) Length of the Investigation

The experiment lasted 77 days, daily weighings as the pigs approached 115 lb liveweight being necessary so that the number of days to gain 70 lb in weight could be accurately assessed.

2.3 RESULTS

2.3/1 HEALTH

Throughout the investigation all pigs remained in good health. However, one pig (Pig A - on 25 % lactose) died the night after completion of the study. Death occurred suddenly, overnight, the afternoon's feed was wholly consumed and there was no indication that death was imminent. Post mortem diagnosis of the cause of death was inconclusive. From the gross appearance of the carcass, septicaemia and toxaemia, probably of anaerobic origin, were suspected but could not be confirmed by bacteriological tests.

As the level of lactose in the diets was being adjusted in accordance with growth performance, severe effects of high lactose levels were not experienced. In association with high lactose levels ingested early in life, one pig (Pig A) showed a pronounced 'pot bellied' appearance. The implication of this will be discussed later.

2.3/2 DUNG CONSISTENCY

Only the results from the first four weeks' observations following treatment introduction are presented in Table 2.3/1 as the dung was of 'normal' consistency in the case of all observations made after this period.

TABLE 2.3/1 OBSERVATIONS OF DUNG CONSISTENCY FOR THE FIRST 28 DAYS AFTER REACHING 45 lb LIVEWEIGHT

Treatment		Dung Consistency					
Pig		Hard	Normal	Soft	Porridge	Watery	Total
A	1	-	28	-	-	-	28
B	2	-	17	6	5	-	28
C	2	-	26	2	-	-	28
D	1	-	13	7	7	1	28

These figures give little indication of a 'diarrhoeal' effect resulting from a high intake of lactose, in fact all voidings after a liveweight of 70-80 lb was reached were classified as 'normal' despite the commencement of lactose feeding at 80 lbs to two pigs.

2.3/3 GROWTH RATE, FOOD INTAKE, AND FOOD CONVERSION EFFICIENCY

As mentioned earlier percentage levels of lactose were reduced as soon as the feed refusal started to rise, an observation invariably accompanied by a decreased growth rate. The figures presented in Table 2.3/2 are therefore a result of several dietary manipulations and cannot be regarded as pertaining to any fixed level of lactose.

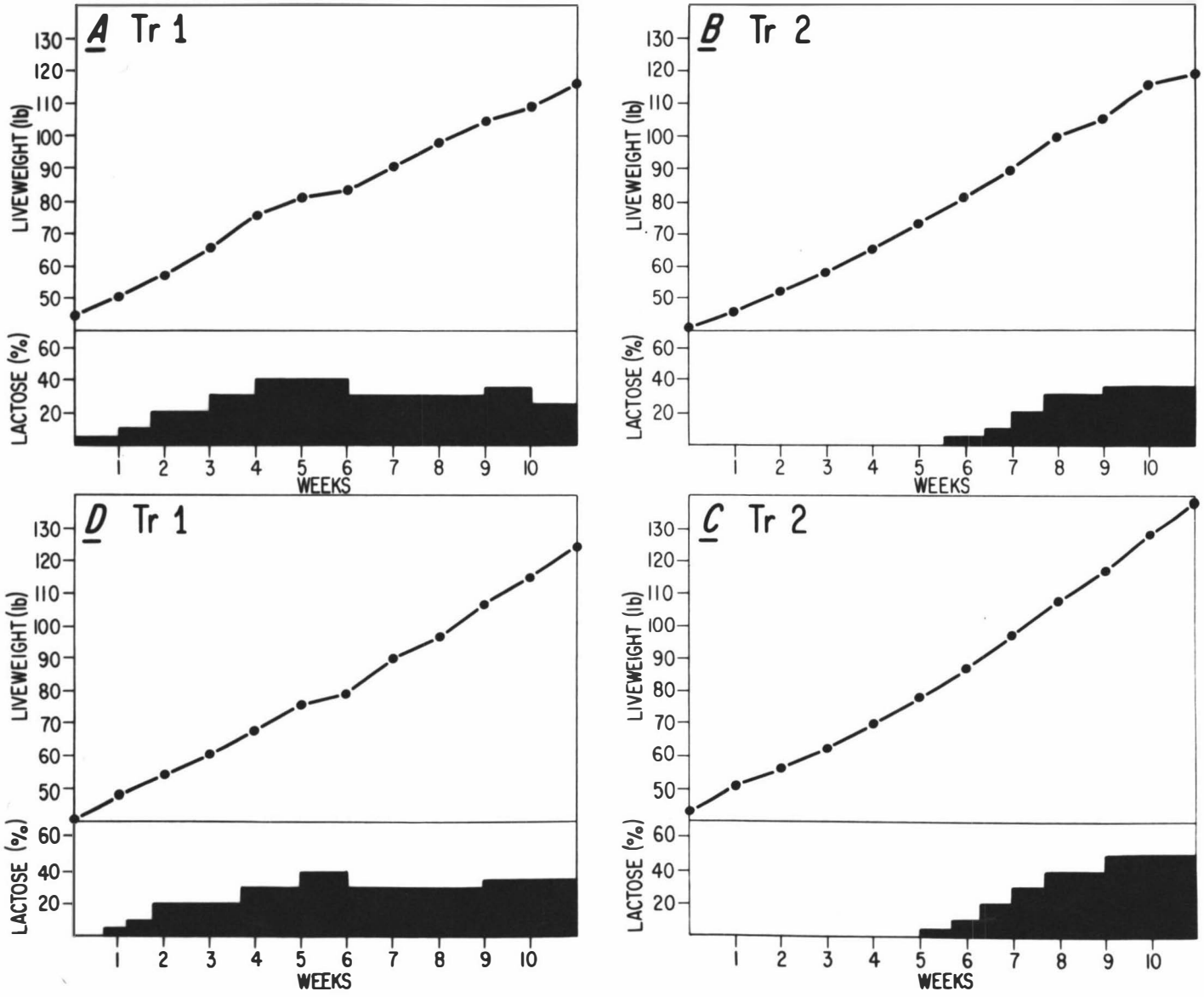


FIGURE 2.3/1 Individual Growth Rates and Levels of Lactose Intake

TABLE 2.3/2 GROWTH RATE, FOOD CONSUMPTION, AND FOOD CONVERSION EFFICIENCY

		Treatment 1		Treatment 2	
		Pig A	Pig D	Pig B	Pig C
(a)	45 lb - 75 lb Liveweight				
	Number of days	27	30	32	30
	Av. Daily Gain (lb)	1.11	1.00	0.94	1.00
(b)	75 lb - 115 lb Liveweight				
	Number of days	51	37	33	29
	Av. Daily Gain (lb)	0.78	1.08	1.21	1.38
(c)	45 lb - 115 lb Liveweight				
	Total No. of days	78	67	65	59
	Av. Daily Gain (lb)	0.90	1.05	1.08	1.19
	Total food consumed (lb)	244.60	216.15	203.15	195.25
	lb Food per lb Liveweight gain	3.49	3.09	2.90	2.79

A clearer picture of individual growth rates in association with varying percentage levels of lactose can be gained from Figs. 2.3/1 2.3/2. A considerable depression in the growth rates of both treatment 1 pigs is evident at the time when the lactose level in the diet reached 40 %. However, in both cases, the previous growth rate was regained when the lactose content of the diet was reduced by 10 %. It is clear from both Fig. 2.3/2 and Table 2.3/2 that all pigs grew at a comparable rate for the first 4 weeks of the investigation. Subsequent to this, Pig C., despite its high level of lactose intake, was noticeably superior in growth rate to the other 3 pigs.

2.3/4 FOOD REFUSALS

A considerable quantity of food was refused by three of the pigs. A summary of the data is presented in Table 2.3/3.

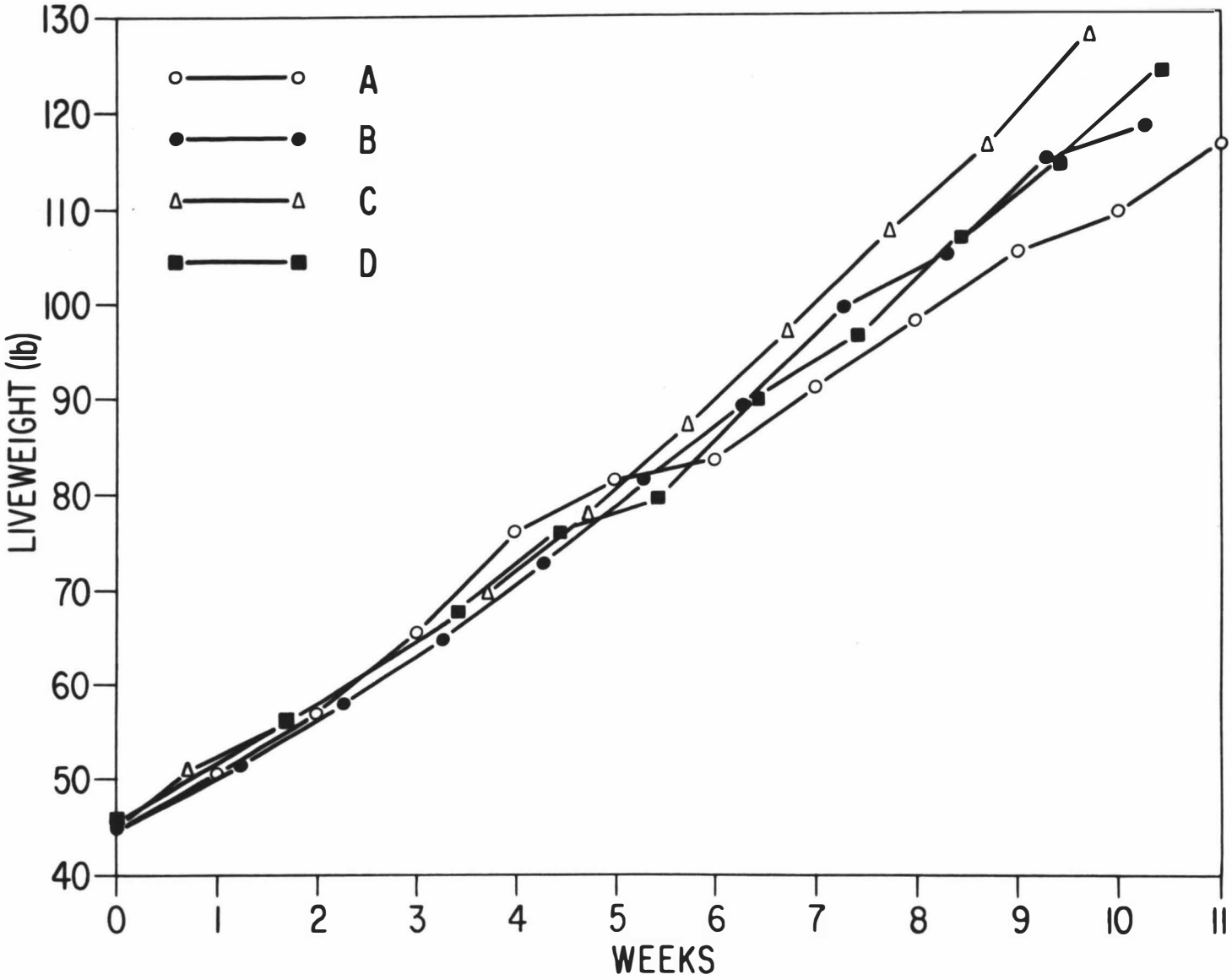


FIGURE 2.3/2 Growth Rates of the Four Experimental Animals

TABLE 2.3/3 Food Refusals

(a)

	TREATMENT 1						TREATMENT 2					
	PIG A			PIG D			PIG B			PIG C		
WEEKS OF EXPT	LACTOSE PERCENT	DAILY INTAKE OF LACTOSE	TOTAL DM FOOD REFUSED	LACTOSE PERCENT	DAILY INTAKE OF LACTOSE	TOTAL DM FOOD REFUSED	LACTOSE PERCENT	DAILY INTAKE OF LACTOSE	TOTAL DM FOOD REFUSED	LACTOSE PERCENT	DAILY INTAKE OF LACTOSE	TOTAL DM FOOD REFUSED
	%	lb	lb	%	lb	lb	%	lb	lb	%	lb	lb
0 - 1	5	0.10	-	5	0.10	-	0	-	-	0	-	-
1 - 2	10	0.25	-	10	0.25	-	0	-	-	0	-	-
2 - 3	20	0.60	-	20	0.55	-	0	-	-	0	-	-
3 - 4	30	0.90	-	20	0.60	-	0	-	-	0	-	-
4 - 5	40	1.35	1.10	30	0.90	-	0	-	-	0	-	-
5 - 6	40	1.40	7.00	40	1.35	0.90	5	0.10	-	5	0.20	-
6 - 7	30	1.10	0.50	30	1.05	0.10	10	0.35	-	15	0.60	-
7 - 8	30	1.15	0.60	30	1.15	0.80	20	0.75	0.50	30	1.20	-
8 - 9	30	1.20	1.70	30	1.20	0.30	30	1.20	3.85	40	1.65	-
9 -10	35	1.40	5.35	35	1.50	0.95	35	1.40	2.45	50	2.10	-
10 -11	25	1.10	2.45	35	1.60	1.10	35	1.60	3.60	50	2.40	-
TOTAL			18.70			4.15			10.40			0.0

(b)

		TR.1		TR.2	
		A	D	B	C
Total Food Refusal - DM	(lb)	18.70	4.15	10.40	-
Total Lactose Consumed 45-115 lb	(lb)	85.75	73.05	37.90	58.35
Total Lactose Consumed before First Refusal	(lb)	23.80	18.10	8.50	58.35*
Days on Lactose before First Refusal		35	32	16	41 *

* Experiment terminated before any refusal occurred.

The results suggested that the onset of a food refusal was associated mainly with the level of lactose intake. For both treatment 1 pigs, refusals were first noted when lactose reached the 40 % level (1.35 lb Lactose daily). Although these refusals were reduced by a 10 % reduction in dietary lactose, they were not eliminated completely, and a subsequent increase of 5 % lactose, caused a further increase in the amounts of food refused. Results from treatment 2 pigs were inconclusive, for although Pig B began to refuse food in half the time, and after approximately half the absolute and percentage amounts of lactose had been fed, compared with treatment 1 pigs, the second pig on treatment 2 (Pig C) did not refuse food, even when the dietary level of lactose rose to 50 %, (2.40 lb Lactose daily).

2.4 DISCUSSION AND CONCLUSIONS

2.4/1 RATION SUITABILITY

So far as could be assessed by the general health of the pigs, the Basal Mixture was nutritionally adequate. In addition the dilution of the meal 1:3 with water, produced a thin slop which appeared to provide sufficient water for the pigs' normal requirements.

2.4/2 DUNG CONSISTENCY

The fact that very few of the dungs were classified as "watery" was not unexpected.

The limited number of pigs used, the relatively short periods over which high (40 %) lactose levels were fed in most cases, and the general tendency for most pigs to refuse feed containing high levels of lactose probably affected the results.

2.4/3 FOOD INTAKE AND REFUSALS

The results indicated that over the liveweight range examined, a level of 1.0 - 1.15 lb lactose could be consumed daily with little or no

depression of intake, but individual variability could result in levels of up to 2.40 lb lactose daily being successfully utilized.

2.4/4 AGE AT WHICH LACTOSE WAS INTRODUCED

Due to the marked differences in growth rate between the pigs in treatment 2, no conclusions were possible on the effects of imposition at 80 lb liveweight compared with imposition at 45 lb.

> "Gus never had a good return from his pigs; they just seemed to exist to get rid of the leftovers and that's all. There's good money to be made out of pigs, I know, and I've been meaning to take an interest in them, but my first love is my dairy herd and I've never been able to get very affectionate towards the piggy part of the farm."
>
> (Frank S. Anthony and Francis Jackson)

CHAPTER 3

SECOND PRELIMINARY INVESTIGATION

3.1 INTRODUCTION

The conclusions that could be drawn from the first preliminary investigation were limited by the small number of animals used, their variability, and the fact that constant adjustments were made to the dietary lactose level. A second preliminary investigation was therefore designed to provide information on the effects of different fixed dietary levels of lactose, and the rate of increase of the lactose content.

3.2 EXPERIMENTAL

The investigation involved four treatments:

Treatment 1 (control)	No lactose
Treatment 2	25 % lactose
Treatment 3	25 % increasing to 50 % lactose over a 4 week period
Treatment 4	50 % lactose

3.2/1 SELECTION OF ANIMALS AND EXPERIMENTAL DESIGN

A randomized block design was used involving four treatments each containing three replicates. The twelve pigs used were all from Landrace X Large White litters and comprised eight gilts and four castrates. Four of the gilts came from one litter, the remaining 8 pigs coming from a second litter. All pigs were weaned at three weeks of age. Following selection, the pigs were randomly allocated to treatments and individual pen positions in the A.P.U., the only limitation being to ensure that each treatment group was represented by two gilts and a hog.

3.2/2 PRE-EXPERIMENTAL FEEDS AND FEEDING PROCEDURES

When selected at 8 weeks of age, all pigs were being fed 'Carry-on' meal pellets similar to those fed before the start of the first preliminary

investigation. In contrast to the earlier study, feeding of the 'Carry-on' meal (mixed 1:3 with water) continued until the treatment rations were introduced.

3.2/3 TREATMENT DIETS AND THEIR INTRODUCTION

The basal meal mixture, the scale of feeding, and the feeding procedure were similar to those used in the earlier investigations, except that the introduction of the experimental diets was made at 50 lb liveweight. Diets were fully established in 5 days. Treatment 3 pigs received a 25 % lactose diet for 11 days. The 35% level was then introduced, this being increased to 45 % after 1 week, and finally to 50 % after a further week.

Three treatment diets were used - their composition being:

Diet A The same Basal Mixture as was used in the first preliminary

Diet B Diet A in which half of the wheat starch was replaced by lactose

Diet C Diet A in which all of the wheat starch was replaced by lactose

Proportionate amounts of Diets A and C were mixed to provide the various lactose percentages required for treatment 3 during the first 4 weeks.

After the experiment had been in progress for almost six weeks, treatments 3 and 4 (now both on 50 % lactose) were producing similar growth rates, and so there seemed to be little point in continuing the investigation on the effects of rate of introduction. As all 6 pigs were on the same treatment diet, it was felt that a further treatment could be imposed upon three of the pigs to enable full utilization of the animals available. Meaney and Sheehy (1965) reviewed a number of investigations in which copper sulphate had been added to diets fed to pigs, and concluded that there was a definite advantage to be gained from the judicious use of the Copper salt. As the main effect of the copper is on the gut microbial population, and in view of the reported effects (Fischer and Sutton, 1949) of lactose on

the gut flora, it was felt that a study of the effect of copper addition to the diet might yield interesting results.

Only treatment 3 pigs received the copper (250 ppm on an air-dry meal basis) and treatment 4 pigs were retained as 'controls'. Supplementation with copper commenced after each pig had been on the treatment diet for 41 days and was continued until the end of the investigation.

3.2/4 MANAGEMENT AND HOUSING

All conditions of management established in the earlier investigation were adhered to. An ambient temperature of 68°F was again desired. The temperature control equipment was now functioning perfectly, and continuous temperature recordings were made with a thermograph. Results of weekly recordings (App. 3.2/1) showed that the average experimental temperature was 70.5°F (Range 65.5 - 75.5°F) and that the number of hours in which this range was exceeded, represented only 1.7 % of the total experimental time.

3.3 RESULTS

The investigation was to proceed until all pigs reached 120 lb liveweight. When it was apparent that all but one of the pigs on treatments 3 and 4 would not reach 120 lb within a reasonable period of time, these pigs were removed from the investigation after 70 days.

3.3/1 DUNG CONSISTENCY

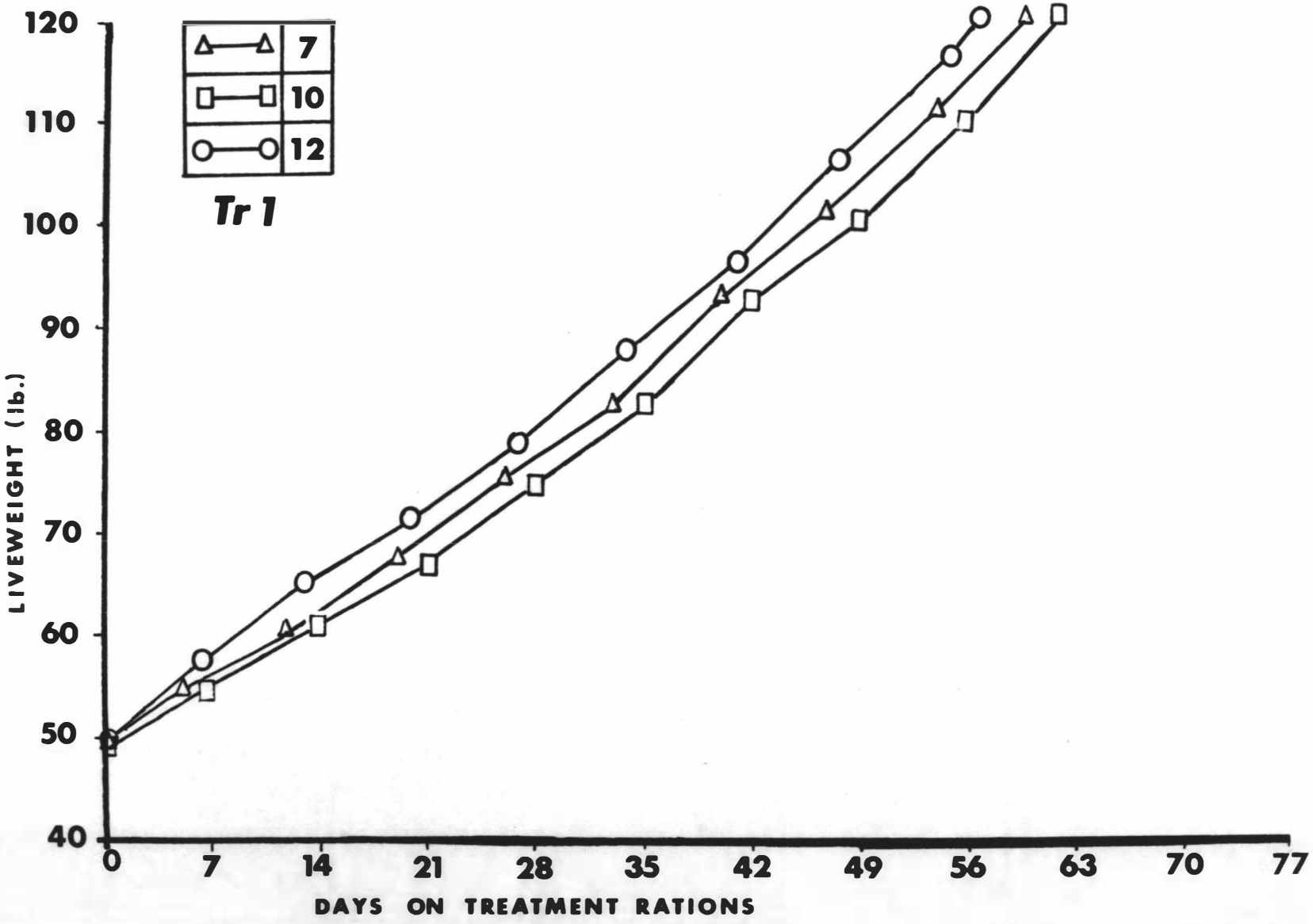
Daily dung observations were recorded as outlined in the earlier investigation and a summary of results obtained over the first eight weeks of the investigation are presented in Table 3.3/1.

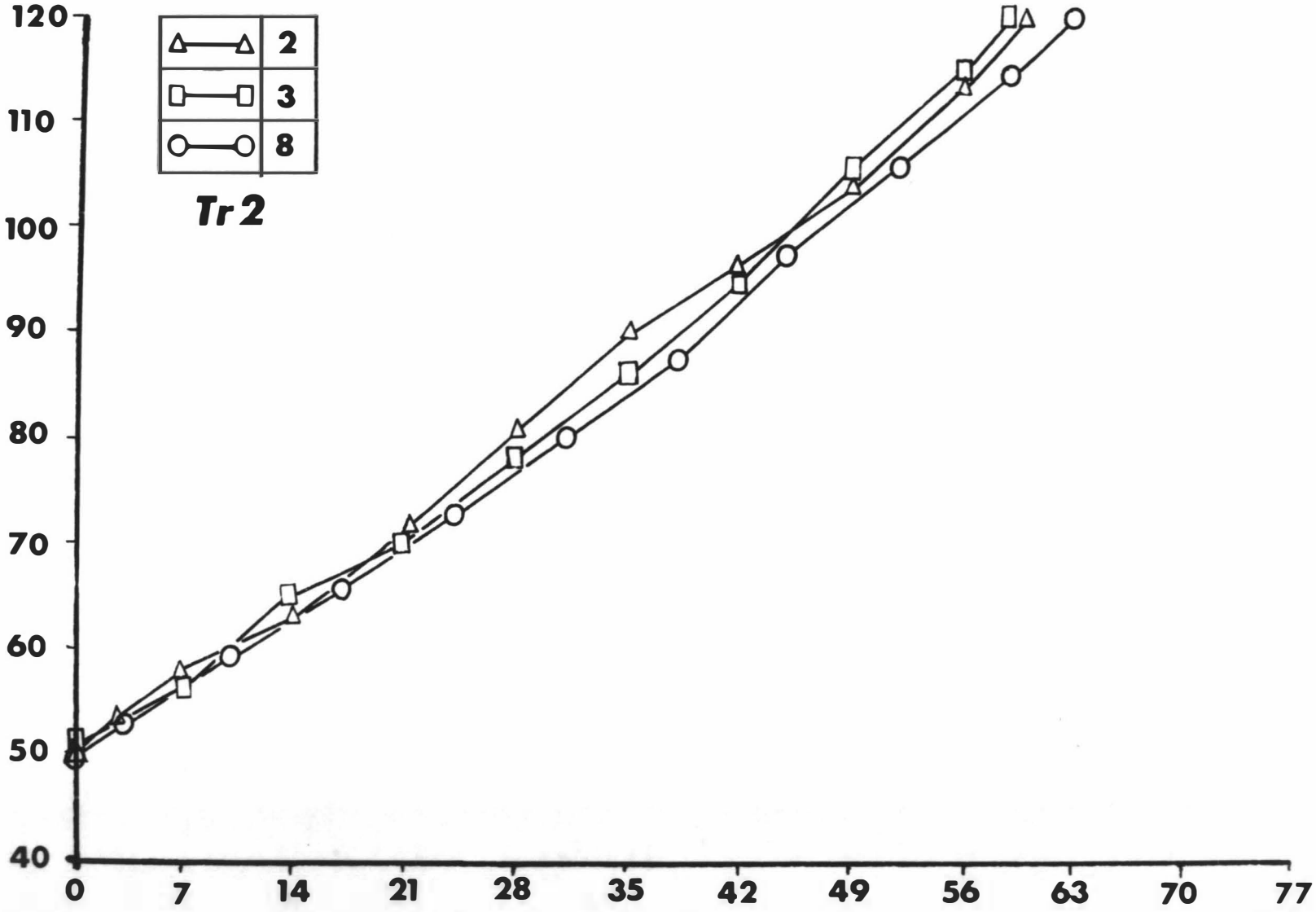
TABLE 3.3/1 RECORDS OF DUNG CONSISTENCY OVER THE FIRST 56 DAYS*

Days	Treatment 1					Treatment 2					Treatment 3					Treatment 4				
	H	N	S	P	W	H	N	S	P	W	H	N	S	P	W	H	N	S	P	W
0- 7	8	13					16	5			1	17	3				4	3	4	10
8-14	9	11	1				19	1		1	3	9	5	3	1		11	5	3	2
15-21	2	19					20	1			6	11	1	1	2		10	4	4	3
22-28		21					21				3	17	1				16	2		3
29-35		19	1	1			21				1	18		1	1		10	8	2	1
36-42		21					21				1	17	1	1	1		12	5	1	3
43-49		21					20	1				19	2				18	2	1	
50-56		19	2				15	6				18	2		1		17	4		
Total	19	144	4	1			153	14		1	15	126	15	6	6		98	33	15	22

* Weekly totals are the sum of observations from three pigs per treatment.

There was clearly a greater incidence of diarrhoea (as indicated by the larger numbers of 'porridge' (P) and 'watery' (W) faeces) in treatments 3 and 4, compared with the other two treatments. The lower number of 'watery' faeces in treatment 3 compared with treatment 4 was probably a reflection of the shorter period of time treatment 3 pigs were on 45-50 % lactose, due to the more gradual rate of lactose introduction carried out. However, over the latter part of the investigation when both treatments were on a 50 % lactose diet, there was still a small number of observations classified as 'soft', 'Porridge' or 'watery' in the treatment 3 group suggesting that the slower rate of introduction may have reduced the pigs' tendency to scour on high levels of lactose.


120
110
100
90
80
70
60
50
40
LIVEWEIGHT (lb.)
7
10
12
Tr 1
0
7
14
21
28
35
42
49
56
63
70
77
DAYS ON TREATMENT RATIONS


120
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77
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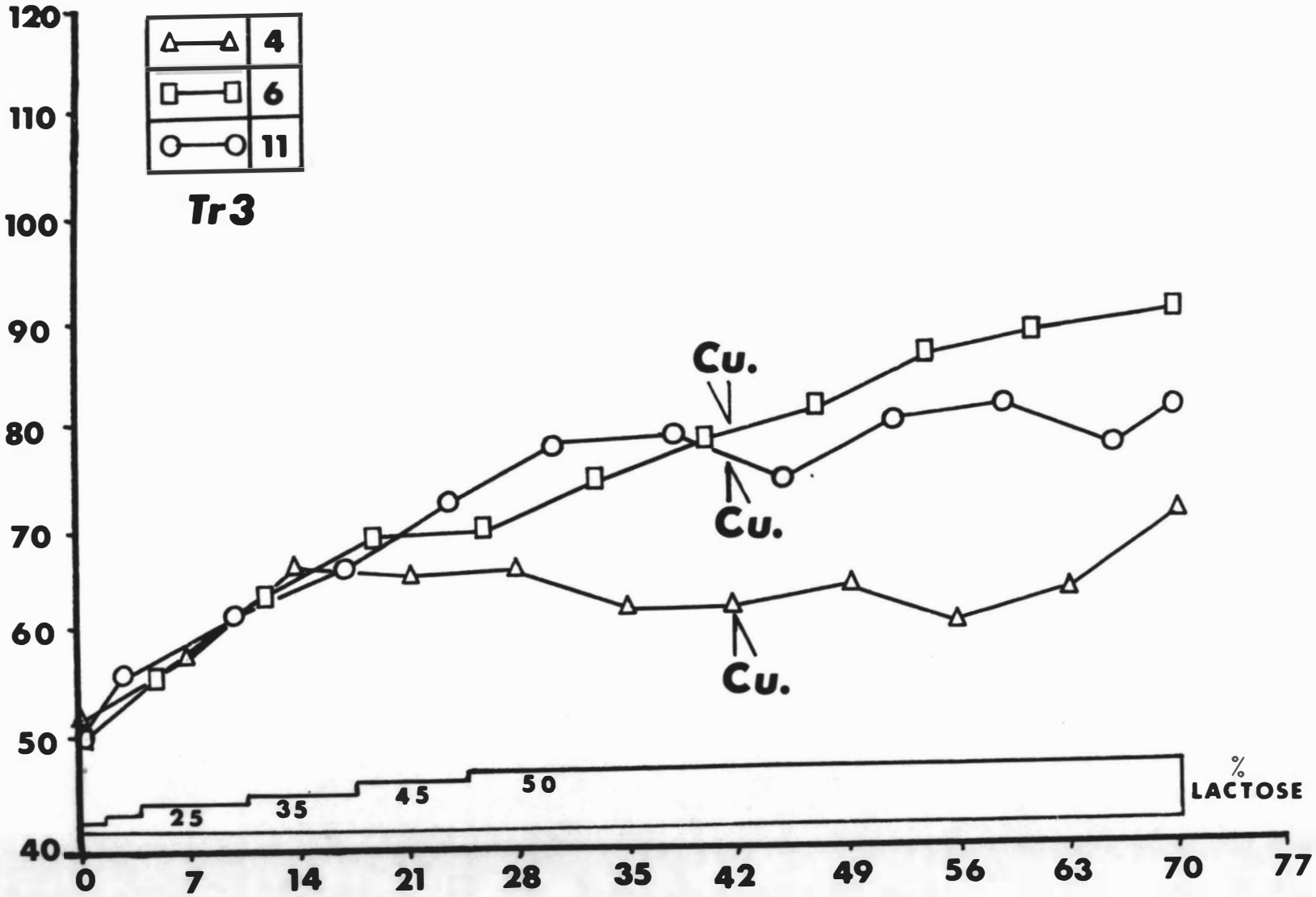
8
Tr 2


120
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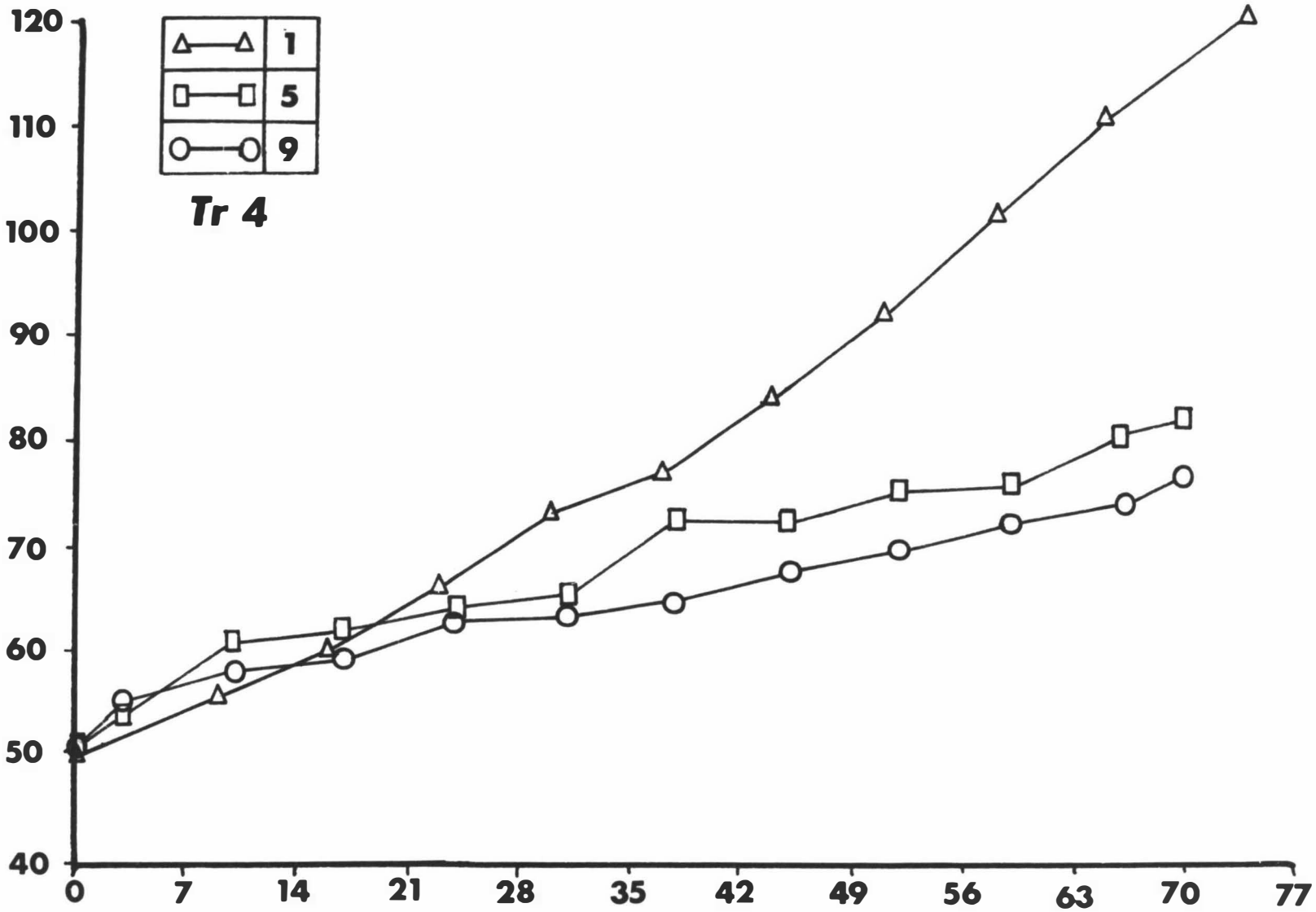
40
4
6
11
Tr3
Cu.
Cu.
Cu.
25
35
45
50
%
LACTOSE
0
7
14
21
28
35
42
49
56
63
70
77

FIGURE 3.3/1 Individual Growth Rates of the 12 Treatment Animals



3.3/2 GROWTH RATE, FOOD INTAKE AND FOOD CONVERSION EFFICIENCY

All pigs on treatments 1 and 2 reached 120 lb liveweight and so full data could be presented. Due to the very poor growth rates of 5 of the 6 pigs in treatments 3 and 4, data for the first 70 day investigation period has been presented. To enable a statistical comparison between all four treatments, mean liveweights after 60 days on the treatment diets have also been presented in Table 3.3/2.

TABLE 3.3/2 MEAN GROWTH RATE, FOOD INTAKE AND FOOD CONVERSION EFFICIENCY FOR A 70 lb LIVEWEIGHT GAIN IN TREATMENTS 1 and 2, FOR A 70 DAY INVESTIGATION PERIOD IN TREATMENTS 3 and 4, AND FOR A 60 DAY PERIOD FOR ALL 4 TREATMENTS.

	50-85 lb Liveweight		85-120 lb Liveweight	
	TR 1	TR 2	TR 1	TR 2
No. of Days	35	34	24	27
Mean daily gain (lb)	1.00	1.03	1.40	1.30

	50 - 120 lb Liveweight	
	TR 1	TR 2
No. of days	60	61
Mean Daily gain (lb)	1.17	1.16
Nett Food Consumption (lb)	198.13	207.10
Mean Daily Food Consumption (lb)	3.32	3.40
lb Food / lb Liveweight Gain	2.83	2.96

	Results After 70 Days	
	TR 3	TR 4
Mean Daily Gain (lb)	0.43	0.59
Nett Food Consumption (lb)	149.30	173.58
Mean Daily Food Consumption (lb)	2.13	2.48
lb Food / lb Liveweight Gain	4.98	4.22

	Liveweight After 60 Days			
	TR 1	TR 2	TR 3	TR 4
Mean ± SE (lb)	120 ± 1.7	119 ± 1.6	77 ± 7.6	85 ± 9.9
* Significance of Difference 5 %	———	———	———	———

* Means not underscored by the same line are significantly different ($P < 0.05$).

Data in Table 3.3/2 and Fig. 3.3/1 illustrate the close similarity in growth rates of pigs on zero and 25 % lactose. Fig. 3.3/1 clearly demonstrated the marked variability experienced when pigs were fed diets containing 50 % lactose. Because of this very large variability the F test of significance (Snedecor, 1961) was unsatisfactory and so an alternative - Dixon's Test (Dixon and Massey, 1957) which takes large differences in treatment variability into account was used to determine significant differences. The analysis (App. 3.3/1) revealed that differences between treatment 1 and treatment 2 (zero and 25 % lactose), and between treatment 3 and treatment 4, were non significant, but the two treatment groups receiving 50 % lactose (3, 4) were both significantly ($P < 0.05$) poorer in liveweight after 60 days following treatment introduction, then treatments 1 and 2.

It was not possible to analyse the effect of copper supplementation statistically, but the average growth curves for treatment 3 and 4 groups

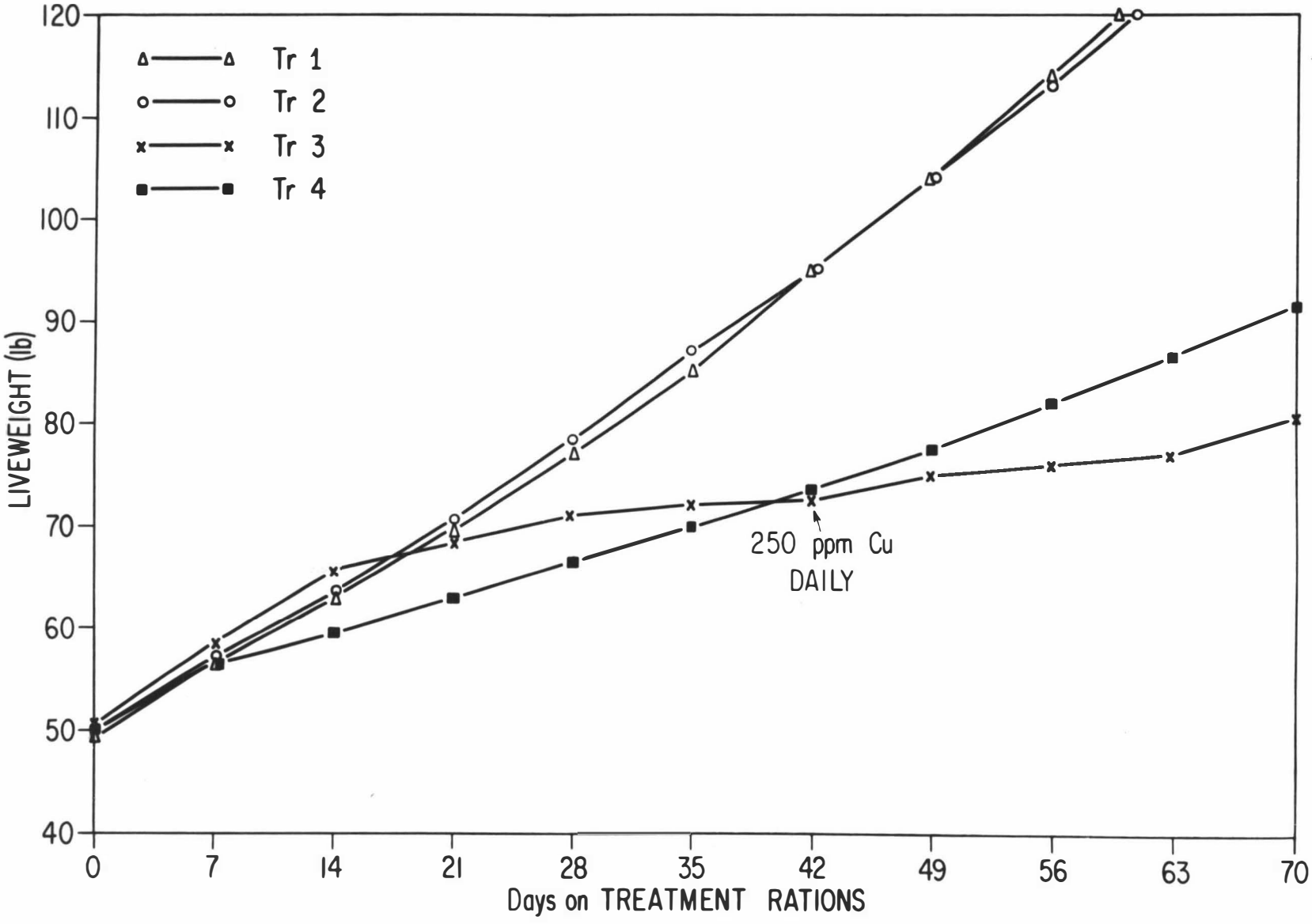


FIGURE 3.3/2 Mean Liveweight Gains of 3 Animals in Each Treatment Group

(Fig. 3.3/2) do not suggest that any beneficial effect in growth rate resulted from its incorporation.

3.3/3 FOOD REFUSALS

In the review of literature it was pointed out that the daily amount of lactose ingested at a given liveweight, rather than the percentage in the diet, was probably the most important factor limiting food intake. When food refusals exceeded 0.5 lb dry matter per day, the amount of lactose (lb) being consumed daily, was determined and the liveweight was recorded. This data, together with data from the treatment 2 group which refused negligible quantities has been presented in Table 3.3/3.

TABLE 3.3/3 (a) LACTOSE INTAKE (lb) AT THE LIVEWEIGHT AT WHICH DAILY FOOD REFUSALS EXCEEDED 0.5 lb DRY MATTER

Pig No.	Treatment	Liveweight lb	Daily Lactose Intake lb	Lactose Per cent %	Food Refused as a percentage of Food offered %
2	2	120.0	1.12 *	25	1.5
3	2	120.0	1.12 *	25	< 1.0
8	2	120.0	1.12 *	25	-
4	3	66.5	1.10	35	39.0
6	3	69.0	1.12	35	22.0
11	3	66.5	1.10	35	35.0
1	4	55.0	1.28	50	6.0
5	4	60.5	1.45	50	25.0
9	4	55.0	1.25	50	35.0

* Daily refusals > 0.5 lb DM did not occur.

(b) DAILY LACTOSE INTAKE (lb) AT 50 and 120 lb LIVEWEIGHT, AND MEAN TOTAL FOOD REFUSAL FOR TREATMENTS 2, 3 and 4

		Liveweight		Mean Total Food Refused (lb)	Mean Total Food Refused as a Percentage of Food Offered %
		50 lb	120 lb		
TR 2	Lactose Intake	0.63 lb	1.13 lb	1.50	< 1.0
3	Lactose Intake	0.63 lb	2.25 lb	70.50	32.0
4	Lactose Intake	1.25 lb	2.25 lb	47.50	22.0

The data presented in Table 3.3/3 (a) for treatment 4 pigs should not be interpreted as showing that these pigs had a higher tolerance, as indicated by the greater daily intake of lactose before food refusals began. Table 3.3/3 (b) clearly shows that this diet even when fed at 50 lb liveweight, already contained 1.25 lb lactose and so at no stage was it as low as 1.10 - 1.12 lb. Table 3.3/3 (b) also shows the magnitude of the treatment 3 and 4 food refusals to be between a quarter and a third of all food offered. The remarkably low level of intake depression noted for No. 1 in treatment 4 was responsible for the lower food refusal percentage in this treatment. The data should therefore be interpreted as resulting from individual variability rather than from the different rates of treatment introduction.

3.4 DISCUSSION

3.4/1 DUNG CONSISTENCY

The higher incidence of watery faeces voided from pigs on the 50 % lactose diets, compared with those from pigs on zero and 25 % lactose, strongly supports the idea that the high lactose content of the whey is an important factor in whey induced diarrhoea. The reduction in the incidence of scours over successive weeks may have been the result of adaption to high

levels of lactose feeding, or to the reduced amounts of lactose ingested as a result of the large food refusals.

3.4/2 GROWTH RATE AND FOOD INTAKE

The comparable growth rates obtained from the diets containing zero and 25 % lactose, suggests that daily lactose intake (lb) in treatment 2 never reached a level which exceeded the pigs tolerance for this sugar. At 120 lb liveweight, a daily intake of 1.12 lb was reached, a level which appeared to be maximal for treatment 3 pigs at a much lighter weight (65-70 lb). The 50 % lactose diet (1.25 lb at 50 lb liveweight) was clearly above maximal even at the time of introduction. This conclusion is supported by the fact that all pigs on treatment 4 started to refuse feed within 10 days following treatment introduction. It is suggested that the refusal of food by pigs in treatments 3 and 4 was a protective measure aimed at limiting the intake of lactose to an acceptable level. This was illustrated in the first preliminary investigation when pigs refused less of the feed offered if the lactose percentage was lowered.

Only one pig clearly exhibited an adaptation to high levels of lactose. This pig, on treatment 4 (No.1), began refusing feed a few days after the treatment diet was introduced and continued to refuse small amounts of feed for 6 weeks. Growth rate was consequently impaired to a lesser extent than was that of the other two treatment 4 pigs which refused much larger amounts of food, (Fig. 3.3/1). After 6 weeks, refusals ceased entirely, despite intakes at this stage of more than 2.0 lb lactose per day. As a consequence liveweight gain improved and over the last 4-5 weeks of the investigation weekly gains were closely comparable with those of treatment 1 and 2 pigs. At 120 lb liveweight 2.25 lb lactose was being consumed without noticeable adverse effects.

3.5 CONCLUSIONS

Sufficient evidence is available to show that, when pigs are fed high levels of dietary lactose, diarrhoea ensues. When a diet containing 25 % lactose is fed over a 50-120 lb weight range according to the feeding scale in Table 2.2/1, the daily intake of lactose (lb) does not appear to reach a level sufficiently high to cause diarrhoea or food intake depression. Clearly the 50 % lactose diet was above the maximum tolerable level (1.1 lb) at 50-60 lb liveweight and so adverse effects would be expected irrespective of the rate of introduction. Copper supplementation did not reduce food refusals and so was not expected to alter the rates of liveweight gain of the treatment 3 pigs. Adaptation to high lactose levels can occur but the means by which this is achieved is not known.

"The only time I have much to do with Piggy is when the wind blows from the south. Then you'd think Piggy Sowby's pigs were right at my back door."....

....."Piggy traipses to town every day and collects the leftovers from restaurants and hotels to feed to his pigs. And what's more, the local storekeeper reckons Piggy lives on the same things as his pigs."

(Frank S. Anthony and Francis Jackson)

CHAPTER 4

MAIN INVESTIGATION, AND THE COLLECTION STUDY

4.1 INTRODUCTION

Two separate experiments were conducted with different primary objectives in mind. The objective in the main investigation was to obtain a more complete picture of the physiological and anatomical effects resulting from high intake levels of lactose. These included, the effect upon growth rate, dung consistency, carcass composition, and caecum development. In the collection study, the biochemical aspects involved in feeding high-lactose diets were investigated. These results have been presented in Part II. The physiological and anatomical data recorded incidental to the biochemical data are presented in this chapter to enable comparisons to be made between the two experiments. This will give some indication of how closely the biochemical results might relate to the physiological results obtained from the main investigation.

4.2 EXPERIMENTAL

All treatment diets for both experiments were prepared from the same consignments of dietary components. As meatmeal often differs appreciably in quality between sacks, the total consignment was mixed thoroughly before being incorporated into the diets.

The same four treatment diets (3 lactose and 1 control) were imposed on a total of 28 pigs in the main experiment and 12 pigs in the collection study. No lactose was fed to the control group in each case. Treatments 2, 3 and 4, received diets containing 15, 30 and 45 % lactose respectively. Liveweight gains were recorded weekly throughout the investigation and at the conclusion of the study, carcass and caecum data were collected.

4.2/1 SELECTION OF ANIMALS AND EXPERIMENTAL DESIGN

A randomized block design was used which contained 7 replicates per treatment in the main investigation, and 3 replicates per treatment in the collection study. Each block consisted of four littermates selected, for their similarity in liveweight at eight weeks, from a variety of crossbred litters. Preference for uniformity in farrowing dates and 3 and 6 week liveweights being greater than uniformity of breed. For the main investigation two blocks (A & B) were derived from a second cross Landrace x (Berkshire x Large White) litter, two blocks (C & D) from a first cross Landrace x Large White litter, and the remaining three blocks (E, F and G) from two first cross Berkshire x Large White litters. All four litters were born within a week of each other and were weaned at three weeks of age. Following selection, one member of each block was allocated randomly to each of the four treatments. As far as possible, treatments were balanced for sex, treatments 1 and 3 containing 4 gilts and 3 castrates, treatments 2 and 4 containing 3 gilts and 4 castrates.

For the collection study, 4 castrates from each of 3 litters born on consecutive days were selected at 8 weeks for their similarity in liveweight. These were born approximately 4 weeks after the litters used in the main investigation, so that both experiments were in progress together for a considerable part of the time. Two litters were of Landrace x Large White origin while the third was from a Large White x (Berkshire x Large White) mating. Each selected member of each litter was randomly allocated to treatment and individual pen positions.

4.2/2 PRE-EXPERIMENTAL FEEDS AND FEEDING PROCEDURES

As feeding procedures used in the second preliminary investigation proved satisfactory no changes were made for the present experiments. However, both the morning and afternoon feeding times in the main investigation were advanced half an hour for convenience.

4.2/3 MANAGEMENT AND HOUSING

Weekly weighings were made before the morning feed and any meal refusals were removed at 8.00 p.m the previous evening to ensure that all pigs were weighed on a comparable stomach 'fill'. More frequent weights were taken as pigs approached 120 lb liveweight, animals being sent for slaughter as soon as possible after this weight was reached. Before dispatch, pigs were permanently marked for carcass identification purposes with a 'slap' marker.

Preliminary comparisons of food refusal dry-matter values determined from 200 and 500 gram samples showed that the more convenient, smaller sample produced identical results. Consequently 200 gram samples of food refused were taken.

Pigs in the main investigation were housed in individual pens in a totally enclosed well insulated Danish-type fattening house equipped with mechanical ventilation. The pigs in the collection study, while not in the metabolism crates, were individually penned in the same room at the A.P.U. used for the two preliminary investigations. This room was adjacent to the metabolism room containing the crates. An ambient temperature of 68°F was thermostatically controlled in all cases, and continuous thermograph recordings were made over the experimental periods.

The temperature in the Test House used for the main investigation averaged 68°F and seldom rose more than 1°F above this level. The normal temperature range was 64 to 68°F and the number of hours at temperatures below 64°F, represented 3.2% of the total number of hours the house was occupied.

The temperature in both the metabolism room and the holding pens averaged 68°F and seldom rose above 70°F or fell below 65°F. The number of hours in which the temperature exceeded these limits was less than 1% for the metabolism room, and 3.4 % for the holding pens, of the total number of hours the rooms were in use.

4.2/4 TREATMENT DIETS AND THEIR INTRODUCTION

Information on the mineral composition of the individual components of the diets, was not available for the two preliminary investigations but came to hand before the diets were prepared for the main experiment and the collection study. If the 'Tasminal' trace element supplement in the 'premix' used in the two earlier experiments was omitted, calculated mineral composition indicated two probable mineral deficiencies. The copper level (5.5 ppm oven-dry meal basis) was approximately one half that normally required for pigs growing over the weight range planned. Although the zinc level (46.00 ppm oven-dry meal basis) may have been sufficient, supplementation of zinc was thought necessary because of the possible effects of the high calcium level (1.26 %) in the mixture.

A second 'premix' was therefore used which was identical to the first but did not contain the trace element supplement. Instead, the necessary copper and zinc additions (ppm on an air dry meal basis) were made when diets were mixed, 10 ppm. Copper (1.82 gms $CuSO_4$ .5 H_2O) and 200 ppm. Zinc (40.0 gms. $ZnSO_4$ .7 H_2O) being added per 100 lb of treatment diet.

Four treatment diets were formulated. Treatment 1 (control) diet was the same as that used on the second preliminary investigation (Diet A) and contained 50 % wheat starch. Substitution of the starch by lactose was made to obtain the remaining three treatment mixtures. The resultant percentage compositions of lactose and wheat starch were:

Diet	Lactose %	Wheat Starch %
Treatment 1	0	50
Treatment 2	15	35
Treatment 3	30	20
Treatment 4	45	5

Proximate analyses were performed on the components of the diets, and on representative samples of the diets used in the collection study. From the results obtained for the components, a proximate composition was calculated, and this compared closely with the actual dietary determinations. The results of all analyses, including the mineral determinations are presented in appendices 4.2/1, 4.2/2.

The experimental diets were all introduced at 50 lb liveweight and over a 5-day period, substituted for the 'carry-on' ration as outlined before. No subsequent changes were made, and daily feeding was again according to Table 2.2/1.

4.2/5 POST SLAUGHTER PROCEDURES

(a) Caecum Examination

Immediately following slaughter, alimentary tracts were removed from the carcasses and labelled. Caecum size relative to the size of the remainder of the tract was subjectively assessed. Each was then stripped of its mesentery and mesenteric fat, tied off with string just distal to the ileo-caecal junction, and removed from the remainder of the tract. After photographing each caecum, that portion containing the ileo-caecal valve and a small piece of colon, was cut off immediately behind the valve. The caecal contents were then emptied out, and each caecum was washed out several times with water to remove all traces of feed residues. Caecae were measured along their dorsal (mesenteric) taeniae, andthen hung upside down to drain. As water displacement provides a convenient measure of tissue volume, the caecae were each totally immersed in 500 mls of distilled water in a 1 litre volumetric cylinder and the consequent rise in water level was recorded. Total wet weights were obtained after splitting each caecum lengthwise and patting it dry with a cloth. Dry weights were obtained following a 48-hour drying period at 100^{o}C.

(b) Carcass Evaluation

The head was removed from each carcass and the carcasses were split longitudinally down the vertebral column. The fillets were removed, but the trotters remained on the carcass. The sides were then placed in a chiller into which a tank $5\frac{1}{2}$ feet high, and 3 feet square, filled with water had been placed.

After approximately 24 hours, flares and kidneys were removed and each side was weighed in air on a 60 x $\frac{1}{10}$ lb spring balance, and again, completely submerged in the tank of water on a 1610 x $\frac{1}{10}$ gram triple beam balance to the nearest 1 gm. The length of each side was then measured from the anterior end of the pubic symphysis to the vascular depression on the 1st thoracic vertebra, and the measurements for each side were averaged. Maximum depth was measured through the chest of each side, the highest value recorded being accepted as the carcass value.

Several linear backfat measurements (excluding the skin) were made using calipers and a millimeter steel rule. Carcass values were obtained by averaging the values recorded for the two sides.

The measurements were:

(i) Depth of fat over the 3rd thoracic vertebra.

(ii) Minimum depth over the mid-back region.

(iii) Maximum depth immediately anterior to the gluteus medius muscle (Max. Loin).

(iv) Minimum depth over the gluteus medius muscle (Min. Loin).

The left side of each carcass was then cut immediately posterior to the head of the 15th thoracic vertebra and millimeter measurements were made of:

(i) The depth of fat directly over the point of maximum eye muscle depth , (C).

(ii) The depth of fat at the distal end of the eye muscle - (K)

(iii) Maximum depth of the innermost layer of fat over the eye muscle - (J).

(iv) Depth of fat at a point along the rib 11 cms. from the cut surface of the thoracic vertebra.

From the anterior half of each left side, a tracing was made of the eye muscle, and eye muscle areas were determined later using a planimeter. The cut surface was also appraised visually and scored for muscle and fat balance, and for the size and shape of the eye muscle, according to a published set of standard photographs.

4.3 RESULTS

4.3/1 HEALTH

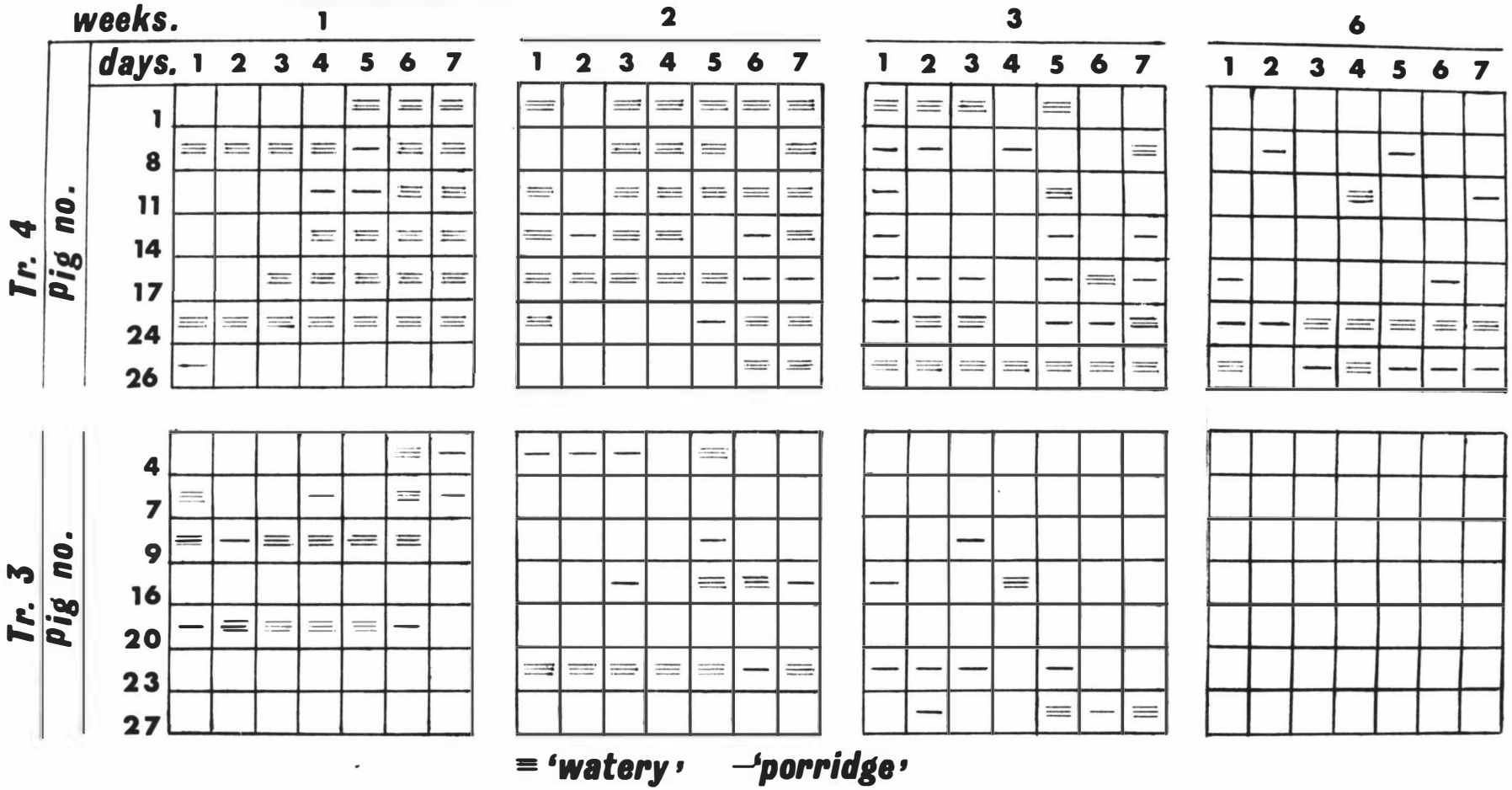
(a) Main Investigation

One treatment 3 pig died after the investigation had proceeded 30 days. Death occurred suddenly overnight, the afternoon ration being entirely consumed. Post mortem examination revealed that an excessive fermentative production of gas in the stomach had produced sufficient pressure on the diaphragm to cause death by asphyxiation. The general health of the remaining pigs was good, and no leg weaknesses were apparent.

(b) Collection Study

One pig (No.4) on treatment 4, stopped growing after 7 days on the 45 % lactose diet, and gained only 3 lb in weight over the next 6 weeks. At this stage it was decided that the pig was of no further use in the investigation and so the animal was changed to the treatment 1 diet. In the next 3 weeks the pig gained a total of 25 lb in weight, and appeared to have recovered from the earlier treatment effects. However in the last week of the investigation, the pig began to regurgitate feed immediately after consuming it. The pig was slaughtered, and autopsy revealed considerable scar tissue in the region of the pars oesophagus. This had resulted in appreciable narrowing (stenosis) at the cardiac sphincter, thereby markedly limiting the normal passage of food into the stomach.

FIGURE 4.3/1 Results of Daily Observations of Dung Consistency for the First, Second, Third, and Sixth Weeks of Treatments 3 and 4 in the Main Investigation



One treatment 3 pig (No.9) at 80 lb liveweight developed what appeared to be acute colonic bloat, and was treated with 500 cc. of paraffin oil. Two days later, it developed a pronounced weakness in both hind legs and refused to stand. A sawdust pad was provided and recovery was complete by the time the pig was due to go into a crate for the final collection at 110 lb liveweight. All other pigs remained in good health throughout the study.

4.3/2 DUNG CONSISTENCY

Results of daily observations of dung consistency for the 1st, 2nd, 3rd and 6th weeks of the main investigation are presented in Fig. 4.3/1 for treatments 3 and 4 only, as there were very few observations of 'watery' faeces on either treatment 1 or 2. There was clearly a greater incidence of 'porridge' or 'watery' faeces on the 45 % lactose diet, all pigs on this treatment diet being similarly affected. By the sixth week a considerable reduction in 'scour' observations had occurred, and these were associated more closely with individuals than with the group as a whole. Scouring was less severe in the 30 % lactose group and although all pigs were affected, scouring seldom continued for more than 1 week, and no scouring was evident in the 5th or the 6th week.

In the collection study, one pig in the treatment 1 group voided an abnormally large number of 'watery' faeces. As a result, a high incidence of 'porridge' or 'watery' faeces was recorded for this group, (Table 4.3/1). With increasing lactose levels there was an increase in the incidence of diarrhoea for the first 4 weeks, but very few observations of diarrhoeal faeces were made in the 5th to 8th week of the investigation.

TABLE 4.3/1 NUMBER OF FAECAL VOIDINGS CLASSIFIED AS 'PORRIDGE' (P) OR 'WATERY' (W) IN EACH TREATMENT GROUP OVER 2 FOUR WEEK PERIODS* FOR THE COLLECTION STUDY

	TREATMENTS							
	1.		2.		3.		4.	
	P	W	P	W	P	W	P	W
WEEK 1.	3	3	2	-	3	5	1	3
2.	3	5	1	3	1	3	-	8
3.	1	6	-	-	1	-	1	2
4.	1	1	-	-	-	2	3	-
TOTAL	8	15	3	3	5	10	5	13
WEEK 5.	-	1	-	-	-	-	2	-
6.	-	1	-	-	-	-	-	-
7.	-	-	-	-	-	-	2	-
8.	1	-	-	-	-	-	1	-
TOTAL	1	2	-	-	-	-	5	-

* Total number of observations per treatment, for each four week period was 84.

TABLE 4.3/2 Liveweight Gain, Food Consumption and Food Conversion Efficiency For Animals in the Main Investigation

	TR.1	TR.2	TR.3	TR.4	Significance of Difference
LACTOSE LEVEL IN THE DIET (%)	0	15	30	45	
LIVEWEIGHT GAIN					
0 - 28 Days					
No. of Pigs	7	7	7	7	
Liveweight Gain (lb) Mean	29.14	27.00	26.71	22.36	(1>3,4 ** (2,3>4 *
± SE	±0.37	±1.32	±0.60	±1.34	
Av. Daily Gain (lb)	1.04	0.96	0.95	0.80	
0 - 56 Days					
No. of Pigs	7	7	5[a]	7	
Liveweight Gain (lb) Mean	67.86	63.00	61.10	41.43	(1>3,4 ** (2,3>4 **
± SE	±0.73	±3.26	±0.41	±5.11	
Av. Daily Gain (lb)	1.21	1.13	1.09	0.74	
Days from 50-120 lb Mean	57	60	63	-	
FOOD CONSUMPTION					
50 - 80 lb Liveweight					
Total Food Consumed (lb) Mean	85.80	90.70	93.80	121.20	(1<4 ** (1<3 * (2,3<4 *
± SE	±1.14	±4.16	±2.50	±9.49	
lb Food Consumed/lb L/wt gain	2.86	3.02	3.13	4.04	
50 - 120 lb Liveweight					
Total Food Consumed (lb) Mean	194.74	205.44	215.66	-	1<3 **
± SE	±1.99	±6.71	±6.04	-	
lb Food Consumed/lb L/wt gain	2.78	2.94	3.08		

(a) 1 Pig died and the results from another pig were removed from the analyses.

* $P < 0.05$

** $P < 0.01$

4.3/3 GROWTH RATE, FOOD INTAKE AND FOOD CONVERSION EFFICIENCY

(a) Main Investigation

A summary of the data for live weight gain and food consumption has been presented in Table 4.3/2.

Growth rate data was analysed on the basis of liveweight gain in 28 and 56 days following treatment introduction. In this way, it was possible to include treatment 4 pigs in the analysis even though only 2 pigs reached 120 lb liveweight.

Only 5 pigs were included in the 56 day liveweight gain data for treatment 3. One pig died after 30 days and another made very slow and eratic growth from 35 days. A test for outlying data (Dixon and Massey, 1957) rejected, at the 1 % level of probability, the liveweight data of this animal at 56 days. ($r_{10} = 0.790$, for six observations $> r_{10} = 0.698$ ($P < 0.01$).

The marked differences in between-treatment variance indicated that the use of an 'F' test for significance might not be satisfactory, and when used for the analysis of the 28 day data for treatments 1, 2 and 3, no significant treatment differences were detected. If treatment 4 was included in the analysis the error variance increased $7\frac{1}{2}$ times (8.25 - 62.30) resulting in an even poorer test of significance between treatments 1, 2 and 3.

By using Dixon's test (Dixon and Massey, 1957) the differences in treatment variance could be taken into account, enabling all treatments to be compared. As treatment means were compared, missing plot values were not required for the two pigs withdrawn from treatment 3.

Variance analysis (App. 4.3/1) revealed that significantly ($P < 0.01$) greater gains in liveweight were made by treatment 1 pigs compared with those on treatment 3 and 4 over both the 28 and 56 day periods. In addition, liveweight gains on treatments 2 and 3 at 28 ($P < 0.05$) and 56 ($P < 0.01$) days were significantly better than those made on treatment 4.

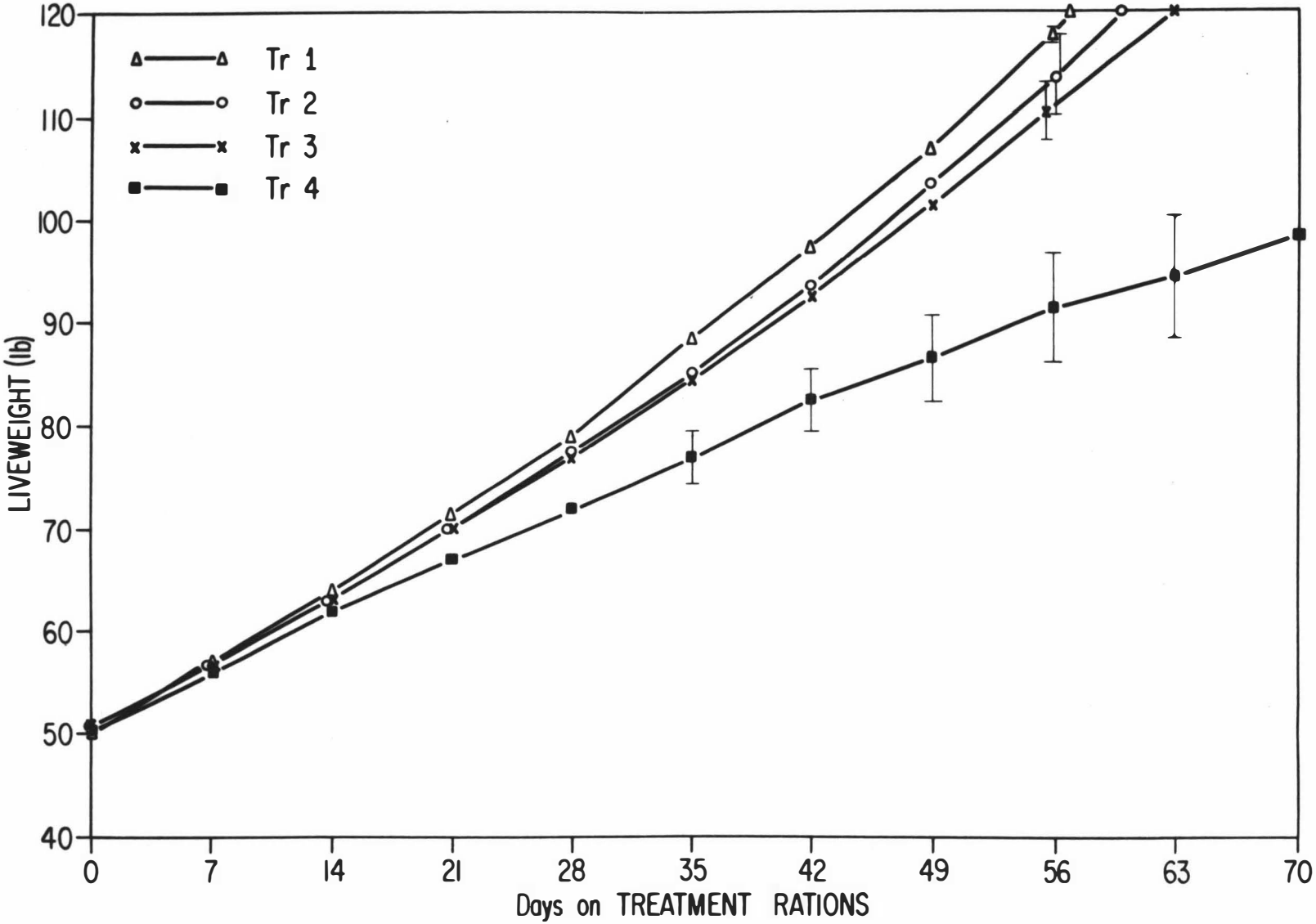


FIGURE 4.3/2 Mean Treatment Growth Rates and Their Standard Errors For Animals in the Main Investigation

Mean growth rates and the standard errors of treatment 4 means are presented in Fig. 4.3/2. The large standard errors on the treatment 4 curve illustrate the considerable variability encountered between animals in this group.

Treatment differences were clearly apparent after 21 days following treatment introduction. By this time, treatment 1 pigs had started to show their superiority while the treatment 4 group was already clearly inferior to the other three groups.

For the same reasons outlined concerning liveweight gains the 'F' test of significance for feed consumption also proved unsuitable, and so Dixon's test was again employed (App. 4.3/2).

It was obvious that the significant treatment differences for food consumption were essentially the same as those for liveweight gain, the only difference being that treatment 3 at 80 lb liveweight differed from treatment 1 at only the 5 % level of probability. Feed conversion efficiency (F.C.E.) worsened as the level of lactose in the diet increased. From 50 - 80 lb liveweight, treatment 4 pigs (45 % lactose) consumed significantly more food than treatment 1, ($P < 0.01$) or treatments 2 and 3 ($P < 0.05$). Treatment 3 pigs (30 % lactose) also consumed significantly ($P < 0.05$) more food than those on the treatment 1 (zero lactose) diet. Over the liveweight range from 50 - 120 lb only three treatment groups were represented. Again the highest level of lactose (treatment 3 - 30 %) produced a significantly lower ($P < 0.01$) F.C.E. than the control diet.

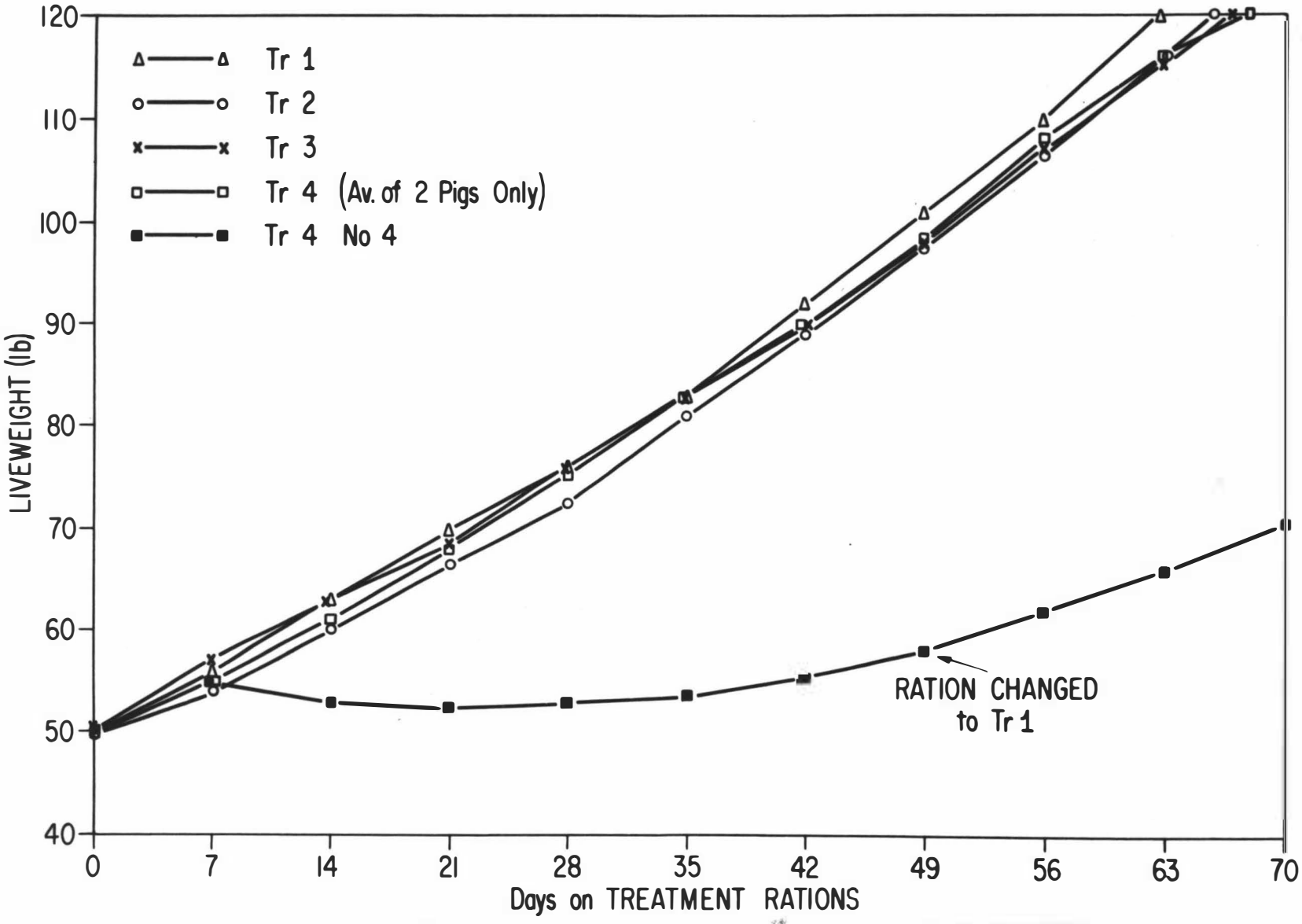


FIGURE 4.3/3

Mean Liveweight Gains for the Four Treatment Groups in the Collection Study

TABLE 4.3/3 Liveweight Gain, Food Consumption, and Food Conversion Efficiency for Animals in the Collection Study

	TR.1	TR.2	TR.3	TR.4	Significance of Difference
No. of Pigs	3	3	3	3/	
LIVEWEIGHT GAIN					
0 - 28 Days					
Liveweight gain Mean (lb)	26.0	23.5	25.8	24.3	N.S.
± SE	1.06	1.06	1.06	1.31	
Average Daily Gain (lb)	0.93	0.84	0.92	0.90	
0 - 56 Days					
Liveweight gain Mean (lb)	61.5	57.5	56.7	58.3	N.S.
± SE	1.87	1.87	1.87	2.30	
Average Daily Gain (lb)	1.10	1.03	1.01	1.04	
Days from 50 - 120 lb Mean	63	66	67	68	
FOOD CONSUMPTION					
50 - 80 lb LIVEWEIGHT					
Total Food Consumed Mean(lb)	90.87	97.82	92.30	96.39	N.S.
± SE	4.6	4.6	4.6	5.7	
lb Food Consumed/lb L/wt gain	3.03	3.26	3.08	3.21	
50 - 120 lb LIVEWEIGHT					
Total Food Consumed Mean(lb)	212.7	218.8	228.8	227.4	N.S.
± SE	4.6	4.6	4.6	5.7	
lb Food Consumed/lb L/wt gain	3.04	3.12	3.27	3.25	

/ All data contains one missing plot value

N.S. ($P > 0.05$)

(b) Collection Study

A summary of the data has been presented in Table 4.3/3, and mean liveweight gains have been presented graphically in Fig. 4.3/3.

With the exception of the one pig on treatment 4 already mentioned, all pigs reached 120 lb liveweight. In contrast to the results gained in the main investigation, within treatment differences in growth rate were very small and permitted statistical analysis using the 'F' test.

Results were again analysed for liveweight differences after 28 (App. 4.3/3) and 56 (App. 4.3/4) days, data for the one treatment 4 pig being replaced by missing plot values. No significant treatment differences were detected at either stage of the study.

Analysis of total food consumption, from 50 - 80 lb liveweight (App. 4.3/5) and from 50 - 120 lb liveweight (App. 4.3/6) also revealed non significant treatment differences.

TABLE 4.3/4 Lactose Intake per day (lb) at the Liveweight at which Daily Food Refusals Exceeded 0.5 lb DM - Main Investigation

TREATMENT 3				TREATMENT 4			
Pig Number	Pig Liveweight	Daily Lactose Intake	Total Food Refused	Pig Number	Pig Liveweight	Daily Lactose Intake	Total Food Refused
	(lb)	(lb)	%		(lb)	(lb)	%
4	120.0	1.32*	-	1	65.0	1.35	11.0
7	120.0	1.35*	-	8	120.0	1.98*	
9	74.0	1.02	10.0	11	65.0	1.35	23.0
16	105.0	1.26	<2.0	14	63.0	1.35	23.0
20		D I E D		17	99.0	1.80	5.0
23	120.0	1.35*	-	24	89.5	1.71	10.0
27	120.0	1.35*	-	26	66.5	1.40	14.0

* Investigation was terminated before a refusal greater than 0.5 lb occurred.

Daily Lactose Intake (lb) at 50 and 120 lb Liveweight.

	LIVEWEIGHT	
	50 lb	120 lb
TR. 2 ...	0.38 lb	0.68 lb
TR. 3 ...	0.75 lb	1.35 lb
TR. 4 ...	1.13 lb	1.98 lb

4.3/4 FOOD REFUSALS

(a) Main Investigation

A summary of the data relating to food refusals has been presented in Table 4.3/4. This shows that only two pigs on the treatment 3 diet refused food, and in one case this was a very small amount. In comparison, the other pig refused more food, began refusing at a lighter liveweight, and was consuming less lactose per day when the refusal began. The 4 remaining animals in this treatment reached 120 lbs without showing any adverse effects, and at this liveweight, approximately 1.35 lb lactose was being ingested daily.

In contrast only 1 pig did not refuse food in treatment 4. This pig consumed 1.98 lb lactose daily at 120 lb liveweight. Fourother pigs began to leave food at 65 lb liveweight when 1.35 - 1.40 lb lactose was offered in the daily ration. Of the remaining two pigs, one began to refuse food at 90 lb liveweight when the daily ration contained 1.71 lb lactose, and intake depression commenced in the other pig at 100 lb liveweight at which time the daily lactose intake was 1.80 lb.

(b) Collection Study

Only one pig (No.4 Treatment 4) refused food continuously. Two pigs showed a noticeable ability to 'adapt' to the lactose diets by regaining their appetites after a period of refusing food, despite continued feeding of the same treatment diet. This is best illustrated by Table 4.3/5. Total food refused by all three pigs, expressed as a percentage of the total food offered, has also been presented.

TABLE 4.3/5 Liveweight and Daily Intake of Lactose (lb) at which Food Refusals Commenced and Ceased - Collection Study

	FOOD REFUSALS				
	COMMENCED		CEASED		
PIG NO.	LIVEWEIGHT	DAILY LACTOSE INTAKE	LIVEWEIGHT	DAILY LACTOSE INTAKE	PERCENT OF TOTAL FOOD OFFERED
	(lb)	(lb)	(lb)	(lb)	(%)
4	53.0	1.15	58.0[a]	0	24.2
6	55.0	0.40	67.0	0.47 (1.13)	3.3
10	55.5	1.17	69.0	1.44	1.3)) 2.5
10	110.0	1.87	120.0[b]	-	1.2)

a Refusals ceased 2 days after the treatment 1 diet (zero lactose) was introduced.

b Investigation was terminated before refusals ceased.

( ) Bracketed value represents the level finally achieved without further food refusals.

4.3/5 CARCASS EVALUATION

A summary of the carcass data analysed for both experiments has been presented in Table 4.3/6.

(a) Main Investigation

Although there was no significant difference in liveweight at slaughter (App. 4.3/7), analysis of variance revealed a highly significant ($P < 0.01$) difference in 'cold' carcass weight (hot carcass weight less 6 %) between treatments 1 and 2, and treatment 3. No significant differences were apparent when carcass specific gravities were analysed (App. 4.3/8), although a trend was evident toward fatter carcasses with increasing lactose levels. Eye muscle areas were significantly ($P < 0.01$) smaller in the treatment 3 carcasses compared with those of treatments 1 and 2, but this

TABLE 4.3/6 Liveweight at Slaughter, 'cold' Carcass Weight 'Killing-Out' Percentage, Specific Gravity (SG) and its Reciprocal (RG), and Eye Muscle Area.

	TR.1	TR.2	TR.3	TR.4	Significance of Difference
MAIN INVESTIGATION					
No. of Pigs	7	7	7/		
Liveweight at Slaughter					
Mean (lb)	126.0	125.2	122.7		N.S.
± SE	1.2	1.2	1.3		
'Cold' Carcass Weight (lb)	91.0	90.4	84.4		1,2>3 **
± SE	0.9	0.9	1.0		
'Killing-out' Percentage	72.2	72.2	68.8		
Specific Gravity (SG) Mean	1.0531	1.0527	1.0523		N.S.
± SE	0.0011	0.0017	0.0014		
Reciprocal of SG (RG) Mean	0.9494	0.9499	0.9504		
± SE	0.0003	0.0016	0.0012		
Eye Muscle area Mean(cm^2)	21.89	22.61	18.64		1,2>3 **
± SE	1.25	2.32	1.43		
Adjusted Eye Muscle area (cm^2)	21.06	21.98	20.10		N.S.
COLLECTION STUDY					
No. of Pigs	3	3	3	3/	
Liveweight at Slaughter					
Mean (lb)	129.5	126.0	127.0	126.0	N.S.
± SE	2.1	2.1	2.1	2.6	
'Killing-out' Percentage					
Mean	73.10	71.23	70.63	69.07	1>3,4 ** 2>4 ** 1>2 * 3>4 *
± SE	0.35	0.35	0.35	0.43	
Specific Gravity (SG) Mean	1.0570	1.0541	1.0545	1.0494	1>(2,3)>4 *
± SE	0.0014	0.0025	0.0023	0.0005	
Reciprocal of SG (RG) Mean	0.9461	0.9487	0.9483	0.9529	
± SE	0.0013	0.0022	0.0020	0.0005	
Eye Muscle area Mean (cm^2)	22.90	21.17	20.00	20.15	N.S.
± SE	1.33	1.10	0.55	0.20	

** Means differ significantly at the 1 % ($P < 0.01$) level of Probability
* Means differ significantly at the 5 % ($P < 0.05$) level of Probability
N.S. Means do not differ significantly ($P > 0.05$)
/ Data contains 1 missing plot value

difference was accounted for almost entirely by differences in 'cold' carcass weight (App. 4.3/9). Examination of all linear fat measurements made on the carcass indicated that no other differences between treatments would reach significance due primarily to large individual variation.

(b) Collection Study

Liveweight at slaughter was analysed (App. 4.3/7) but differences were found to be non significant between treatments. In view of the high error variance detected in this analysis, and the correspondingly large individual variation apparent in 'cold' carcass weight data, it was felt that a more reliable test of significance could be obtained by the analysis of 'killing-out' percentages. (App. 4.3/7) Highly significant ($P < 0.01$) differences were found to exist between treatment 1, and treatments 3 and 4, and between treatment 2, and treatment 4. Significant ($P < 0.05$) differences were also detected between treatments, 1 and 2, and between treatments 3 and 4.

Statistical analysis of specific gravity measurements (App. 4.3/8) showed that significantly ($P < 0.05$) fatter carcasses resulted with increasing levels of lactose in the diet, but no differences (App. 4.3/9) were detected between treatments for eye muscle areas (cm^2). Examination of all linear fat measurements showed that in most cases treatment differences were not sufficiently large to warrant statistical analysis. This was due to the small number of animals per treatment and to the individual variability of the animals as illustrated in Table 4.3/7.

TABLE 4.3/7 Linear Fat Measurements (mm) for the Carcasses from the Collection Study

Measurement Taken At	TR.1		TR.2		TR.3		TR.4 ≠	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
3rd Vertebra	28	26-31	25	24-27	25	23-29	25	23-29
Mid Back	14	13-15	14	12-15	14	13-15	14	13-16
Max Loin	24	23-24	23	21-24	23	22-23	21	20-24
Min Loin[a]	15	14-17	12	11-13	13	12-13	13	13-14
'C'	14	13-15	14	13-14	14	14-15	14	14-15
'K'	20	19-22	19	16-23	20	17-23	20	19-20

a - Analysis of Variance (App. 4.3/7) showed differences to be NS ($P > 0.05$)

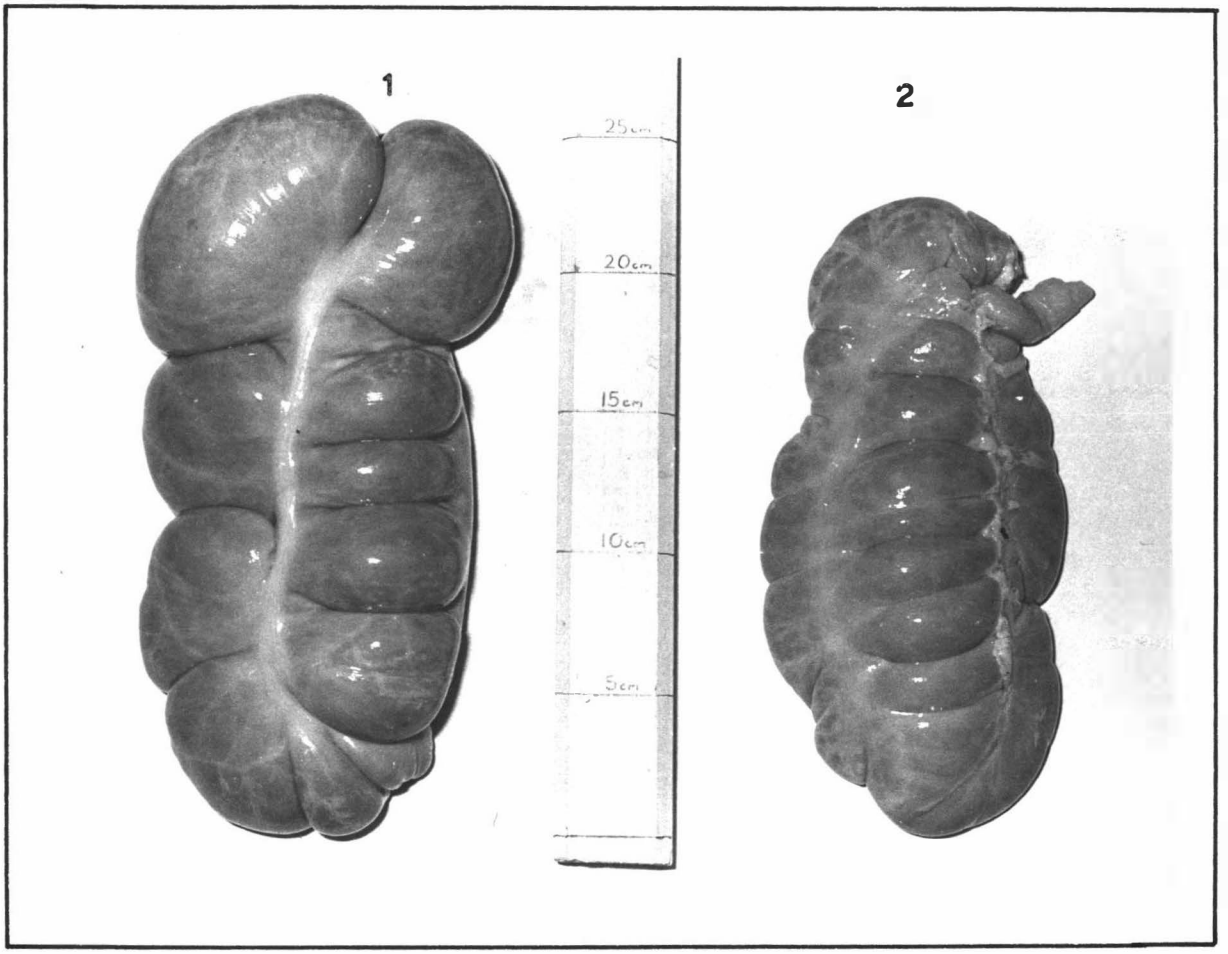
≠ - Data contains one missing plot value

4.3/6 CAECUM EXAMINATION

Examination of the caecum data from the main, and collection investigations showed that the experimental error variances for both investigations were similar, and so all data was analysed together. A summary of the data has been presented in Table 4.3/8. In both wet weight and tissue volume, the mean values for treatments 2 and 3 were significantly ($P < 0.01$) larger than those of treatment 1. In dry weights, treatment 3 was again superior ($P < 0.01$) to treatment 1, but treatment 2 just failed to differ significantly from treatment 1 at the 5 % level. A significant ($P < 0.05$) difference was also recorded between treatments 3 and 2 in tissue volume.

The differences between representative treatment 1 and 3 caecae can be clearly seen in Fig. 4.3/4. Data available from the four treatment 4 pigs which reached 120 lb liveweight suggested that even greater effects could have been expected from the diet containing 45 % lactose. Mean wet

FIGURE 4.3/4 Representative Caecae From Treatment Three (1) and Treatment One (2)



and dry caecum weights were 128.3 and 28.8 gms respectively and mean tissue volume was 141.3 mls.

Highly significant ($P < 0.01$) block differences in caecum dry weight, and significant ($P < 0.05$) block differences in caecum wet weight were also recorded, and have been presented in Appendix 4.3/10.

TABLE 4.3/8 Mean Caecum Measurements for 3 Treatment Groups : Wet Weight, Dry Weight, Tissue Volume, and Length - Combined Data From Both Investigations

	TR.1	TR.2	TR.3	Significance of Difference
No. of Pigs	10	10	10/	
Caecum Wet Weight (gms)	81.85 ± 2.53	110.90 ± 2.53	117.90 ± 2.67	2,3 > 1 **
Caecum Dry Weight (gms)	22.95 ± 0.84	25.45 ± 0.84	26.75 ± 0.89	3 > 1 ** 2 > 1 (*)[a]
Caecum Tissue Volume (mls)	91.00 ± 3.43	122.50 ± 3.43	134.5 ± 3.61	3 > 2 * 2,3 > 1 **
Caecum Length (cms)	25.55 ± 1.09	26.25 ± 1.09	27.90 ±1.15	NS

** Means differed significantly at the 1 % ($P < 0.01$) level of probability

* " " " " 5 % ($P < 0.05$) " " "

(*)[a] Means just failed to differ significantly at 5 % ($P > 0.05$)

NS ($P > 0.05$)

/ Data contains one missing plot value

4.4 DISCUSSION

4.4/1 THE EFFECT OF HIGH DIETARY LEVELS OF LACTOSE ON DUNG CONSISTENCY

In the second preliminary investigation, and the two present experiments, there was a progressive increase in the incidence of diarrhoea with increasing levels of lactose, especially over the first 4 weeks. Subsequently, only the pigs on the highest levels (45-50 %) of lactose continued to show a considerable degree of scouring, and even in their case, there was a marked

reduction in incidence. The results suggested that a considerable degree of adaptation (as defined by Lawrence et al, 1956) to the high lactose feeding had occurred. However this cessation of diarrhoea was seldom accompanied by any noticeable improvement in the rate of liveweight gain, clearly showing that a large part of the growth depression noted on the high lactose diets was not being caused by the diarrhoea as such.

Only limited information is available on the extent to which diarrhoea contributes to the poorer growth rates seen when pigs are fed diets containing high levels of lactose. This is from experiments in which whey in various forms has been fed, and so must be interpreted with care in view of the possible effects of the other whey components. Krider et al (1949) fed pigs dried whey, and concluded that, although a pronounced diarrhoea resulted, this had little or no effect on growth rate or food conversion efficiency. Similar conclusions can be drawn from a report by Dunkin, (1959) in which large treatment differences in the incidence of diarrhoea resulting from the use of fresh whey, were not reflected in similar differences in liveweight gain.

The present results on the incidence of diarrhoea in growing pigs, 50-120 lb liveweight, supplement those published by Becker and Terrill (1954) who fed diets containing up to 50 % lactose to growing pigs over the liveweight ranges from 45-80, and from 125 to 160 lb liveweight. At all liveweights the 45-50% lactose diet appears to produce a comparatively high incidence of diarrhoea, compared with that recorded at lower (25-30 %) levels of lactose.

Conflicting reports have been published on the affects of various forms of whey on dung consistency, and tend to emphasise the point made earlier that the other whey components may enhance or reduce the laxative effects of lactose.

Two separate experiments, using fresh whey diets containing up to 52 % lactose, were conducted by Dunkin (1961). In the first experiment where pigs were group housed but individually fed (Dunkin pers comm.) a high incidence of diarrhoea was reported, but in the second experiment, using individually penned pigs, the scouring was described as negligible. However it is unlikely that such noticeably different results were due entirely to housing conditions. Becker et al (1957) fed a dried whey diet containing 40 % lactose to growing pigs and reported a marked diarrhoea, but very little scouring was reported by Dunkin (1963 a) when he fed growing pigs dried whey diets containing up to 60 % lactose.

From this discussion it is clear that lactose per se when fed at high levels to growing pigs will result initially in considerable diarrhoea. Whether this has any appreciable effect on the growth rate of the pig has not been satisfactorily determined and will be dealt with more fully after the data on the biochemical effects of lactose ingestion have been presented.

4.4/2 GROWTH RATE, FOOD INTAKE, AND FOOD CONVERSION EFFICIENCY

The results from the main investigation show that better liveweight gains from 50 - 120 lb could be achieved when diets contained 50 % wheat starch, than when the starch was replaced by lactose at three different levels. Substitution resulting in a 15 % lactose diet, was sufficient to cause a 3 day delay in the time taken to reach 120 lb, while the 30 % lactose diet caused a further 3 day delay. As all pigs represented in these results either consumed all the food offered, or refused only negligible quantities of food, it could be concluded, that the replacement of wheat starch, pound for pound by lactose, adversely affects the growing pig's rate of growth. Negligible food refusals were also experienced in the collection study but here no significant differences in liveweight gain between all treatment groups, were apparent. Furthermore, almost identical rates of growth were achieved by control (50 % wheat starch) and treatment 2 (25 % lactose) groups in the second preliminary investigation.

These results suggest an opposite conclusion, namely that the control diet, and the diets containing up to 25 - 30 % lactose can be used with comparable efficiency.

Assuming for the moment, that the results obtained in the main investigation (due to the greater numbers of animals used) are a more reliable indication of the effects of increasing the lactose percentage in the diet at the expense of wheat starch, an explanation is required for the conflicting results obtained in the second preliminary and collection investigations. As food consignments, and the 'premix' used in the second preliminary investigation were different from those used in the main and the collection investigations, it can be argued that the results obtained may have been a reflection of these differences. In addition (although even less likely) the ratio of 25 % wheat starch, 25 % lactose (25:25) may have been more suitable nutritionally than the two ratios (15:35, 30:20) used, in the main investigation.

However these two points do not explain the different results obtained between the collection study and the main investigation. Proximate analysis showed the diets to be very similar, and the wheat starch : lactose ratios in the diets were identical. Feeding procedures were strictly comparable, and mean ambient temperature differences were small.

The only major difference between the main investigation and the two smaller experiments was in the housing. The two smaller experiments were conducted in the A.P.U. in which less than 20 pigs had been housed before the current investigations began. The main investigation was conducted in a test house at the piggery and had been used almost continuously for about 10 years.

A possible explanation for the conflicting results obtained is that there was ahigher incidence of subclinical disease in the test house, and that progressively greater stress was imposed on the pigs by increasing the dietary levels of lactose. As a result, the subclinical infection had a

more pronounced effect on the liveweight gains of the treatment 3 animals on 30 % lactose compared with animals on the 15 % lactose or the control diets.

The major factor contributing to the poorer growth rates of pigs on the 45 % (main investigation) and 50 % (second preliminary investigation) lactose diets, was the very pronounced depression in food intake. When pigs on these lactose levels refused very little or no food, growth rates were comparable with, or only slightly poorer than the liveweight gains made by animals on the other treatment diets. That these pigs were clearly in a minority is illustrated by the fact that, of the 16 pigs fed either 45 or 50 % lactose, only 2 consumed all the food that was offered, one refused very small amounts, (2.5%) and a fourth refused approximately 6 % of the total food given. The close association between the amount of food refused and the liveweight gain is shown in Table 4.4/1.

TABLE 4.4/1 Summary of data from the Main Investigation and the Second Preliminary Investigation illustrating the relationship between the amount of food refused (as a percentage of the total food offered) and the liveweight of the animals after 60 days on the lactose diets.

MAIN INVESTIGATION (45 % LACTOSE)

Pig Number	8	17	24	1	26	14	11
Food Refused (%)	0	5	10	11	14	23	23
Liveweight After 60 Days (lb)	119.0	108.0	93.5	91.0	86.5	78.5	78.0

SECOND PRELIMINARY INVESTIGATION

	25 - 50 % LACTOSE			50 % LACTOSE		
Pig Number	6	11	4	1	5	9
Food Refused (%)	22	35	39	6	25	35
Liveweight After 60 Days (lb)	87.0	80.0	62.0	105.0	77.0	71.5

A most noticeable increase in within treatment variability in the rate of liveweight gain accompanied the 45 - 50 % lactose diets in both the main and the second preliminary investigations. Table 4.4/1 shows that this variability was a reflection of the marked differences in the amount of food refused by individual animals.

Because food refusals were so closely associated with the reduced rates of liveweight gain, a more detailed examination was carried out into the possible factors involved in this food intake depression. Calculations were made of the daily amount of lactose ingested and the liveweight of the animals, at the time food refusals were first noted. The resultant data suggests that pigs begin to refuse food as soon as the amount (lb) of lactose offered daily in the diet reaches a level above which the efficient utilization of the sugar is no longer possible. This level has been termed the 'Maximum Tolerable Intake' (M.T.I.), and is dependent on both the dietary lactose percentage and the scale of feeding used. Furthermore, under systems of feeding in which the daily ration increases with increasing liveweight it may be necessary to express 'M.T.I' at a particular liveweight. This depends on whether the conclusion reached by Walker, (1959) 'that the baby pig is born with a fixed amount of lactase which does not change with age', can be extended to include animals older than the 5 week maximum age used in forming this conclusion. Data presented by Walker, (1959) for lactase activity in pigs 5 and 8 weeks of age, and conclusions drawn by DeGroot and Hoogendoorn, (1957) for lactase activity in pigs over 2 months old, suggest that such an extension is possible, but this requires further verification. Clearly, if the total amount of lactase does not change over a considerable weight range, the 'M.T.I.' would also be a constant value for the animal at any liveweight within this range.

The 'M.T.I.' hypothesis outlined above assumes that it is an amount rather than a percentage of dietary lactose which is important in initiating food refusals. The validity of this assumption could be easily tested by feeding two diets containing different percentages of lactose (25 % and 35 %) at two scales of feeding (100 % and 120 %). If the assumption were correct the 'M.T.I.' would be recorded at the lowest liveweight for the 120 % / 35 % diet and at the highest liveweight for the 100 % / 25 % diet, these two diets contributing the most and the least lactose respectively, at any particular liveweight. This situation was not quite achieved in the main investigation, where the 30 % diet contributed 1.35 lb lactose per day just prior to the termination of the experiment at 120 lb liveweight. The same amount of lactose had been reached at 65 lb liveweight using the 45 % diet, and had produced a marked depression in food intake in 4 of the 7 pigs in this group. Had the experiment been continued to enable daily lactose intakes above 1.35 lb to be contributed by the 30 % diet, it is possible that adverse effects would have been observed.

The hypothesis has considerable practical bearing on experiments in which large quantities of whey are fed to growing pigs. Braude et al (1957; 1958; 1959 a) reported a considerable decline at approximately 150 lb liveweight, in the growth rates of pigs fed whey ad lib. In work carried out at Massey University (Dunkin, pers comm.) a similar effect, accompanied by a noticeable increase in within treatment variability, has been noted in several whey feeding investigations at 90 - 110 lb liveweight. The depression in growth rate and the marked increase in individual variability suggest that the diets may have been providing a quantity of lactose which was above the pigs' 'M.T.I', the adverse effects being noted earlier in the N.Z. work because greater amounts of whey were fed at any particular liveweight than were used under U.K. conditions.

The results obtained in the present investigations together with published results, suggest that a number of factors may affect the 'Maximum Tolerable Intake' of lactose. The more important factors are:-

(a) Individual Animal Variation

(b) Feeding Routine

(c) Protein Level and Protein Source

(d) Enzyme Activity and Adaptation

(e) Level of Dietary Fat

(a) Individual Variability

This is best illustrated by the fact that, in the collection study one pig began refusing food when the daily ration contained 0.4 lb lactose, while 3 times this amount was ingested with no adverse effects, by a pig at the same liveweight (55 lb) in the main investigation.

(b) Feeding Routine

In view of the importance of lactase activity in the utilization of lactose, it is suggested that animals fed diets containing high levels of lactose may make more efficient use of the sugar if it is fed in small quantities throughout the day, than if the same quantity of food were given in two feeds per day. Twice-a-day feeding of a high lactose diet would result in a 'flooding' of the Mucosal lactase with lactose, and as a consequence, large amounts of the sugar could escape hydrolytic cleavage and absorption. If much smaller amounts were ingested several times a day, the amount of lactose reaching the intestinal mucosa after each feed, is unlikely to be in excess of the lactase capacity, and so very little would escape hydrolysis and absorption. This could explain the conflicting results obtained by Dunkin (1961) in which group housed, individually fed pigs in the first trial produced more pronounced adverse effects from the whey diet, than individually penned pigs in the second trial. Under the group housing conditions pigs had a limited time in which to consume their

food, intake was therefore more rapid and so more likely to 'flood' the lactase than under individual pen conditions.

(c) Protein Level and Protein Source

Growth experiments with rats were conducted by DeGroot and Engel, (1957) using feed mixtures incorporating a variety of protein components, which included acid and rennet casein, skimmed milk powder, and soya bean oil meal. In other experiments, growth responses were determined on 25 % lactose diets containing 10, 20 and 30 % casein. The authors concluded from the results, that modifying the type and the amount of the protein component in the diet did not result in a noticeable improvement in growth response. However, more recent work with rats (Février and Rérat, 1964) suggests that the level of protein in a lactose diet may be important. These authors reported that intake and growth depression was less severe when a high lactose high protein (16 % herringmeal) diet was fed than when the high lactose diet contained only 8 % herringmeal protein. Although work has been conducted with pigs to examine the effects of lactose on protein utilization (Section 3.2 Part II) the reverse effect namely the influence of protein level or source on lactose utilization has been neglected. The fact that, in many cases up to 2.0 - 2.5 lb of lactose has been successfully fed per day using whey diets, (Dunkin 1961; 1963 a, b) suggests that milk proteins may be more suitable for lactose diets than either meat or fish meal proteins, or plant proteins such as soya bean oil meal.

(d) Enzyme Activity and Adaptation

Although the cause of the depressed food intake is still unresolved, it is reasonable to suppose that greater lactose hydrolysis would be associated with lower food refusals. As the level of lactase activity in the animal determines the rate and magnitude of lactose hydrolysis, it

is possible that the differences in the 'Maximum Tolerable Intake' of the animals reported above, are largely a reflection of differences in lactase activity. In general once food refusals commenced, food intake depression continued until the termination of the investigation. Only 3 animals recovered their full appetite after a period of depressed food intake. In these cases it is suggested that the animals increased their lactase activity sufficiently to enable them to cope with the lactose levels being ingested, i.e. they adapted to the lactose diet. This adaptation was reflected in the establishment of a new, higher 'M.T.I.' as seen clearly in all three animals. In the pig from the second preliminary investigation adaptation raised the amount from 1.28 lb lactose daily to greater than 2.25 lb. In a second pig the amount rose from 1.17 to 1.87, and in the third animal refusals began when small amounts of lactose (0.4 lb) were ingested, but adaptation finally resulted in 1.13 lb lactose per day being consumed without incident.

(e) Level of Dietary Fat

Two early reports from Wisconsin (Anon.1937 a, b) suggested that fat may be required in skim milk diets fed to pigs, but in recent years this aspect of lactose utilization appears to have been totally neglected. Work conducted with rats fed lactose diets in which a variety of fats and oils were added, were reviewed by Deuel Jr. (1957). The results (Geyer, Boutwell, Elvehjem and Hart 1946; Nieft and Deuel Jr. 1947) suggested that the addition of up to 30 % fat to high lactose diets (44-48 % lactose) markedly reduced galactosuria probably by decreasing the rate at which galactose was absorbed from the small intestine. As no growth rates were recorded, it is not possible to judge whether these urinary energy losses even on the diets not containing fat, were sufficiently large to affect liveweight gains.

4.4/3 THE EFFECT OF HIGH LEVELS OF DIETARY LACTOSE ON CARCASS CHARACTERISTICS

The lack of published data on this subject, and the conflicting results obtained in the present experiments, make it very difficult to discuss this aspect of lactose utilization.

The linear fat measurements in the main investigation showed no consistent trend in carcass fatness with increasing dietary lactose. In the collection study, a slight trend was apparent toward leaner carcasses on the high lactose diets. This was particularly evident in values for minimum loin, and in this measurement statistical analysis showed treatment differences to approach significance at the 5 % level.

No significant differences were found in specific gravity measurements for the carcasses from the main investigation, but a very slight trend in the treatment means suggested that control animals were leaner than animals on 15 % or 30 % lactose. Significant ($P < 0.05$) treatment differences in specific gravity were found in the data obtained from the collection study, and in this analysis the control animals again had the leanest carcasses and progressively fatter carcasses were evident with each increase in dietary lactose percentage. Linear fat measurements therefore suggested that lactose in the diet increased carcass lean, while specific gravity measurements indicated that the lactose diets produced fatter carcasses. Assuming that both methods provide an equally reliable assessment of carcass fatness, the results obtained suggest that no appreciable effects result from lactose incorporation into the diet.

4.4/4 THE EFFECT OF HIGH LEVELS OF DIETARY LACTOSE ON CAECUM DEVELOPMENT

The effect of lactose intake on caecum size was most striking. Much larger caecae, as indicated by wet and dry weights, and tissue volume measurements, resulted from as little as 15 % lactose in the diet. Even

greater differences were experienced when pigs were fed the 30 and 45 % lactose diets. Lawrence et al (1956) studied the importance of caecal development in rats fed high levels of lactose, and concluded that caecum distension was accompanied by a reduction and cessation in diarrhoea. The results obtained from the present investigations suggest that a similar situation exists in pigs and that the marked reduction in diarrhoea noted over the experimental period, was possibly a reflection of caecal enlargement. The fact that some pigs on 45 % lactose continued to scour until the experiment ended, suggested that their lactose intake was greater than could be 'handled' by their caecae, despite the enlargement which had occurred. Similar effects were reported by Lawrence et al (1956) when rats were fed a 50 % lactose diet.

Ohio workers have shown that increased bacterial activity accompanies caecal distension (Fischer et al 1949; Lawrence et al 1949). Dahlqvist and Thomson (1964) studied this source of lactase activity in rats and concluded that it could play an important part in the hydrolysis of lactose which escaped degradation in the small intestine. The fact that removal of lactose by bacterial lactase reduced diarrhoea, suggests that lactose per se (Fischer and Sutton 1949) is responsible for the laxation. If diarrhoea was secondary to the irritating effects of lactic acid - a view expressed by Friedland, (1965), more pronounced scouring would be expected after bacterial lactose hydrolysis, because more lactic acid would be produced.

Lee and Moinuddin (1958) reported that caecal development in rats fed lactose diets, was accompanied by parallel development in the colon and rectum. Observations made of the alimentary tracts from pigs on the lactose diets, also suggested that caecal development was accompanied by similar increases in the size of the colon and rectum. These increases could explain the highly significant decrease in mean 'killing-out' percentages found with increasing levels of lactose.

4.5 CONCLUSIONS

High dietary levels of lactose will cause diarrhoea, but a considerable degree of adaptation is possible and this may be largely due to the caecum development noted.

Chronic diarrhoea does not appear to have a marked effect on liveweight gains.

At the two lower levels of lactose used (15 % and 30 %) food intake is rarely depressed, but a depression in growth rate may still result. This suggests that the digestion and/or the metabolic processes may be altered as a result of lactose incorporation into the diet.

On high levels of lactose (45 %) reduced food intake is responsible for much of the poor growth and efficiency of food utilization.

No conclusive evidence was obtained to show that lactose incorporation into the diet at any of the levels used, had any appreciable effect on carcass characteristics.

Lactose diets cause a marked increase in caecum development in the pig, and this may be accompanied by similar increases in the colon and the rectum.

PART II

BIOCHEMICAL ASPECTS INVOLVED IN THE UTILIZATION OF LACTOSE BY THE GROWING PIG

CHAPTER 1

THE ABSORPTION AND UTILIZATION OF LACTOSE AND ITS MONOSACCHARIDE DERIVATIVES - A REVIEW

1.1 ABSORPTION OF LACTOSE BY THE INTESTINAL MUCOSA

Only a small quantity of the total carbohydrate ingested by most higher monogastric animals is in the form of simple sugars. In general the small intestine is presented with a mixture of disaccharides resulting either directly from the diet, or indirectly as a result of the degradation of more complex polysaccharides by the action of salivary, gastric and pancreatic secretions. These must be further hydrolysed to their constituent monosaccharides if the body is to make effective use of them. Investigations with humans (Borgstrom, Dahlqvist, Lundh and Sjövall 1957, Dahlqvist and Borgstrom 1961) and *in vitro* work by Miller and Crane (1961) provide considerable evidence to show that this final cleavage occurs within the cells of the intestinal mucosa.

1.2 ENZYMIC HYDROLYSIS OF LACTOSE

(a) Introduction

Lactase (β galactosidase, E.C. No. 3.2.1 23) (I.U.B. 1961) exhibits a strict glycon specificity (Wallenfels and Malhotra, 1961) for the β galactoside structure and is therefore the main enzyme involved in the hydrolysis of lactose (4-O-β-D. galactopyranosyl-D glucopyranose), (Clamp, Hough, Hickson and Whistler 1961). However, some confusion exists concerning this specificity. (See β-glucosidase E.C. No. 3.2.1 21, Dixon and Webb 1964). Monod, Cohen-Bazire and Cohn (1951) showed that the β galactosidase from *E. coli* could only hydrolyse disaccharides which were β galactosides. On the other hand there is considerable evidence (Dahlqvist 1960, 1961 a,b,c) to suggest that the β galactosidase and

β glucosidase activities in the pig, and in the human (Dahlqvist 1962) were associated in a single enzyme, intestinal lactase.

(b) Development of Intestinal Lactase Activity in the Pig

Limited work with pigs (Sprague, Ullrey, Waddill, Miller, Zutaut and Hoefer 1963) has shown that a marked increase in activity (per gram of mucosa) occurs over the last few days of pre-natal life, rising to a maximum level at birth. Several workers have confirmed the presence of high lactase activity per gram of mucosa (DeGroot and Hoogendoorn, 1957; Walker, 1959; Dahlqvist, 1961) or per kilogram bodyweight (Bailey, Kitts and Wood 1956) at birth, but reported that the level diminished considerably after the first three weeks of life. Subsequently activity remained at a constant, lower level over adult life (Dahlqvist 1961 b,c). However, the total activity, as the pig grows, does not change (Walker 1959), only the activity per gram of tissue, either wet, (DeGroot and Hoogendoorn 1957, Hartman, Hays, Baker, Neagle and Catron, 1961; Dahlqvist, 1961 b,c) or dry (Sprague et al 1963) decreases, and because of the low relative growth rate of the intestine, activity per Kilogram bodyweight (Bailey, Kitts and Wood 1956) decreases more.

(c) The Location of Lactase Along the Alimentary Tract

Decreased lactase activity has been reported in the distal segments of the pigs small intestine. (Bailey et al 1956, DeGroot and Hoogendoorn 1957). Although DeGroot and Hoogendoorn (1957) concluded that maximum lactase activity occurred in the duodenum, more recent work (Walker 1959, Dahlqvist 1961 c, Sprague et al 1963) showed that the medial areas, specifically the upper jejunum, contained the greatest lactase activity and that this decreased proximally in the duodenum and distally in the ileum and colon. A similar localization has been reported for humans (Dahlqvist 1962) dogs (Cajori 1935) and rats (Dahlqvist and Thomson 1964).

All workers have reported zero activity in the stomach and colon and very low activity in the ileum.

These results are essentially in agreement with observations on the intestinal site of lactose absorption in the human (Borgstrom et al 1957, Dahlqvist and Borgstrom 1961). However, considerable lactose absorption was reported (Dahlqvist and Thomson 1964) in the large intestine of the rat. This apparent discrepancy could result either from an accumulation of bacterial lactase activity in the caecum (discussed in full in Part I), or from the presence of more than one β galactosidase in the rat (Doell and Kretchmer 1962) located in different sites along the tract.

(d) Factors Influencing the Level of Lactase Activity in the Animal

(i) Substrate Ingested

Fischer and Sutton (1949) reviewed studies on lactose adaptation in the adult rat, and postulated that lactase production was increased in response to high levels of dietary lactose.

Prolonged feeding of lactose to rats, was reported to increase lactose absorption (Fischer and Sutton 1953) and lactase activity (Fischer and Patton, 1955; Fischer, 1957; Girardet, Richterich and Antener 1964). This increase in total β galactosidase activity was attributed (Fischer and Patton, 1955; Fischer, 1957 a) to the accompanying increase in growth of the small intestinal mucosa. For this reason, no increases were reported when activity was expressed per gram of intestinal mucosa (DeGroot and Hoogendoorn, 1957; Hartman et al 1961). In contrast, Fischer, Sutton, Lawrence, Weiser and Stahly (1949) reported no increase in total lactase activity in rats fed lactose.

More recently Doell and Kretchmer (1962) have reported that evidence from rabbits with mammary glands removed, did not support the hypothesis

(Herzenberg, and Herzenberg 1959) that β galactosidase in the foetal tissue was induced by circulating lactose.

(ii) Bacterial Lactase Activity of the Caecum

Although induction of intestinal lactase activity is unresolved, it has been suggested (Dahlqvist and Thomson 1964) that the increased bacterial lactase activity reported in the caecum of rats fed high lactose diets (Lawrence, Weiser, Stahly, Fischer and Sutton 1949, Lawrence, Fischer, Sutton and Weiser 1956, and Dahlqvist and Thomson 1964) may play an important part in the digestion of lactose.

1.3 ABSORPTION AND METABOLISM OF THE MONOSACCHARIDE COMPONENTS OF LACTOSE

(a) Introduction

Cleavage of the β - D - (1 $\longrightarrow$ 4) linkage of lactose, produces equal quantities of the monosaccharides glucose and galactose. Any further metabolism of lactose after hydrolysis must therefore be that of its constituent monosaccharides.

(b) The Absorption of Glucose and Galactose

It is not proposed to review at length, the literature relating to the site, specificity and mechanisms of intestinal absorption since several excellent reviews (Crane 1960, Crane 1962, Isselbacher and Senior 1964, Csaky 1965) and two recent texts (Wilson 1962, Wiseman 1964) adequately cover this field of discussion.

Until Verzár and McDougall (1936) proposed that active absorption was responsible for the increased rates of glucose and galactose uptake, over other hexose sugars, it was generally believed that intestinal absorption of sugars was by simple diffusion, and that 'selective permeability' (Hèdon 1900 Cited by Crane 1960) accounted for the

differences in absorption rates. Since the active transport principle was proposed, two hypotheses of carbohydrate absorption have been advanced. The first, the 'phosphorylation' concept whereby all sugars required a linkage with a phosphate radical before absorption could take place, was proposed by Magee and Reid (1931). Although subsequent work showed the concept to be based on incorrect assumptions, the hypothesis was nevertheless favoured by Verzar and McDougall (1936) in their review on carbohydrate absorption. More recently, Crane (1962) proposed an alternative mode of intestinal absorption based on observations made initially by Riklis and Quastel (1958), that active transport of sugar was completely dependent on the presence of Na^+. This alternative, the 'Sodium Pump' hypothesis, has considerable experimental support. (Csaky and Thale, 1960; Csaky, 1961; Barry, Mathews, Smith and Wright, 1962; and Bihler and Crane, 1962). The hypothesis (Crane 1962) is that glucose associates with a mobile carrier and Na^+ ions, forming a complex which 'traverses the apparent diffusion barrier to the inner side where it dissociates releasing glucose and Na^+ into the cytoplasm', the Na^+ being returned to the extra-cellular medium by means of a Na^+ active transport system, the glucose remaining within the cytoplasm (Crane, 1965; Curran, 1965).

(c) Glucose and Galactose Metabolism

Irrespective of how the two monosaccharides are transferred into the bloodstream, it is clear that glucose and galactose, despite their structural similarity exhibit markedly different metabolic effects. Glucose, by way of glucose -1- phosphate, is rapidly utilized either as a free energy source or as a precursor in the biosynthesis of glycogen. Galactose is less readily utilized by the body tissues (Verzár and McDougall 1936). Poorer growth rates compared with control animals, and early death, have been reported from a number of experiments in which

rats were fed diets containing large proportions of galactose (Guha 1931, Scheunert and Sommer 1956, DeGroot and Engel 1957, Heggeness 1959). All reports showed that galactose could not provide sufficient energy to support life, indeed Guha (1931) reported that death was the result of virtual rather than actual starvation, as the rats continued to consume food until shortly before death.

(d) The Importance of Galactose in Neonatal Nutrition

Galactose comprises half the total carbohydrate initially available to the neonatal animal but it is unlikely that the major avenue of utilization of this monosaccharide is through the glycolytic pathway. If the sole purpose of galactose were to provide energy via this pathway, it could be beneficially replaced by glucose, which is a more readily available substrate for the pathway. Consideration of the alternative avenues of galactose utilization in the neonatal animal is therefore desirable.

Considerable glycogen synthesis occurs in rat liver slices after birth, and this increases to a maximum at 10 days of age (Ballard and Oliver 1964). By far the greatest portion of galactose ingested is utilized as a source of glycogen in the rat liver after the first 7-10 days of life (Segal, Roth and Bertoli, 1963; Ballard and Oliver, 1964) and it appears that in view of the considerable utilization of galactose in children from 8 hours to 7 days old (Haworth and Ford, 1963; Cornblath, Wybregt and Baens, 1963; Haworth, Ford and Robinson, 1965; Anyon and Clarkson, 1965), similar liver glycogen synthesis could also occur in humans. This preferential galactose uptake (Ballard and Oliver 1964) occurs during a clearly defined period, poor utilization (evidenced by high peripheral blood galactose) being recorded from birth up to 8 hours of age, (Anyon and Clarkson 1965) and from 8 days on into adulthood (Haworth and Ford 1963).

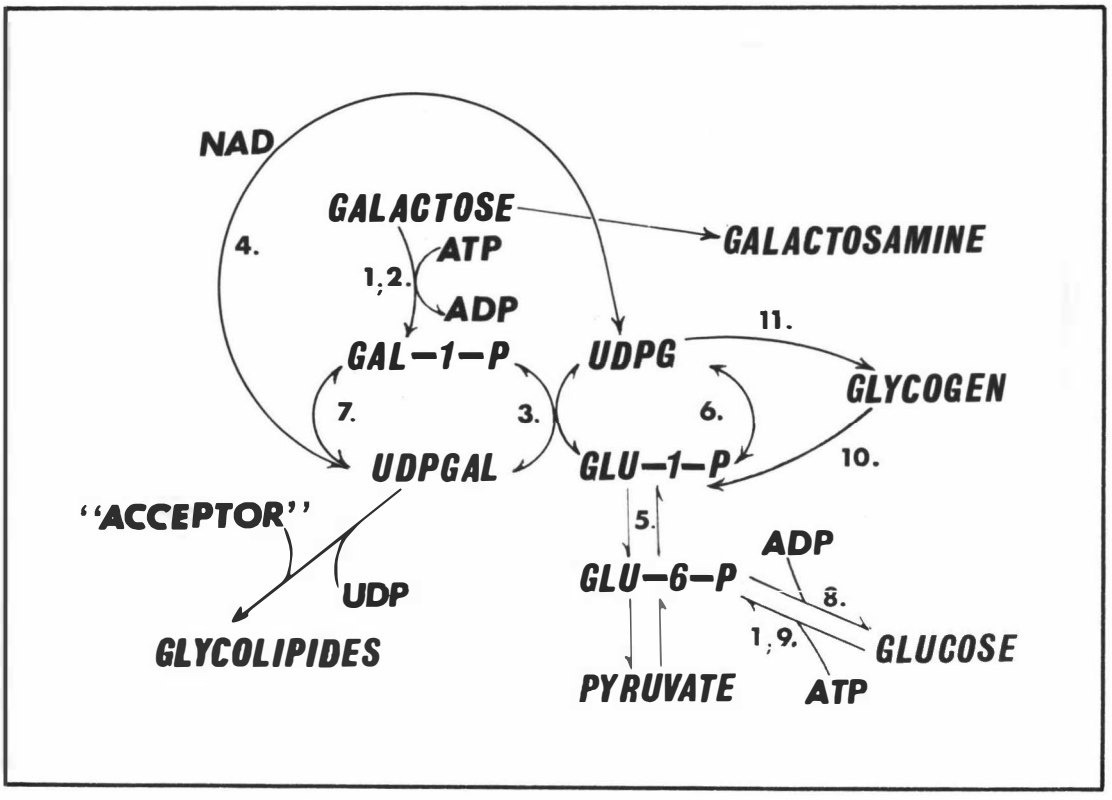


FIGURE 1.3/1

PATHWAYS OF GALACTOSE METABOLISM

(Modified from Pesch and Topper, (1963) and Dawkins, (1966))

ENZYMES INVOLVED

1. HEXOKINASE
2. GALACTOKINASE
3. HEXOSE 1 PHOSPHATE URIDYL TRANSFERASE
4. UDPGALACTOSE-4-EPIMERASE
5. PHOSPHOGLUCOMUTASE
6. UDPG PYROPHOSPHORYLASE
7. UDPGAL. PYROPHOSPHORYLASE
8. GLUCOSE 6 PHOSPHATASE
9. GLUCOKINASE
10. α GLUCAN PHOSPHORYLASE
11. UDPG α GLUCAN GLUCOSYLTRANSFERASE

ABBREVIATIONS USED

ADP ... ADENOSINE DIPHOSPHATE
ATP ... ADENOSINE TRIPHOSPHATE
GAL-1-P ... GALACTOSE-1-PHOSPHATE
GLU-1-P ... GLUCOSE-1-PHOSPHATE
GLU-6-P ... GLUCOSE-6-PHOSPHATE
NAD ... NICOTINAMIDE ADENINE DINUCLEOTIDE
UDP ... URIDINE DIPHOSPHATE
UDPG... URIDINE DIPHOSPHOGLUCOSE
UDPGAL ... URIDINE DIPHOSPHO-GALACTOSE

It is still uncertain why galactose should be preferred to glucose as the substrate for glycogen synthesis over this particular period. An early report (Bell 1936) that galactose produced a larger, 18 carbon (cf. 12 carbon from glucose) glycogen which was more slowly broken down, may be of consequence, particularly in view of the pronounced neonatal hypoglycaemia of new born infants which usually lasts for approximately 7 days (Shelley and Neligan 1966).

Two considerably smaller avenues of galactose utilization have been identified. One is the production of galactosamine (Chondrosamine) which is a major component of Chondroitin Sulphate (Muir 1961), a structurally important sulphated polysaccharide of the eye (Maurice 1962) and of bone and cartilage (Eastoe 1956). The second is the production of glycolipides (Cerebrosides, Cerebron Sulphate, polycerebrosides, strandin (ganglioside)), which are essential for the normal development and maintenance of the brain and nervous system. Uridine -di-phospho-galactose (UDP-Gal) is the primary hexose donor in glycolipide biosynthesis (Burton, Sodd and Brady 1958, Moser and Karnovsky 1959) but these authors' experiments with glucose -U-C^{14}, galactose-1-C^{14} phosphate, and UDP Gal-1-C^{14} (Burton et al 1958) and Glucose-1-C^{14}, Galactose-1-C^{14} (Moser and Karnosky 1959) showed that Glucose can substitute for galactose as the hexose source of the galactose in the UDP-Gal (see also Varma, Schwartz and Simpson, 1962). This is due to the body's ability to convert glucose to galactose even at an early age (Cornblath, Wybregt and Baens 1963).

It is evident from Fig. 1.3/1 that the major pathway for galactose utilization after the initial ten day neonatal period, is through the Glycolytic Pathway, by way of glucose - 6 - phosphate. Presumably the small amounts of galactose required for either Chondroitin Sulphate or Glycolipide production are either 'chanelled off' from ingested galactose, or produced by the body from glucose by epimerization.

(e) The Importance of Lactose as a Source of Galactose

If it is assumed that galactose is important in the neonatal diet, the form in which it is presented (as a component of lactose) requires consideration. If galactose alone, is presented to the gut, due to its rapid uptake (Cori 1925, Verzar and McDougall 1936), a pronounced galactosaemia ensues, (Folin and Berglund 1922), (Haworth and Ford 1963). This results in considerable loss of the monosaccharide in the urine due to the very low renal galactose tolerance (Folin and Berglund 1922). Obviously the rate of uptake must be considerably reduced if galactose losses are to be prevented. When equal amounts of glucose and galactose were fed simultaneously, or when an equivalent amount of lactose was given, reductions were reported in both blood sugar levels (Bodansky, 1923; Corley, 1927; Coryell and Christman, 1943; Haworth, Ford and Robinson, 1965) and in sugar excretion levels (Folin and Berglund 1922), in comparison with results obtained by feeding galactose alone. Furthermore Carleton, Misler and Roberts (1955) measured labelled CO_2 production and radioactivity retention in the carcasses of rats administered a variety of labelled sugars, (D Gal-1-C^{14}, D Glu-U-C^{14}, D Glu-1-C^{14}, D Lactose-1-C^{14}). This work showed that greater C^{14} retention could be achieved by feeding either lactose, or a mixture of glucose and galactose, in preference to feeding either monosaccharide alone.

Glucose appears to inhibit the absorption of galactose (Corley, 1928; Crane, 1960; Haworth et al, 1965, Koldovský, Muzyčenková, Hahn, Heringová and Jirsova, 1965). Moreover, it has been shown that glucose, (Stevenson, 1912; Cajori, 1935) and possibly galactose (Wallenfels and Malhotra 1961) inhibit intestinal lactase activity. This negative feedback, whereby the products of hydrolysis (glucose and galactose) inhibit the hydrolytic cleavage of the substrate (lactose) results in a decrease in the rate of galactose absorption. This accounts for reports (Folin

and Berglund 1922, Haworth et al 1965) that a much larger blood sugar increase accompanies the administration of equal amounts of glucose and galactose compared with that seen after the administration of an equivalent amount of lactose.

It is clear then, that to ensure minimal losses of galactose, both glucose and galactose, preferably in equal amounts, and in a form which is not too rapidly absorbed, must be presented to the gut. Lactose meets these requirements.

CHAPTER 2

COLLECTION PROCEDURES

2.1 INTRODUCTION

Several indirect methods available for the determination of digestibility were considered (Clawson, Reid, Sheffy and Willman, 1955; Cunningham, 1959; Nordfeldt and Kihlén, 1965) but as analysis of urine as well as faeces was desired the total collection procedure (Robinson, Morgan and Lewis 1964 a, b; Robinson, Prescott and Lewis, 1965) was employed.

2.2 ADOPTED PROCEDURE

Determination of the calorific, nitrogenous and lipid contents of the composite faeces were carried out, and in conjunction with a proximate analysis (AOAC 1965) of the feeds offered (Appendix 5.2/1) assessment was made of the effects of different dietary lactose levels on energy, ether extract, and protein digestibility. Nitrogen determinations of composite urine samples enabled an assessment of Nitrogen retention.

With the facilities available the estimation of metabolizable energy was not possible. However, analyses of the urinary filtrates for total reducing sugars, were carried out. In order to assess the contribution faecal excretion made to the total sugar loss, total reducing sugar was estimated on protein free filtrates obtained from faecal samples. As some of the unabsorbed lactose could be converted by the lower gut flora to lactic acid, the faeces filtrates were also analysed for their lactic acid content.

2.3 MATERIALS AND METHODS

(a) Design of the Metabolism Crate

The metabolism crates used in the study were a modified version of those used at the Rowett Research Institute (Jones pers. comm.). Similar

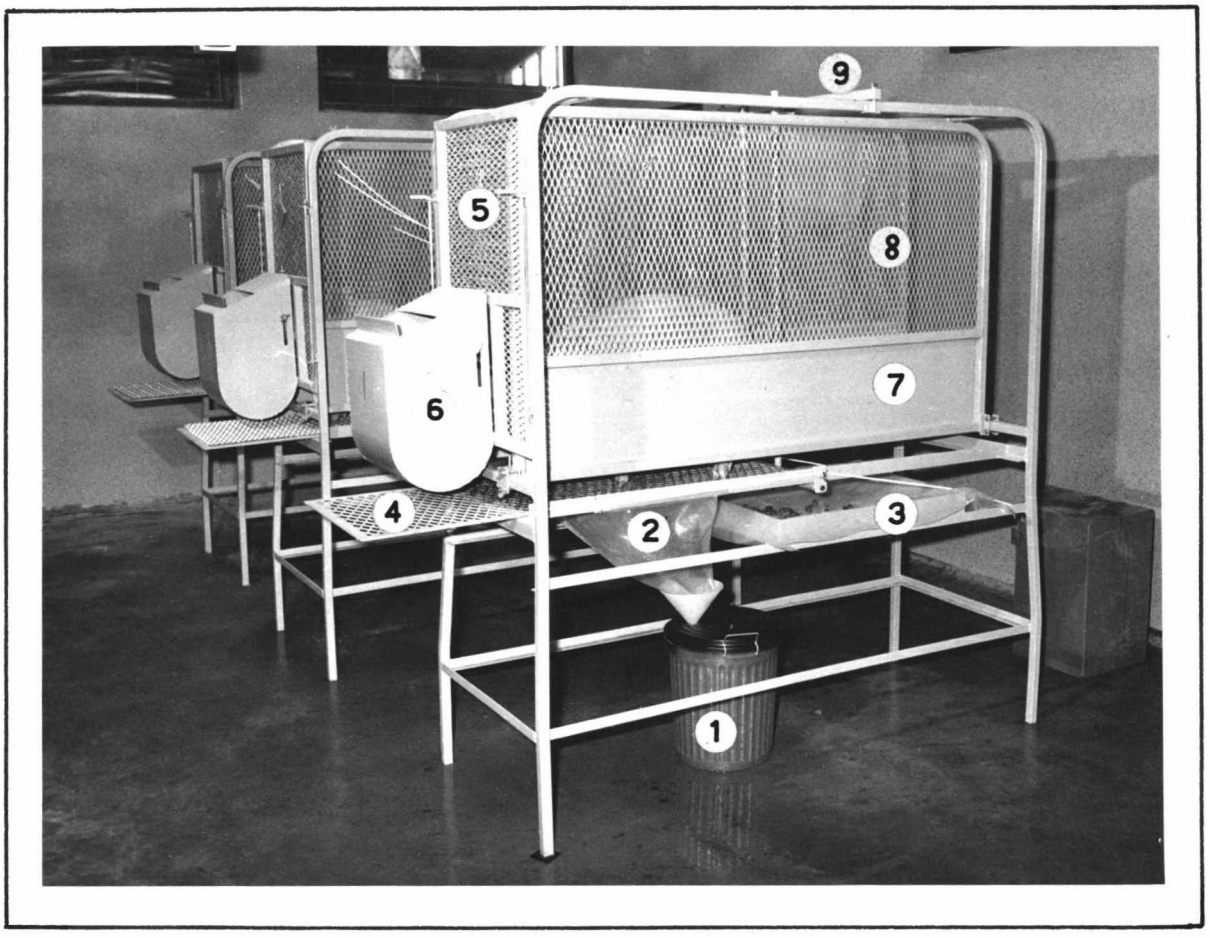


FIGURE 2.3/1

The Metabolism Crate and Collection Equipment

1. Polythene Collecting Bin.
2. Polythene Urine Apron
3. Dung Tray with Polythene Sheet.
4. Expanded Metal Floor
5. Expanded Metal Front hinged on both sides to enable ready access to the pig.
6. Removable Food Trough.
7. Sheet metal strip
8. Expanded metal fully adjustible side.
9. Adjustible backstop enabling adjustments in crate length

crates have since been reported used at the University of Nottingham School of Agriculture by Robinson et al (1965). In view of differences in construction Fig. 2.3/1 has been presented.

(b) Preparation for Collection

(i) Preparation of Animals

All pigs were housed in individual pens adjacent to the room in which the metabolism crates had been placed. Similar ambient temperatures were maintained (68°F) in both rooms. Previous work (Carr pers. comm.) indicated preliminary training to accustom the pigs to crate conditions was not necessary. All pigs were weighed each Tuesday to enable ration adjustment as mentioned previously, and those pigs whose liveweight and rate of growth suggested that 60 lb weight would be exceeded within a week, were placed in individual crates. A two day preliminary period was followed by a collection period lasting 5 days, (Lassiter, Terrill, Becker and Norton 1956). This meant that the pigs were removed from the crates after exactly one week and so could be weighed with any pigs not on collection, on the Tuesday. Although this procedure was very convenient, results of total reducing sugar analyses performed on the faecal samples collected, indicated with few exceptions, higher figures for the first day in comparison with the remaining days.

Consequently, in the two later collections (at 85 and 110 lb liveweight), meal adjustment was made six days before the pigs were placed in the crates. This meant that the same meal level was maintained for two weeks. In addition it was felt that an extra day in the crate before collection, was desirable. This resulted in all pigs being in the crates for a total of eight days for collections B and C, collection being carried out over the last five days as before.

(ii) Preparations for the Collection of Faeces

Faeces were collected on heavy duty polythene sheeting placed inside the metal faeces trays positioned behind the pigs.

Adjustments were made to the crates during the two days preceeding collection to minimise urinary contamination of the faeces.

(iii) Preparations for the Collection of Urine

Urine was collected under toluene in polythene bins placed directly beneath the crates. Over the two preliminary days adjustments were made to the position of the apron to minimise urinary losses, urinary contamination of faeces, and contamination by feed spillage. The lower end of the apron was placed inside the collecting funnel into which had been wedged a piece of wire gauze and a square of glass fibre. This arrangement allowed urine to freely pass through but retained any skin tissue, hair and faeces. Adjustments to the position of the feed spillage tray beneath the feed trough prevented urinary contamination, spillage being very small due to the consistency of the ration fed.

(c) Collection Routine

(i) Collection of Daily Samples

A total collection procedure was adopted to study the digestibilities of the four feeds in use. 24-hour voidings were collected for five consecutive mornings, at 7.00 a.m. before the pigs were fed.

Faeces were transferred from each polythene sheet into numbered plastic buckets, tared prior to the collection, and any faeces sticking either to the crate or to the sheet were scraped into the bucket. After weighing, each 24-hour sample was mixed in a commercial cake mixer, between 5 and 10 mls of toluene (depending on the amount of faeces present) being mixed in as a preservative. Between three and five minutes sufficed to produce a smooth homogeneous paste from which one quarter of the total weight was taken to provide duplicate D.M. samples. These were dried at 100°C for 48 hours in a hot air oven to give daily dry-matter figures. One half of the total weight of the paste was taken and stored frozen at -12°C to form part of a composite wet sample for the five day collection period. From the remaining faecal paste 100 grams were taken for the preparation of protein

free filtrates.

Daily voidings of urine were weighed in the tared bins, thoroughly stirred, and one tenth of the volume stored under toluene at -12°C to form part of a composite urine samples for the five day collection. In addition a 10 ml. sample was taken, mixed with 0.2 ml. toluene and set aside for urinary filtrate preparation.

(ii) Preparation of Composite Samples

Composite samples of faeces were treated in a similar way on the day following the completion of a collection period. The samples were thawed overnight at 4°C and each was thoroughly mixed. From the resultant paste, two 500 gm samples were taken and dried at 100°C for 48 hours in a forced draught oven. From the remainder, 100 gms were taken for subsequent sample preparation andthe rest was placed in plastic bags, frozen and stored at -12°C in reserve, should a repeat analysis be necessary. No daily dry matter samples were kept, after their weights had been recorded, but the two composite dry matter samples from each collection after weighing were placed in plastic bags and stored at -12°C until grinding was possible.

Composite samples of Urine, when thawed, were thoroughly mixed and sampled as before for deproteinization.

Additional samples for Nitrogen analysis were taken and the urine returned to the 4°C cooling room in case any repeat determinations were necessary.

(d) Preparation of Protein Free Filtrates

In order to minimise proteolytic and bacterial degradation of sugars in the samples, protein free filtrates were prepared as soon as possible after daily sample collection.

The 100 gm faecal paste samples taken daily were each blended with 100 mls of water and 100 mls of acetone (Ford and Haworth, 1963), to which 5 mls of toluene was added. This produced a thick slurry which showed no

apparent settling over a 5 minute period. From each slurry 3 ten gram samples were pipetted into tared evaporating dishes and weighed quickly. The dishes were then dried at 100°C for 22 hours to enable calculation of moisture and dry matter contents of the slurry. At the same time one 10 ml. sample was centrifuged and the clear aqueous supernatent, containing sugars and other water soluble components, retained.

From this supernatent and the urine samples, 1 ml. of solution was withdrawn and deproteinized using $ZnSO_4$ and $Ba(OH)_2$ according to the method of Nelson (1944). The protein free filtrates (P.F.F.) were frozen in stoppered polystyrene vials and stored at -12°C until analysed for total reducing sugar and lactic acid concentrations.

The same faecal slurry provided samples for Nitrogen determination thereby eliminating the need for 2 sets of dry matter determinations.

2.4 PRELIMINARY STUDIES

(a) Estimation of the Precision of Dry Matter Determinations

To determine the precision of dry matter procedures triplicate determinations were made on five samples covering the normal range of D.M. experienced. Samples were dried at 100°C for 24 hours.

Results were:

Mean	A. 8.28	B. 9.61	C. 10.14	D. 11.67	E. 12.09
± SE	± 0.00	± 0.03	± 0.04	± 0.17	± 0.01

The data indicated that the method was sufficiently precise to enable accurate assessments of D.M.%.

(b) Effect of Drying Time on Dry Matter Determinations

As 22 hours was the maximum time available for daily dry matter determinations due to limited oven space, four drying times were examined (22, 24, 46, 48 hours) to see if the 22 hour period gave a sufficiently accurate result. Each drying time was represented by six 10 gram

replicates, all 24 replicates being taken from the same mascerate. Results were:

	Drying Time (Hours)			
	22	24	46	48
Mean DM %	8.33	8.32	8.28	8.27
± SE	± 0.035	± 0.034	± 0.035	± 0.027

Although the longer drying period gave slightly lower D.M. percentages, these were not significantly different from the estimated value determined after 22 hours.

(c) Effect of Evaporation from Faeces Mascerates

While preliminary work was being carried out it was noticed that the faeces mascerates lost weight after transfer into the dry-matter dishes. To examine the extent and duration of this loss, six 10 gram samples were transferred to dry matter dishes and were weighed immediately after transfer and then at 5, 10 and 15 minutes after transfer. Mean losses (mgms) were:-

0 - 5 minutes	28.9 ± 1.4
0 - 10 "	59.7 ± 3.5
0 - 15 "	85.3 ± 4.5

The large evaporative loss continued for at least 15 minutes and so all subsequent samples were weighed immediately after transfer to the dry-matter dishes.

CHAPTER 3

SAMPLE ANALYSIS

3.1 TOTAL REDUCING SUGAR ANALYSIS OF URINARY AND FAECAL PROTEIN FREE FILTRATES

3.1/1 REVIEW OF LITERATURE

(a) Urinary Sugar Studies in the Pig

Very little work has been published on either the sugar components, or concentrations, in the urine of the pig. Urinary glucose investigations were carried out by Carlson and Drennan, (1912-13), Hanawalt, Link and Sampson, (1947) and Link, (1953) to establish a renal glucose threshold for pigs weighing up to 120 lb liveweight, but no other sugars were examined. Working with weanling pigs, Fisher (1931-32), reported finding as much as 35 grams of sugar in the urine daily, over a three-day period in which rations containing 65 % lactose were fed. In work with growing pigs fed lactose, Dunkin (1957) reported that 'snap' samples of urine indicated increased reducing sugar activity with increasing treatment levels of lactose, but no figures were given. More recently Kidder, Manners and McCrea, (1963) measured urinary fructose losses from sucrose-fed piglets 6-13 days old, and concluded that the fructose loss in the urine represented only a small proportion of the fructose ingested, and a still smaller fraction of the energy content of the dietary sucrose.

(b) Faecal Sugar Studies in the Pig

Although several workers have reported scouring in pigs fed high levels of dietary lactose (Johnson, 1949; Becker and Terrill, 1954), or lactose-containing milk by-products (Krider, Becker, Curtin and Van Poucke, 1949; Becker, Terrill, Jensen and Hanson, 1957), no assessments have been made of sugar losses (if any) which occur in association with the diarrhoea.

Recently Salo (1965) carried out sugar analyses on the faeces of pigs fed diets consisting predominantly of wheat-bran and skim milk. From his studies he concluded that both lactose and starch were 100 % digestible. However, no mention was made of any analyses being performed on diarrhoeal faeces. Evidence suggests that diarrhoea in humans is accompanied by considerable sugar loss. Recognising the value of faecal examination for the diagnosis of mono- and disaccharide intolerance, Ford and Haworth (1963) analysed the stools from 61 children ranging from 1 day to 14 years of age. In the stools of one group of children suffering from acute diarrhoea, the levels of total reducing sugar and total glucose were found to be two times the levels found in two other groups of children voiding stools of 'normal' consistency.

In view of the noticeable lack of knowledge on the extent to which ingested sugar is lost from the pig in the urine and the faeces, under normal and diarrhoeal conditions, a comprehensive study of these avenues of energy loss was undertaken.

3.1/2 METHODS OF ANALYSIS

(a) The Total Reducing Sugar Reaction

The Nelson - Somogyi method for the determination of blood glucose (Nelson, 1944; Somogyi, 1945) was used. One ml. samples of the blank (Distilled Water), Glucose standards (40, 80, 120, 200 mg/100 ml.) or protein free filtrates from faeces and urine, were added to 1 ml. portions of the copper reagent (Somogyi, 1952) in sugar tubes which were then loosely stoppered with glass marbles to prevent evaporation. The tubes were randomly distributed throughout a rack and placed simultaneously into a boiling water bath into which 150 ml. of polyethylene glycol had been added to stabilize the boiling point and to reduce splashing. The samples were boiled for exactly 20 minutes (Salo 1965), continuous agitation

of the water in the bath over this period ensuring a uniform temperature across the bath. As soon as the 20 minutes had elapsed, all samples were simultaneously removed from the water bath, allowed to cool slightly in air, and were then thoroughly cooled in a tank of cold water.

After cooling, 1 ml. of Arsenomolybdate solution (Nelson 1944) was added to each sugar tube, the mixture was shaken, and the contents diluted with distilled water to 10 ml. After vigorous mechanical agitation to ensure complete mixing, the optical densities of the samples and standards were read against the blank at 660 mμ (Nelson, 1944) in a Unicam SP500 Spectrophotometer using 10 mm glass absorption cells. All samples were represented in triplicate, as were the four standard sugar solutions and the distilled water blank, in each set of determinations.

(b) Calculation of Results

Preliminary studies established the linearity of the line relating optical density to sugar concentration over a range of sugar concentrations expected in the sample determinations. Linear regression equations were therefore calculated for each set of determinations from the standard sugar solutions included. The regression coefficients and correction terms so resolved, were applied to the optical density figures for the samples, to enable the sugar concentrations of samples to be calculated. Where comparison between two sets of determinations was desired, an 'F' test of heterogeneity of the regressions was applied, and where non-significant results were obtained, the sugar concentrations from the two sets of determinations were compared. For this reason strict adherence to the procedure outlined was essential.

3.1/3 PRELIMINARY DETERMINATIONS

The Nelson - Somogyi method of sugar determination has been recently criticised (Dygert, Li, Florida and Thoma, 1965) for its lack of precision.

In view of the criticisms put forward, recovery and repeatability determinations were essential. Furthermore, no information was available on the effects of collection, preservation and storage of samples on their sugar levels.

For these reasons, the following determinations were made :

(a) Sugar recovery.

(b) Sugar repeatability determination.

(c) Changes in the sugar concentration of protein free filtrates stored for zero and 56 days at $-12^{o}C$.

(d) Changes in the sugar concentration of urine stored intact for four different periods of time.

(e) The effect of the storage of faeces at $-12^{o}C$ for five days, on their total reducing sugar concentrations.

(a) Sugar recovery

Sugar recoveries were determined on protein free filtrates from a sample of dung and urine.

The sugar concentrations determined, were expressed as a percentage of that calculated from the sugar concentrations of the original filtrates and the standard glucose added. Results of four analyses on each filtrate (App. 3.1/1) were:

Sample A (Dung) Mean Recovery 100.0 % Range 92.5 - 108.0 %

Sample B (Urine) Mean Recovery 100.3 % Range 97.2 - 104.3 %

(b) Sugar Repeatability Determination

Protein free filtrates were prepared from one dung and one urine sample. From each filtrate 18 sugar determinations were made. Values

obtained for the two samples are presented in full in the appendix (App. 3.1/2) and a summary of the data is presented in Table 3.1/1.

TABLE 3.1/1 Sugar Repeatability Determination: Results of 18 Analyses of two protein free filtrates

	Sample A (Dung)	Sample B (Urine)
Mean (mg/100 ml)	58.4	103.6
± SE	2.58	1.73
Coefficient of Variance	18.7 %	7.08 %

Dygert et al (1965) reported relative standard errors, using the same method, much larger than 3 % for samples with low reducing sugar concentrations. In view of the large treatment differences noted in 'snap' urine samples the method was accepted as being sufficiently repeatable to permit determination of treatment differences.

(c) Changes in the Sugar concentration of protein free filtrates stored for zero and 56 days at -12°C.

To ascertain whether deproteinized samples could be safely stored frozen without incurring sugar loss, protein free filtrates were prepared from ten different urines. A portion of each was analysed immediately and the remainder was stored at -12°C for 56 days. A second determination on each was then carried out. An F test of heterogeneity of the two regressions gave F = 0.01 for 1:6 degrees of freedom (N.S.) (App. 3.1/3).

Mean values for the two sets of determinations were:

A (Fresh) 67.04 ± 7.73 mg/100 ml.

B (56 days at -12°C) 66.89 ± 8.74 mg/100 ml.

It was concluded that no change in sugar concentration occurred in the deproteinized samples stored under the conditions described.

(d) Changes in the Sugar concentration of urine stored intact for four different periods of time.

The extent of losses in sugar concentration prior to deproteinization were investigated. Based on the collection procedure already outlined, samples were collected and deproteinized (A) at urination, (B) after 24 hours at room temperature (68°F), (C) after 5 days (120 hours) storage at -12°C, and (D) after 10 days (240 hours) storage at -12°C. This procedure was repeated on two successive days with the same four pigs. Results of the two series determined separately are presented in the appendix (App. 3.1/4). (The markedly lower set of values for all time intervals from the second series should not be interpreted as illustrating daily variability as the two regressions differed significantly ($P < 0.01$). A summary of results are presented in Table 3.1/2.

TABLE 3.1/2 Means and their standard errors, of sugar concentrations of urine samples stored for four periods of time

Hours	0	24	120	240
Mean (mg/100 ml)	46.4	43.9	51.2	53.0
± SE	10.8	7.9	10.4	13.3

It was concluded that no significant change in urinary sugar concentration occurred under the storage conditions employed.

(e) The effect of the Storage of Faeces at -12°C for five days, on their total reducing sugar content.

Seven faecal samples were collected and a faecal paste of each was prepared. From these, a representative sample was taken, homogenized and a protein free filtrate prepared. The remainder of the faecal pastes were stored at -12°C. After 5 days storage, these samples were thawed, and a portion deproteinized. The two sets of protein free filtrates were then developed together and analysed for total reducing sugars. Analysis of

variance (App. 3.1/5), revealed a highly significant ($P < 0.01$) difference in the mean sugar contents of the two sets of samples. The mean ($\pm$ SE) for the samples deproteinized fresh was 31.66 $\pm$ 6.79 mg/100 ml. and for the samples stored for 5 days 69.61 $\pm$ 6.79 mg/100 ml. This difference could not be accounted for by differences in mascerate dry matters.

3.1/4 SAMPLE ANALYSIS FOR TOTAL REDUCING SUGARS

The significantly higher results for sugar concentrations in faeces stored for five days frozen, meant that if filtrates were prepared from the five-day mixed dung samples, sugar values considerably higher than the true five-day mean would be obtained. To overcome this, daily deproteinizations of both dung andurine were continued in both collection B and C, and the proportionate amounts of each days protein free filtrate were mixed to provide the composite sample.

Composite urine samples were obtained by combining urine filtrates prepared daily in proportion to the total amount of urine voided each day. In preparing dung composite protein free filtrates both the dung dry matter output, and the dry matter of the mascerate prepared daily for each sample were taken into account, using the formula:

$$\frac{\text{Total D.M. (Dung) Voided}}{\text{D.M. \% of Mascerate Produced}}$$

(a) Daily Variability in Sugar Output

For the first collection only, (Collection A) Total reducing sugar determinations were performed on each total daily sample of dung and urine, to assess daily variability. Unfortunately the determination procedures were not well standardized and most of the regression coefficients for the daily determinations were clearly different. However, two separate determinations (Days 4 and 5) which included 8 pigs (Nos 2-9) were found to be similar, and so data from these pigs for the two days mentioned, has been presented in Table 3.1/3.

TABLE 3.1/3 Variability in Total Reducing Sugar Output (milligrams) on two days of a five-day collection period

Pig No.	Treatment	Dung		Urine		Total	
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
5	1	61	51	81	386	142	437
7	1	114	150	27	130	141	280
2	2	70	82	519	412	589	494
3	2	79	51	194	382	273	433
6	2	71	100	260	679	750	779
8	3	54	86	1209	1096	1263	1182
9	3	92	100	1272	1086	1364	1186
4	4	15	126	1742	1334	1757	1883
	Mean (Mg)	70	93	633	688	785	834
	± SE	10	12	230	153	217	191

Although the results of two days analyses are very limited both with respect to number of days and number of pigs, the individual figures show little variability in most cases, for both Dung and Urine.

(b) Relative Proportions of Total Reducing Sugar Loss in the Dung and Urine

The daily variability studies showed that only a small proportion of the total loss was associated with the faeces. In addition there was less treatment influence on the dung sugar level compared with that of the urine. As a result of these studies, the figures obtained from Collection C (Appendix 3.1/6) were analysed so that the relative proportions of the total loss could be ascertained. The data showed that, of the total sugar voided, 94.4 ± 0.9 % was found in the urine and only 5.6 ± 0.9 % was lost in the faeces of 'normal' consistency.

(c) The Effects of Increasing Lactose Intake on the Amount of Urinary Sugar Loss

Analysis of variance (App. 3.1/7) of the results from four treatment groups at two liveweights (60.0 ± 0.95 lb and 110.82 ± 0.79 lb) revealed highly significant treatment and collection effects upon urinary sugar output. Mean values for the four treatments and two collections are presented in Table 3.1/4. Raw data, and an F test of heterogeneity of regressions giving F = 0.01 for 1:6 degrees freedom (NS) are presented in App. 3.1/7.

TABLE 3.1/4 Mean ± SE of total urinary sugar loss (gms) for two 5-day collection periods (at 60 and 110 lb liveweight) and for four treatment groups.

	COLLECTIONS		TREATMENTS			
	A	C/	1	2	3	4/
1 % **						
Mean	4.156	7.921	2.045	4.854	7.361	9.893
± SE	± 0.349	± 0.365	± 0.655	± 0.655	± 0.655	± 0.699
5 % *						

** Means not overscored by the same line are significantly ($P < 0.01$) different.

* Means not underscored by the same line are significantly ($P < 0.05$) different.

/ Data contains one missing plot value.

(d) Total Sugar Loss in Relation to Lactose Intake - Sugar 'Balance'

To assess the proportion of ingested lactose lost in the faeces and urine a rough balance between lactose intake and total reducing sugar outgo was calculated. This could only yield maximum 'percentage loss' values as no account was taken of any contribution from alternative carbohydrates

in the diet, such as starch. However, considering starches rapid digestion to produce glucose which is readily absorbed and utilized, it is unlikely that any substantial contribution arose from this source. Only a low reducing activity was recorded in the urine from pigs receiving no lactose. In view of results obtained by chromatography (to be presented later) most of this activity was probably caused by non-sugar reducing substances such as uric acid and nucleoprotein normally present in the urine. These would not alter the differences between treatments but might tend to increase all values slightly. Data from Collection C involving sugar intake and excretion are presented in the appendix, (App. 3.1/8). The results show that less than 1 % of the total lactose intake was lost as reducing sugar in the urine and faeces.

(e) The effect of Diarrhoea on Faecal Sugar Loss

Unlike the pigs in the main investigation, very little diarrhoea was recorded in the collection study. This was unfortunate as it was hoped that information concerning the extent of sugar loss under diarrhoeal conditions could be collected. Only one pig scoured while on collection, and this occurred on one day only. Analysis of a sample of the faeces, revealed a very high level of total reducing sugar. Calculation showed that almost 5 grams (4.86 grams) of sugar had been lost via the faeces on this one day. This, in comparison with a urinary loss of 0.75 grams, represented 86.6 % (cf. 3.1/4 (b)) of the total sugar loss occurring from the body on that day.

3.1/5 DISCUSSION AND CONCLUSIONS

The significant increase in faecal sugar resulting from storage, frozen at -12°C for 5 days, was unexpected. As the Nelson - Somogyi colour reaction depends on the oxidation, in alkaline solution, of sugars containing a reducing group (and consequently on the reduction of

the cupric ion to the cuprous form) one explanation is that non-reducing carbohydrates such as starch underwent bacterial digestion to yield the reducing sugar glucose.

No definite conclusions on the degree of variability in daily sugar excretion are possible. Such information would be of importance in deciding the minimum number of days required to obtain an accurate assessment of sugar losses, or carbohydrate digestibility. If the results presented for two days, were typical of the day to day variation, the five-day collection period used would have been adequate.

Although analyses indicated that approximately 95 % of the sugar loss occurred in the urine, and that the urinary level was significantly influenced by the level of lactose in the treatment ration, the sugar 'balance' clearly showed that total reducing sugar losses occurring in the faeces and urine were of minor nutritional importance to the animal. Less than 1 % of the total lactose ingested was lost (as total reducing sugar) in the faeces and urine.

More work is required to establish the degree of sugar loss occurring when diarrhoeal conditions predominate. Although absolute levels were comparatively low in the faeces analysed, this avenue of loss could have been important to some of the animals in the main investigation whose food consumption was little more than would be required for maintenance and who were scouring repeatedly.

3.2 APPARENT DIGESTIBILITY AND RETENTION OF NITROGEN

3.2/1 REVIEW OF LITERATURE

Dried skim milk has been shown to be superior to soybean oil meal (SBOM) as a primary source of protein in baby pig diets (Lewis, Catron, Liu, Speer and Ashton, 1955; Hudman, Speer, Ashton and Catron, 1955; Hays, Speer, Hartman and Catron 1959; Sewell 1963; Combs, Osegueda,

Wallace and Ammerman, 1963; Pekas, Hays and Thompson 1964).

The better growth rates made by baby pigs on dried skim milk is due at least in part to their greater ability early in life, to digest the milk protein (Lloyd, Crampton and MacKay, 1957). With increasing age, an increased ability to digest alternative sources of protein, results in similar liveweight gains by animals on most types of protein by about 7 weeks of age (Lloyd and Crampton 1958; Hays et al 1959; Combs et al, 1963; Pekas et al, 1964).

Although small improvements in liveweight gain and food conversion efficiency have been reported by supplementing a diet containing SBOM with methionine to correct an amino acid deficiency (Hays et al, 1959; Sewell, 1963) no changes in the nitrogen digestibility were reported. Furthermore, the performance of pigs on diets containing skim milk protein was still significantly superior to that of the pigs on the methionine supplemented diets. Hays et al, (1959) and Sewell, (1963), implied that methionine was the main amino acid contributing to any amino acid imbalance in the SBOM diets and therefore concluded that the imbalance in general was not to any major degree, responsible for the poorer digestibility of the SBOM protein.

Recently Pekas et al (1964) have shown that, whereas dried skim milk protein is largely degraded by intestinal and gastric secretions SBOM protein is considerably dependent on pancreatic secretions for its breakdown. This suggested that slower development of pancreatic secretions after birth could account for the poorer digestibility of the SBOM protein seen over the early weeks of life.

Although both the amino acid imbalance, and the dependence on pancreatic secretion probably play a small part in the poorer digestibility of the soybean protein, it is clear that lactose has a major influence on growth performance and protein degradation in the baby pig. The results obtained with dried skim milk suggested and Hudman et al (1955) showed,

that the presence of lactose in a diet containing casein as the primary protein source resulted in a marked improvement in the rate of gain of these pigs, compared with gains made by pigs on diets in which lactose was excluded.

Lactose action is not limited solely to milk proteins, significant improvements in growth being reported when lactose was added to diets containing soybean protein (Lewis et al 1955; Sewell and West 1965). As meatmeal and pollard (3:2) contributed the protein used in the present investigations, determinations of apparent nitrogen digestibility and nitrogen retention were made, to assess the effects of different levels of lactose, on the utilization of protein in this form.

3.2/2 METHOD OF ANALYSIS

Duplicate wet faecal samples from collections B and C (85 lb and 110 lb liveweight) were obtained as outlined earlier (Section 2.3, Part II), Feed, urine and faeces samples were analysed for nitrogen content by the Kjeldahl method (A.O.A.C.)(1965) but mercuric sulphate (Hiller, Plazin and Van Slyke, 1948) was used as a catalyst, and the ammonia was collected in boric acid (Meeker and Wagner, 1933).

Samples were transferred to the Kjeldahl digestion flasks by three procedures according to their consistency. Meal samples were accurately weighed (2gm for mixed meals, 0.5 gms for meatmeal) on filter paper and placed in the digestion flasks. Faecal homogenates were weighed (20 gms) on a tared watch glass and washed into flasks with distilled water. Urine (5 ml.) was transferred to the flasks using a 5 ml. bulb pipette.

3.2/3 RESULTS

Statistically significant treatment differences in daily intake of digestible energy ($P < 0.10$) and Nitrogen ($P < 0.05$) were detected at 85 lb liveweight but not at 110 lb liveweight (Table 3.2/1) (App. 3.2/1; 3.2/3). Although the differences arose as a consequence of the treatments imposed,

they were used as concomitant variables in covariance analyses (Cochran, 1957), with the limitations outlined by Smith, (1957) in mind. This procedure enabled the calculation of the statistical significance of the regressions between D.E. or Nitrogen intake, and Nitrogen digestibility or Nitrogen retention. (App. 3.2/2; 3.2/4)

In all covariance analyses the pooled error regressions were non-significant. In some of the analyses the relationship between the variables differed widely in magnitude and sign between treatments, and in these cases the pooled error regression, evenif it had been significant would have been irrelevant to the problem.

At 85 lb liveweight, data from one pig on treatment 3 could not be obtained (Section 4.3/1 Part I) and data from one pig on treatment 4 was discarded due to the pigs poor performance and very high feed refusal. At 110 lb liveweight only 2 pigs remained on treatment 4, and during the collection, one of these refused approximately 20 % of the feed offered. For this reason, all data concerning treatment 4 at the heavier weight was omitted from statistical analysis.

Analysis of apparent Nitrogen Digestibility percentage (App.3.2/5) involving three treatments (1,2 and 3) at two liveweights (85 lb and 110 lb) produced a significant ($P < 0.10$) treatment x Liveweight interraction (Table 3.2/2). At 85 lb liveweight, treatment 1 and 3 pigs, compared with treatment 2 pigs, showed a significantly ($P < 0.05$; $P < 0.10$, respectively) poorer ability to digest dietary protein. However, due to a marked improvement in protein digestibility noted for treatment 1 pigs, no differences were detected between treatments 1 and 2 at 110 lb liveweight. Treatment 3 improved only slightly with the increase in weight and was significantly ($P < 0.10$) poorer than treatment 1 at 110 lb liveweight.

TABLE 3.2/1 Mean daily D.E. and Nitrogen Intakes, Apparent Nitrogen Digestibility, and Nitrogen Retention.

85 lb LIVEWEIGHT	TREATMENT MEANS 1	2	3	4	± SE[a]	Significance of Difference
No. of Pigs	3	3	3 ≠	3 ≠		
Daily DE Intake (Kcals)	4,860	4,900	5,000	4,780	37 (45)	3>1,4 +
Daily Nitrogen Intake(gms)	38.77	39.27	41.44	40.14	0.31 (0.38)	3>1,2,4 *
						4>1 *
Nitrogen Digestibility(%)	69.88	76.42	71.52	[70.24]	1.29 (1.59)	2>1,3 +
Nitrogen Retention(NR)(%)	20.26	28.37	32.20	31.76	2.13 (2.60)	3,4>1 *
Daily NR (i) gms	7.83	11.14	13.33	12.76	4.0 (4.9)	2,3,4>1 *
(ii) gms/lb live-weight	0.092	0.131	0.157	0.150		
(iii) gms/kg live-weight	0.203	0.289	0.346	0.331		
110 lb LIVEWEIGHT						
No. of Pigs	3	3	3	2		
Daily DE Intake (Kcals)	5,880	5,900	5,880	[5,740]	58	NS
Daily Nitrogen Intake(gms)	46,90	47.44	48.84		0.47	NS
Nitrogen Digestibility(%)	77.02	76.06	73.07	[68.41]	1.29	1>3 +
Nitrogen Retention(NR)(%)	27.68	29.64	32.31		1.58	NS
Daily NR (i) gms	12.98	14.06	15.77		3.2	NS
(ii) gms/lb live-weight	0.118	0.128	0.143			
(iii) gms/kg live-weight	0.260	0.282	0.315			

* Means differ significantly at the 5 % ($P<0.05$) level of probability

\+ Means differ significantly at the 10 % ($P<0.10$) level of probability

NS ($P>0.05$)

≠ Data includes one missing plot value

a Values in brackets denote SE for means containing one missing plot

[ ] Means enclosed in square brackets not included in the analysis

TABLE 3.2/2 Summary of means and their standard errors for a treatment x age interraction in the apparent digestibility of crude protein nitrogen

	B x 1	B x 3	C x 3	C x 2	B x 2	C x 1
No. of Pigs	3	3 ≠	3	3	3	3
5 % *						
Mean	69.88	71.52	73.07	76.06	76.42	77.02
± SE	1.29	1.59	1.29	1.29	1.29	1.29
10 % +						

≠ Data contains one missing plot value

* Means not overscored by the same line differ significantly at the 5 % ($P < 0.05$) level of probability.

\+ Means not underscored by the same line differ significantly at the 10 % ($P < 0.10$) level of probability.

At 85 lb liveweight (App. 3.2/6) all pigs receiving lactose in their diets retained significantly ($P < 0.05$) more protein nitrogen (gms) than the control group receiving no lactose. However, at 110 lb liveweight (App. 3.2/7) no significant differences were detected between treatments 1, 2 and 3. All Nitrogen digestibility data, and Nitrogen retention data including a single value for treatment 4, are presented graphically in Fig. 3.2/1.

3.2/4 DISCUSSION AND CONCLUSIONS

The results show that, as the dietary lactose level was increased the digestibility of the protein (41 % Pollard / 59 % meatmeal protein) decreased. This was evident for all treatments at 110 lb liveweight and for treatments 2, 3 and 4 at 85 lbs liveweight but for unaccountable reasons, treatment 1 did not conform to this generalization at the lower weight.

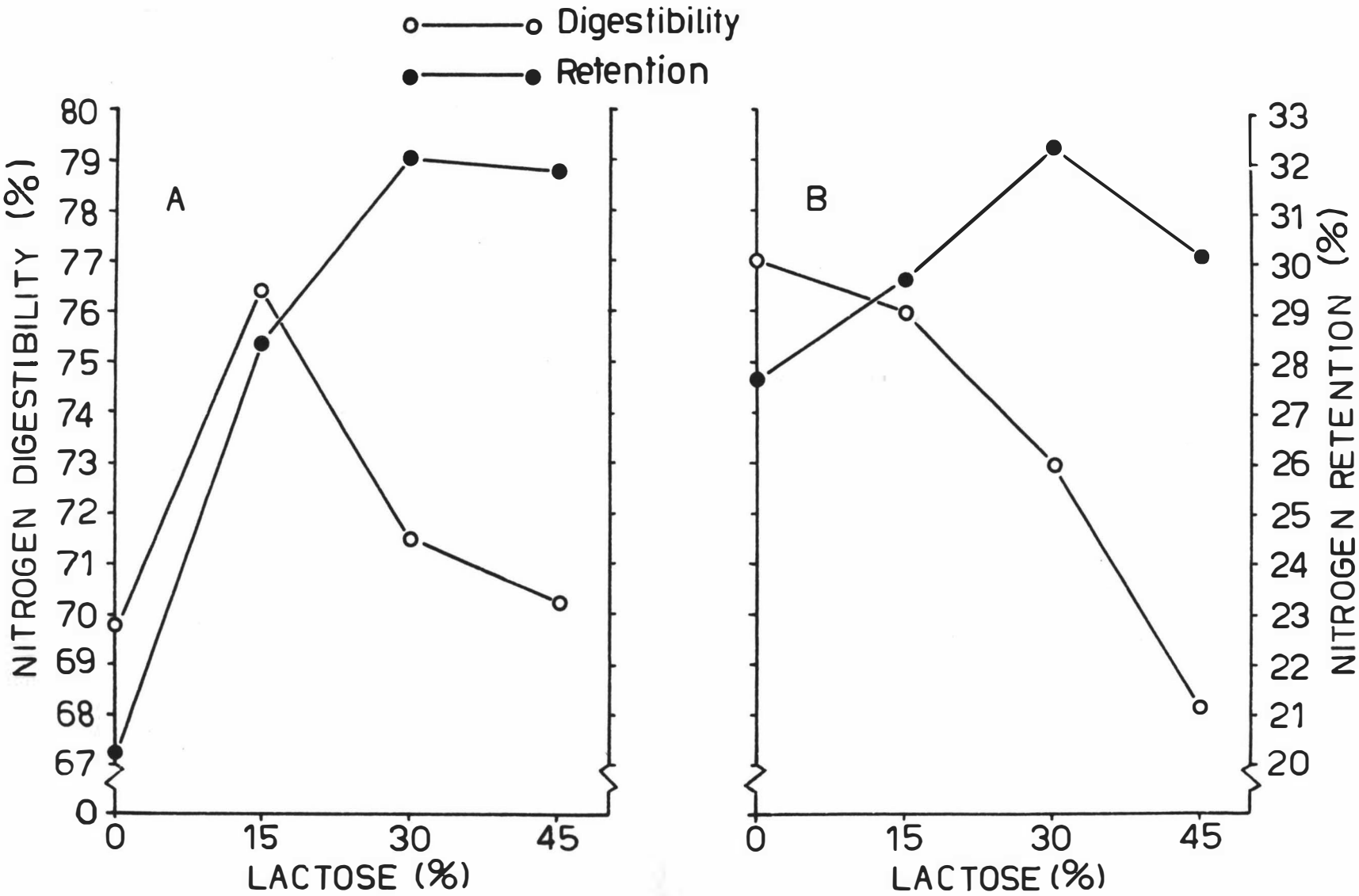


FIGURE 3.2/1

Nitrogen Digestibility (%) and Nitrogen Retention (%), (A), at 85 lb Liveweight, and (B) at 110 lb Liveweight, for Diets Containing 0, 15, 30, and 45 % lactose

In view of the decreasing digestibility as lactose levels increased, the increase in Nitrogen retention (per cent and absolute) was unexpected. Clearly the very low retention evident at 85 lb liveweight for treatment 1, was in part a reflection of the depressed protein digestibility, but the elevated retention seen for both treatments 3 and 4 occurred despite decreased digestibility. Although the significantly greater intake of nitrogen detected in treatment 3 (Table 3.2/1) could be responsible for greater absolute retention, it is unlikely that this would influence percent retention. Furthermore, no differences in calorie or protein intake were detected between treatments 1, 2 and 3, at 110 lb liveweight but increased nitrogen retention was still evident, but non significant.

Although more work is required to confirm these findings, the results suggest that the form in which energy is provided in the diet ('Calorie quality') may be an important determinant in the utilization of the other components in the diet. In the present investigation dietary carbohydrate was provided either by starch (Treatment 1) or by various proportions of starch and lactose. As starch is more rapidly digested than lactose (Table 3.3/2 Part II; Cunningham, Friend and Nicholson, 1963) increasing levels of dietary lactose would result in greater amounts of energy passing into the lower gut. Here this energy surplus could stimulate microbial growth, thereby increasing faecal endogenous nitrogen and so produce an apparent decrease in protein digestibility. Furthermore, lactose, by virtue of its slow degradation, would provide a more steady supply of energy to the bloodstream and hence the tissues, this being a factor of possible importance in maintaining protein anabolism. The overall effect would be a decrease in apparent nitrogen digestibility, and an increase in nitrogen retention, as seen in the results obtained.

The possibility that the mean apparent nitrogen digestibility value obtained for treatment 1 pigs at 85 lb liveweight was not a valid result, must be stressed, although there appeared to be no reason to reject it.

Further work along similar lines is clearly necessary before it is possible to draw any definite conclusions on the influence of lactose on (meatmeal/pollard) protein digestibility and retention.

3.3 APPARENT DIGESTIBLE ENERGY DETERMINATIONS

3.3/1 REVIEW OF LITERATURE

A knowledge of the available energy of dietary components is important in formulating a balanced diet. In recent years, the inadequacy of TDN values to provide this information has resulted in considerable research to determine by direct methods, the DE and ME values of feeds. From work with chicks (Hill, Anderson, Renner and Carew Jr., 1960; Potter and Matterson, 1960) and pigs (Diggs, Becker, Terrill and Jensen, 1959; Diggs, Becker, Jensen and Norton, 1965) a number of ME and DE values have been assembled for cereals and other foodstuffs including dried whey and lactose. Values were calculated in all cases by feeding the test substance as a percentage of a control diet for which ME or DE values had previously been determined. All results were therefore affected to some degree by the so-called associative effects evident in work by Skipitaris, Warner and Loosli (1957) and Pond, Lowrey and Maner (1962). This problem was largely overcome by Robinson, Prescott and Lewis, (1965) by feeding pigs (120 - 200 lb liveweight) the test cereal as 97.5 % of the total daily ration, the remaining 2.5% comprising essential vitamins and minerals. However this can only be done when the test feed is nutritionally adequate in all major dietary components.

All treatment diets used in the present study contained 50 % of a common meatmeal/pollard mixture. It was therefore possible to determine what effect lactose substitution for wheat starch in the remaining 50 %, had on the energy digestibility of the four treatment diets.

3.3/2 METHOD OF ANALYSIS

The dried composite faecal samples from the three collection periods were ground through a 1 millimeter mesh in a rotary mill. Between 1 and 2 gram duplicate samples of the dung and the feeds were pelleted, dried at 100°C for 16 hours, and weighed. The samples were then combusted in a random order, in an Adiabatic Bomb Calorimeter, a 1 % error of estimate being desired, but errors of up to 2 % were accepted.

3.3/3 RESULTS AND DISCUSSION

It was hoped that substitution of wheat starch for lactose on an equal weight basis might also prove to be an isocaloric substitution. The gross energy values presented in Table 3.3/1 illustrate the degree to which the four treatment diets differed in their caloric value.

TABLE 3.3/1 Gross and apparent Digestible Energy Values of the four treatment Diets (K cal/kg)

	TREATMENTS			
	1	2	3	4
	GROSS ENERGY			
Replicate (a)	4381	4325	4275	4190
Replicate (b)	4354	4311	4246	4151
Mean	4368	4318	4260	4170
	DIGESTIBLE ENERGY			
No. of Pigs	3	3	3 ♂	3 ♂
Liveweight 60 lb	3600 ± 74	3471 ± 60	3465 ± 100	3364 ± 77
85 lb	3616 ± 11	3625 ± 11	3495 ± 18	3444 ± 18
110 lb	3614 ± 29	3602 ± 31	3514 ± 14	3378 ± 4
Mean of 3 collections	3611 ± 23	3566 ± 23	3491 ± 30	3395 ± 41
Significance at 0.1 % (a)	—	—	—	—
1.0 %	—	—	—	—
5.0 %	—	—	—	—

≠ Data contained one missing plot value.

(a) Means not underscored by the same line differ significantly.

Analysis of the results obtained for apparent Digestible Energy (App. 3.3/1) showed that large treatment differences existed (Table 3.3/1). This was unfortunate in that direct comparison between treatments for many other digestibility analyses would have to be made with the caloric differences in mind. The similar downward trend in Gross Energy (G.E.) values suggested that the apparent Digestible Energy values were largely the result of G.E. differences. Consequently, the coefficients of energy digestibility (per cent) were analysed (App. 3.3/2) thereby taking into account differences in Gross Energy intake.

A summary of results are presented in Table 3.3/2

TABLE 3.3/2 Block, Treatment and Collection Means of the Coefficients of energy digestibility (%) for the four treatment diets

Block	Mean ± SE %	Signif. of Diffce	Treatment	Mean ± SE %	Signif. of Diffce.	Collection	Mean ± SE %	Signif. of Diffce.
H	82.23 ± 0.25		1	82.67 ± 0.29		A	81.12 ± 0.38 ≠	
I	83.11 ± 0.26 ≠	I>J **	2	82.83 ± 0.29		B	82.78 ± 0.40 ≠	B > A **
J	80.93 ± 0.29	H>J *	3	81.49 ± 0.31 ≠	1,2>3,4 *	C	82.37 ± 0.38 ≠	C > A *
			4	81.36 ± 0.36 ∅				

** Means differ significantly at the 1 % (P < 0.01) level of Probability

* Means differ significantly at the 5 % (P < 0.05) " "

≠ Mean includes one missing plot value

≠ " " two " " "

∅ " " three " " "

Table 3.3/2 reveals that, although differences between treatments 1 & 2 and 3 & 4, were small nevertheless they were significant at the 5 % level of probability. Although this suggests that the highly significant results

presented in Table 3.3/1 were due largely to G.E. differences, it shows that true differences in the apparent digestibility of the lactose and wheat starch existed. Because treatments 3 & 4 had the highest percentages of lactose and the lowest digestibility coefficients, the results clearly show that lactose is less completely digested than wheat starch. Cunningham, Friend and Nicholson (1963) arrived at the same conclusion using corn starch as a comparison.

3.4 APPARENT DIGESTIBILITY OF ETHER EXTRACTS

3.4/1 REVIEW OF LITERATURE

A recent report (Kern Jr, Struthers Jr and Attwood 1963) showed that high levels of faecal fat occurred in a human suffering from lactose intolerance. This, together with personal observations made on the appearance of the faeces voided by pigs unable to utilize lactose efficiently, suggested that high dietary lactose levels might affect the absorption of the ether extract fraction in diets fed to pigs.

3.4/2 METHOD OF ANALYSIS

Two, 2 gram sub-samples from the ground, dried faeces samples prepared for the energy determinations, were extracted with anhydrous ether (B.P. 34 - 35°C) for 8 hours in a Soxhlet apparatus. After extraction, the ether was distilled from the flasks and these were then placed in an oven at 100°C for one hour to drive off the last traces of the solvent. Flasks were then cooled in a desiccator, weighed, washed out thoroughly with ether, dried in the oven and again cooled in the desiccator. A second weighing provided a weight difference representing the ether extract fraction which was then expressed as a percentage.

3.4/3 RESULTS

Treatment means of apparent ether extract digestibility (a. D.E.E.) and the energy characteristics of the diets are presented in Table 3.4/1.

TABLE 3.4/1 Gross Energy, apparent Digestible Energy, Percent ether extract, and apparent digestibility of the ether extract, of four treatment diets.

	Tr. 1	Tr. 2	Tr. 3	Tr. 4
Gross Energy KCAL/KG	4,368	4,318	4,260	4,170
D.E. KCAL/KG	3,611 ± 23	3,566 ± 23	3,491 ± 30	3,395 ± 41
Ether Extract %	3.70	3.68	3.65	3.63
a. D.E.E.	71.39 ± 2.10	68.98 ± 2.10	64.12 ± 2.23	66.12 ± 2.23

Analysis of variance (App. 3.4/1) showed that the incorporation of lactose into the pigs' diets by the substitution of wheat starch, had no significant effect on the apparent digestibility of the ether extract fraction of the diets. However, there was a highly significant litter x age (liveweight) interraction. Results of the interraction are summarised in Table 3.4/2.

TABLE 3.4/2 Means and Standard Errors for a litter x Age Interraction in apparent ether extract digestibility.

	A x J	A x H	C x J	C x H	A x I	B x H	B x J	B x I	C x I
No. of Pigs	4	4	4	4	4	4	4	4	4
1 % **									
Mean	43.18	63.20	66.88	70.02	71.10	71.25	72.02	74.72	76.50
± SE	2.38	2.38	2.75	2.38	2.38	2.38	2.38	2.75	2.38
5 % *									

Collection A .. 60.00 ± 0.95 lb l/wt. Block H .. Large White x (Berkshire x Large White)

Collection B .. 85.15 ± 0.52 lb l/wt. Block I .. Landrace x Large White

Collection C .. 110.82 ± 0.79 lb l/wt. Block J .. Landrace x Large White

** Means not overscored by the same line are significantly different at the 1 % level of probability.

* Means not underscored by the same line are significantly different at the 5 % level of probability.

/ Data contains one missing plot value.

The results showed pigs on Block J at 60 lb liveweight, to have a much poorer ($P < 0.01$) ability to utilize the ether extract fraction in the diet. A small, but significant ($P < 0.05$) age effect was apparent in Block H between values obtained at 60 and 85 lbs liveweight, but not between values obtained at 60 and 110 lb liveweight. Litter effects were apparent. At 60 lb, litter I was more capable of digesting the ether extract fraction of the diet, than litters H ($P < 0.05$) and J ($P < 0.01$). No significant differences were apparent at 85 lbs, but litter I gave significantly ($P < 0.05$) higher a.D.E.E. values than litter J at 110 lb liveweight.

3.4/4 DISCUSSION AND CONCLUSION

The influence of age on ether extract digestibility has been reported by Lloyd, Crampton and MacKay (1957) from determinations made with baby pigs at 3 and 7 weeks of age. Although differences were not significant, due to a high variability reported within age groups, results presented showed appreciably better utilization of dietary ether extract at 7 weeks of age. Results obtained in the present work would seem to suggest that development of digestive ability may continue for as long as 10 weeks (60 lbs).

The treatment mean a.D.E.E. values obtained with diets containing approximately 4 % ether extract, are comparable with published figures (Clawson, Blumer, Smart Jr and Barrick 1962) of 47, 73 and 75 % for a ration (Gross Energy 4,008 Kcal/Kg) containing three levels (0, 5 and 10 %) of fat supplementation respectively. More recently Bayley and Lewis (1965) have reported values for apparent digestible fat ranging from 60 - 76 % for three sources of fat comprising 5 % of the total diet. It was concluded that the results did not support the suggestion that lactose inclusion

into diets for growing pigs affects their ability to digest the ether extract fraction of those diets.

3.5 LACTIC ACID ANALYSIS

3.5/1 REVIEW OF LITERATURE

In humans diarrhoea resulting from lactose intolerance is invariably accompanied by a marked increase in faecal lactic acid (Weijers, Van de Kamer, Mossel and Dicke 1960, Kern, Struthers and Attwood 1963, Struthers, Singleton and Kern 1965, Kern and Struthers 1966, Torres-Pinedo, Lavastida, Rivera, Rodriguez and Ortiz 1966).

Although there have been several reports of lactose induced diarrhoea in pigs, no reports of tests for lactic acid in the faeces are available.

Friend, Cunningham and Nicholson (1963) studied the levels of both lactic acid and the volatile fatty acids (v.f.a.^{s}) in sections of the alimentary tract of the pig, but unfortunately, only levels of the v.f.a.^{s} were studied in earlier work (Friend, Cunningham and Nicholson 1962) with pig faeces. Results of work on the distribution of lactic acid over the length of the gut (Friend et al 1963, Alexander and Davies 1963) showed maximum lactic acid absorption to occur in the small intestine, very little change in lactic acid concentration being apparent distal to the caecum. Lactic acid in the faeces, is therefore a reflection of either impaired intestinal absorption, resulting from lactose action in some way, or of an increased production of lactic acid in the lower gut areas from which absorption is relatively poor. It is likely that both factors are involved. Although no reports are available on the effects of lactose induced diarrhoea on the intestinal absorption of lactic acid, work reported earlier (Part II, Section 3,1/1) in which high faecal sugar levels were attributed to impaired sugar absorption, suggest that lactate malabsorption could also occur. The second possibility, increased production of lactic acid, is

supported by work by Wilbur (1959), and Wilbur, Catron, Quinn, Speer and Hays (1960) who reported an increase in lactobacilli resulting from a lactose diet.

3.5/2 METHOD OF ANALYSIS

Lactic acid was determined by the method of Ryan (1958). Several preliminary determinations with standard lactic acid solutions, to establish a regression coefficient to the linear component, showed the method to be working satisfactorily. A linear regression equation $Y = 8.302\ X \pm 0.012$ was calculated. It was found advisable to store the semicarbazide and phosphate buffer solutions separately and to mix them immediately prior to each set of determinations, as the semicarbazide became inactive if stored at pH 7.

When sample, lactic acid concentrations were determined against a water blank (2 ml distilled water in place of the 2 ml deproteinized sample) abnormally high readings were obtained, a dilution of 1: 1000 (v/v) with distilled water being necessary before the O.D. values were low enough to enable a reading to be made. The samples were then determined against a reagent blank (2 ml sample used but the 0.4 ml ceric sulphate solution in 6 N. H_2SO_4, was replaced by 0.4 ml of 6 N. H_2SO_4 alone.)

Samples which, when read against the water blank, yielded very high values, when read against the reagent blank, gave zero, negative or very small positive values. The basis of the reaction involves the formation of a coloured (under ultra violet light) semicarbazone from a reaction between semicarbazide and the acetaldehyde formed by the oxidation of lactic acid by acidified ceric sulphate. As ceric sulphate was absent from the reagent blank, the high optical density (O.D.) values produced in these samples must have arisen from an alternative source of aldehyde or ketone derivatives. It was possible to rule out the method of deproteinization as the cause by preparing fresh protein free filtrates by the usual method.

These produced only slightly positive O.D. values when measured against a water blank. It then appeared obvious that the preservative was responsible. Samples were tested with and without toluene. Very high values were produced by the samples with the toluene. It was concluded that toluene readily underwent oxidation to produce both benzaldehyde and benzoic acid, both of which were capable of reacting with the semi-carbazide.

An alternative method of lactic acid determination (Barker and Summerson 1941) was considered, but because this also depended on an acetaldehyde reaction, it appeared to suffer from the same disadvantages. Continued use of the reagent blank, was not satisfactory as this resulted in a considerable loss in the accuracy with which true lactic acid concentrations could be determined. Because at this stage of the work all faeces samples had had toluene added, estimation of lactic acid was abandoned.

3.5/3 DISCUSSION AND CONCLUSIONS

The very small values obtained when samples were determined against a reagent blank, indicated that it was unlikely that lactic acid was present in any appreciable quantities. However, the faecal filtrate from the single pig which scoured badly over one collection period was not included in these determinations and so the possibility that there was a greater concentration of lactic acid in this faecal sample cannot be ruled out. It is clear that analysis of fresh samples is to be preferred, but if storage is unavoidable, care should be taken in the choice of a preservative.

CHAPTER 4

CHROMATOGRAPHIC SUGAR ANALYSIS

4.1 FAECAL AND URINARY ANALYSIS

4.1/1 INTRODUCTION

The results of reducing sugar analyses reported earlier provided information on the total reducing sugar losses in faeces and urine. In order to determine the form in which sugar loss from the body occurred, deproteinized faecal or urinary samples were investigated by chromatography.

4.1/2 REVIEW OF LITERATURE

(a) Urinary Filtrate Analysis

Early studies on sugar metabolism involving the analysis of urine for total reducing sugars, used only very crude methods (i.e., yeast fermentation) for the identification of the individual sugar components (Watkins, 1928; Winter, 1931; Deuel Jr, 1936). Chromatographic separation of the reducing sugars in urine was first carried out by Williams (1954) and since that time a considerable amount of work with urine, for both metabolic and diagnostic purposes, has involved paper chromatographic separation of sugars (Haworth and McCredie, 1956; Haworth and McDonald, 1957; Umbarger, 1962; Gryboski, Thayer, Gabrielson and Spiro, 1963; Signoretti, Parisella and Tucciarone, 1964). The principle factor limiting its wider use in routine clinical analysis is that it is very time consuming. This can be largely overcome by adopting thin-layer procedures which yield results in a few hours, whereas paper chromatography usually involves at least a day.

(b) Faeces Filtrate Analysis

Chromatographic analysis of faecal sugars is still in the early stages of development. The method was first used in carbohydrate intolerance studies by Durand, Martino and Lamedica (1961) to identify by

its presence in the faeces, the sugar responsible for a patient's chronic diarrhoea. When an absolute lactase deficiency existed, only lactose was found in the stool chromatographic pattern, but if the enzyme deficiency was partial, the monosaccharides glucose and galactose were also reported found. Similar procedures have been used by Auricchio, Prader, Murset and Witt (1961) and Auricchio, Dahlqvist, Murset and Parker (1963) to investigate sucrose intolerance and by Lindquist and Meeuwisse (1962) investigating a case of monosaccharide malabsorption. Ford and Haworth (1963), and Baar and Bickel (1964) pointed out that the tendency to concentrate on patients exhibiting a disaccharide intolerance had resulted in little or no information on the normal faecal sugar picture in children. From a total of 71 stools examined by paper chromatography Ford and Haworth (1963) concluded that a variety of mono- and disaccharides are to be found in the faeces, their presence and quantity being largely dependent upon the diet.

4.1/3 METHOD OF ANALYSIS

(a) Introduction

Although very few publications are available on the separation of sugars by thin-layer chromatography, it was decided that the advantages in time saved, both in developing the plates and in the number of samples which could be handled at one time, justified its use. Glucose and galactose, by virtue of their structural similarity are difficult to separate by paper chromatography, and careful choice of solvents is necessary to ensure adequate separation. As several successful separations on paper had been reported (Opienska - Blauth, Madecka - Borkowska, and Borkowski, 1952; Roberts, 1957; Colombo, Corbetta, Pirotta, Ruffini, and Sartori, 1960) it was decided that a cellulose film would be most likely to produce adequate separation.

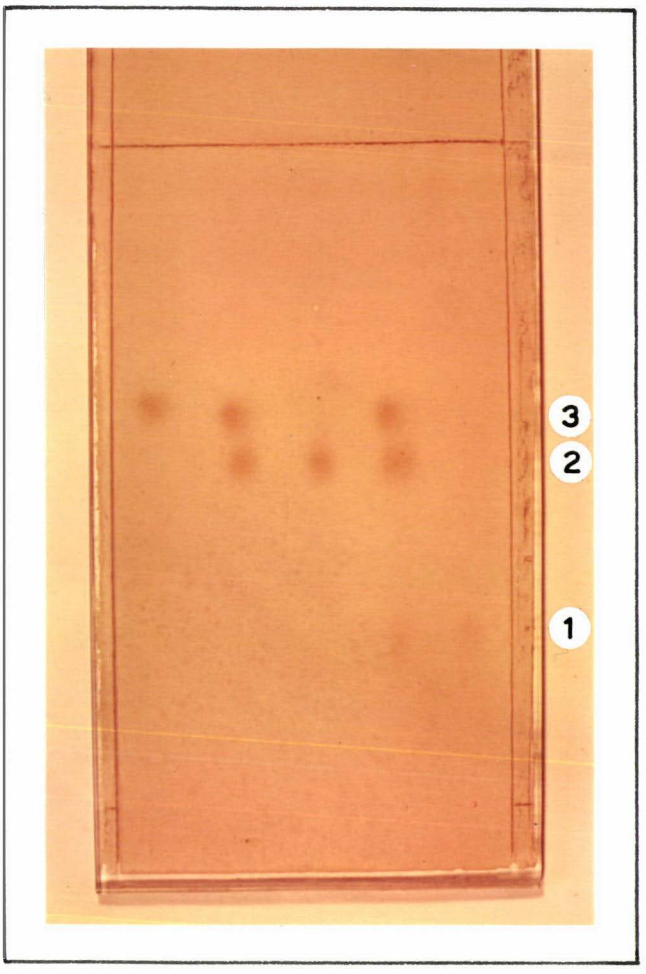


FIGURE 4.1/1

Thin-layer Chromatographic separation of Glucose, Galactose and Lactose

Development: Ascending, at 4°C for 6 hours - 2 runs
Solvent: Isopropanol-Ethyl Acetate-Water (7:1:2)
Spray: Aniline Phosphate
(1) Lactose (2) Glucose (3) Galactose

(b) Preparation of Plates

Plates (10 cm. x 20 cm.) were thoroughly washed in a strong detergent solution and well rinsed with boiling water. They were then washed several times with distilled water and finally wiped with a soft paper tissue soaked in methanol. A slurry prepared by blending 15 gms cellulose M.N. 300 (Machery Nagle & Co., no binder) with 90 ml. of deionized water - methanol solution (5:1 v/v) (Vomhof and Tucker 1965) was spread on the plates as a 250 μ layer and oven dried after evaporation of surplus fluid at room temperature.

(c) Preparation of Samples

Preliminary determinations showed that the sugar in the samples was at too low a concentration to allow detection without overloading the chromatoplates. Consequently, a 50 fold increase in concentration was achieved by freeze drying 10 ml. of each sample and adding 0.2 ml. of distilled water to the dried residue.

(d) Choice of Solvents

Little information was available on solvents for the separation of glucose and galactose on cellulose layers. From current literature several likely solvents were chosen, two were tested, and one resulted in excellent separation after the plates were developed twice (Fig. 4.1/1). This was a mixture of Isopropanol - Ethyl Acetate - Water (7:1:2) used by Adachi (1965) for the separation of glucose, galactose and lactose on layers of Keiselgel.

(e) Development Procedure

Samples were applied 2 cm. above the bottom of the plates using a 10 cmm. graduated micropipette. In every case the two outside samples were standard solutions containing 2.0 μg. of glucose, galactose and lactose. Volumes of faecal and urinary samples applied, were adjusted according to calculated sugar concentrations, so that approximately 2.5 μg.

and 5.0 μg. respectively of sample was applied in all cases. When large volumes were required, the samples were added in 0.2 μl. increments and the area dried, using a hair drier.

Four plates were developed at one time in each of 2 solvent saturated tanks lined with filter paper. The tanks were placed in a cool room kept at a constant 4°C to improve the resolution (Truter 1963), and were developed in an ascending manner for 6 hours (approximately 15 cms). The plates were then removed, and the solvent front was marked. After 15 minutes drying time, they were returned to their respective tanks, developed until the previous solvent front was reached, removed, and allowed to dry.

(f) Spraying Procedure

Preliminary work indicated that neither aniline phosphate or benzidine was satisfactory for the detection of both hexoses and lactose in the amounts present in this procedure. The cellulose layers were therefore divided in half, the upper area being sprayed with aniline phosphate (Bryson and Mitchell 1951) to detect hexoses, the lower area, with benzidine (Horrocks 1949) to detect lactose. The plates were then placed for 10 minutes in an electric oven maintained at 110°C.

4.1/4 RESULTS AND DISCUSSION

The data on total reducing sugar concentrations of faecal and urinary protein free filtrates was examined so that samples with sufficiently high sugar concentrations could be selected. A total of 4, 5, 3 and 3 faecal, and 6, 9, 10 and 15 urinary protein free filtrates were used from treatments 1, 2, 3 and 4 respectively.

Standard glucose and lactose solutions containing 0.2, 0.4, 0.8, 1.2, 1.8, 2.0 μg. sugar were developed under identical conditions to those used for the samples. The amounts of each sugar present in the sample spots were then assessed by a visual comparison with the spots obtained

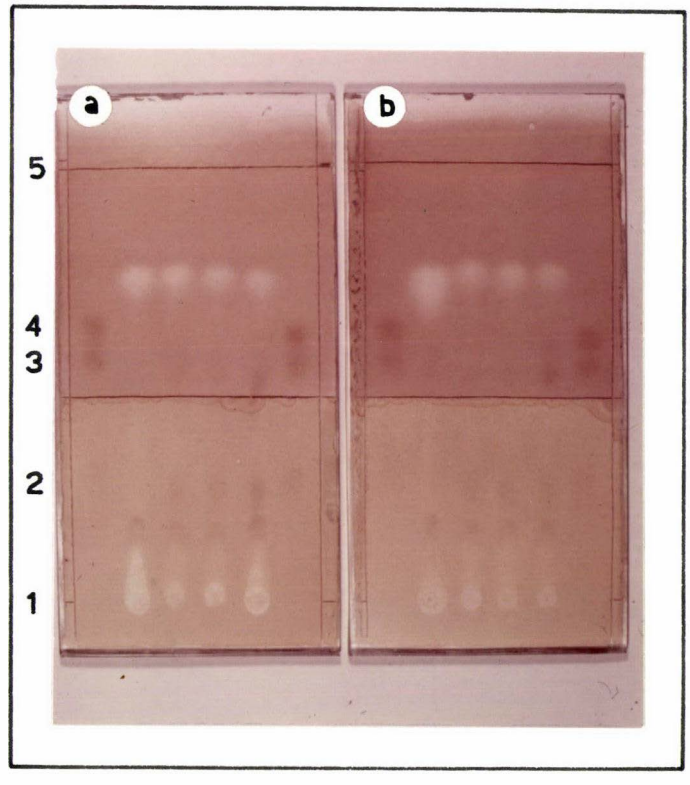
FIGURE 4.1/2

Thin-layer Chromatoplates of Urinary Filtrates

Four sample spots on each plate from Left to Right correspond to Treatments 1 to 4.

Plate (a) Samples collected at 60 lb Liveweight.
Plate (b) Samples collected at 110 lb Liveweight.
Development: Ascending, at 4°C for 6 hours - 2 runs
Solvent: Isopropanol-Ethyl Acetate-Water (7:1:2)
Spray: Upper half. Aniline Phosphate
Lower half. Benzidine

(1) Sample Origin (2) Lactose
(3) Glucose (4) Galactose
(5) Solvent Front



from the standard solutions. Quantities of sugar for each sample spot were comparable between samples as a result of equal volumes being applied to the plates.

(a) Urinary Filtrate Analysis

Results of the chromatographic analysis of urinary filtrate sugars are summarised in Table 4.1/1.

TABLE 4.1/1 Quantities of Glucose, Galactose and Lactose (µg.) present in urine samples, identified by thin-layer chromatography

		TREATMENTS			
		1	2	3	4
Glucose	Mean µg.	0.10	0.04	0.28	0.21
	Range µg.	0.0-0.4	0.0-0.4	0.0-1.2	0.0-1.8
Galactose	Mean µg.	-	0.70	0.42	1.07
	Range µg.	-	0.0-2.2	0.0-1.8	0.0-2.0
Lactose	Mean µg.	-	0.40	0.93	1.01
	Range µg.	-	0.0-1.8	0.0-2.5	0.0-2.5
Galactose + Lactose	Mean µg.	-	0.55	0.68	1.05

The data shows that very small amounts of glucose were lost by all treatment groups. Due to the wide variability in individual determinations, the results of galactose and lactose assessed independently show little treatment effect. However, the mean values of the two sugars illustrate an increase in amount with increasing levels of lactose as was reported earlier (Section 3.1 Part II) using total reducing sugar analysis.

Within treatment groups (Fig. 4.1/2) there appears to be a decrease in urinary lactose loss with increasing age. This suggests that mucosal hydrolysis increased either with age, or with prolonged feeding of lactose as has been suggested by Fischer and Sutton (1953), Girardet, Richterich and Antener (1964). Unfortunately it was not possible to obtain informat-

ion on the effects of lactose in the diet on the development of the intestinal mucosa. This has been reported to result in increased galactosidase activity (Fischer and Patton, 1955; Fischer, 1957 a) which would account for the increased mucosal hydrolysis.

(b) Faeces Filtrate Analysis

Results of the chromatographic analysis of faecal samples are presented in Table 4.1/2.

TABLE 4.1/2 Quantities (µg.) of Pentose from Faeces filtrates identified by thin-layer chromatography

	TREATMENTS			
	1	2	3	4
No. of Determinations	4	5	3	3
Pentose (µg.) Mean	1.30	0.64	1.33	0.80
Range	0.2-2.0	0.4-1.2	0.8-2.0	0.4-1.2

In no case did glucose or galactose quantities exceed 0.2 µg., the mean being approximately 0.1 µg. in every case. Lactose was not detected.

The data showed that little glucose and galactose loss, and no apparent lactose loss occurred in the faeces. A pentose was clearly the predominant sugar present. While performing preliminary chromatographic determinations using the samples prepared from faeces stored for zero and 5 days at -12°C (Section 3.1), it was noticed that greater pentose concentrations accompanied the samples prepared after 5 days storage frozen. It appears likely, therefore, that the increases noted in the total reducing sugars after storage were the result of increases in the concentration of this pentose.

Only one case of diarrhoea occurred, and analysis of samples taken indicated very high levels of total reducing sugars. When chromatographed

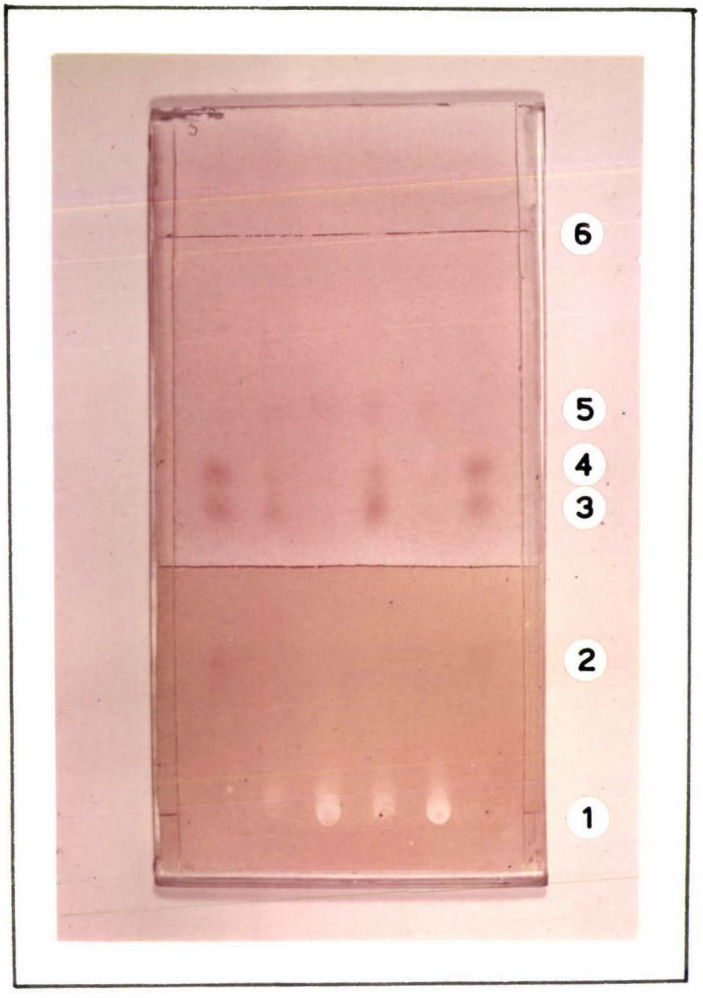


FIGURE 4.1/3

Thin-layer Chromatoplate of Filtrates Prepared from 'Normal' And Diarrhoeal Faeces

Development: Ascending, at 4°C for 6 hours - 2 runs
Solvent: Isopropanol-Ethyl Acetate-Water (7:1:2)
Spray: Upper half. Aniline Phosphate
Lower half. Benzidine

(1) Sample Origin (2) Lactose
(3) Glucose (4) Galactose
(5) Pentose (6) Solvent Front

Sugar Spots from Left to Right are: Sugar Standards; Diarrhoeal Faeces Filtrate; 'Normal' Faeces Filtrate; Diarrhoeal Faeces Filtrate; 'Normal' Faeces Filtrate; Sugar Standards.

with a corresponding sample taken from the same pig at a later collection, the plate (Fig. 4.1/3) revealed that, in addition to the pentose, very high concentrations of glucose and galactose, but not lactose, were lost from the faeces. As mucosal hydrolysis of lactose would result in absorption of the monosaccharides from the alimentary tract, it appears likely that the lactose was hydrolysed in an area of the gut in which glucose and galactose could not be absorbed. It is suggested that cleavage occurred in the caecum and (or) the colon (Section 4.3/5 Part I), where the resulting monosaccharides by osmotic (Lindquist and Meeuwisse 1962), or some other means, affected water reabsorption thereby causing the diarrhoea.

4.2 IDENTIFICATION OF THE FAECAL PENTOSE

The occurrence of a pentose sugar in the faeces of children was suggested by Ford and Haworth (1963) who wrote:-

> "In a number of stool samples unidentified bands were seen on the chromatography papers. Some of these were in the position in which pentoses are usually found, but a specific pentose was not identified with certainty. The nature of these substances warrants further investigation."

In addition, Baar and Bickel (1964) hydrolysed the oligosaccharides in children's faeces and reported the presence of a pentose in the hydrolyzate, but no attempt was made to identify it.

4.2/1 METHOD, RESULT AND DISCUSSION

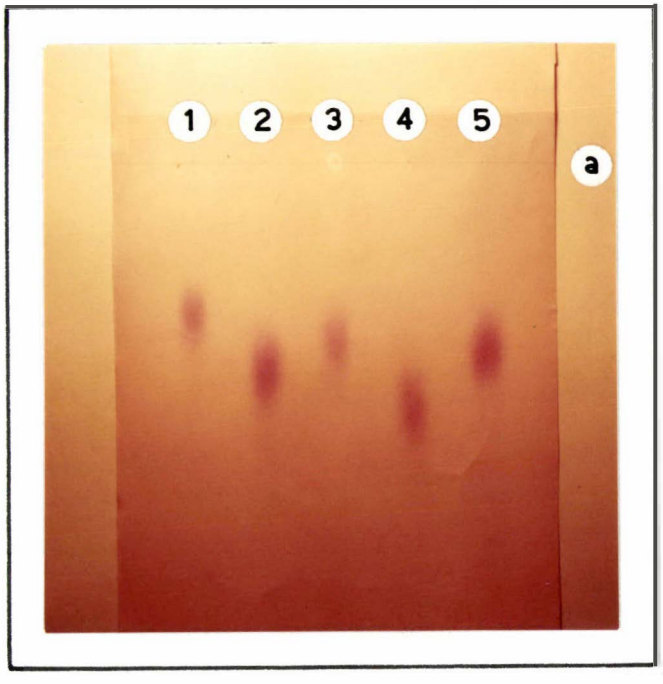
Faecal filtrates, and four standard pentose solutions (L. Arabinose, D. Lyxose, D. Ribose, L. Xylose) were applied to Whatman No.1 filter paper. The paper was developed in a descending manner for 24 hours using n Butanol - Glacial Acetic Acid - Water (4:1:1) (Grimmett and Richards 1964). After drying, the paper was sprayed with freshly prepared phloroglucinol (Borenfreund and Dische 1957) which produces green coloured spots with keto-pentoses, and purple spots with aldo-pentoses. The paper was dried at room temperatures and then heated for two minutes at 90°C. From the

FIGURE 4.2/1

Faecal Pentose Identification using Paper Chromatography

Development: Descending, at room temperature for 24 hours
Solvent: Butanol-Glacial Acetic Acid-Water (4:1:1)
Spray: Phloroglucinol

(1) = L. Arabinose (2) = D. Lyxose
(3) = Faeces Filtrate Sample
(4) = D. Ribose (5) = L. Xylose
(a) = Sample Origin



position and colour reaction of the spots (Fig. 4.2/1) it was possible to tentatively identify the unknown pentose as xylose.

Xylose has been reported found in the urine of children (Haworth and McCredie, 1956; Haworth and McDonald, 1957), and later, Ford and Haworth (1963) reported a pentose in the faeces of children, but the possible relationship between the faecal and the urinary pentose observations was never discussed.

D. Xylose is the principle product of the hydrolysis of Xylan, a polysaccharide which occurs in practically all plants and closely follows starch in distribution (Whistler and Smart 1953). Xylan is poorly digested by the pig (Salo 1965) but a number of micro-organisms are reported (Whistler and Smart 1953) to be able to decompose the polysaccharide. The presence of Xylose in the faecal samples may therefore, be the result of microbial activity in the large intestine. Furthermore the presence of Xylan in the stored faeces reported earlier (Section 3.1/3(e) Part II) could have resulted in the measured increase in total reducing sugar as a result of faecal microbial action.

PART III

GENERAL DISCUSSION, POSSIBLE TOPICS FOR FUTURE RESEARCH, AND SUMMARY OF RESULTS.

CHAPTER 1

GENERAL DISCUSSION

1.1 INTRODUCTION

With one exception, all pigs in the collection study, regardless of treatment, grew at a comparable rate, and consumed statistically similar amounts of food. As a result, no difficulties were experienced in obtaining collection data at 60, 85 and 110 lb liveweight for all treatment groups. Had the collection animals on the 45 % lactose diet refused as much food, and grown as poorly, as many of the animals on the same diet in the main investigation, it is unlikely that more than one collection (at 60 lb liveweight) could have been made. However, certain advantages would have also been gained if the animals in the collection study had produced treatment differences similar to those produced by the animals in the main investigation. This is particularly true in the case of treatments 1, 2 and 3, in which (in the main investigation) statistically significant differences in liveweight gain resulted from factors other than a depressed food intake. Such factors might well have been detected had the collection animals also shown this growth rate effect.

1.2 DISCUSSION OF RESULTS

The biochemical, physiological and anatomical aspects involved in the utilization of high dietary levels of lactose are best discussed in relation to Fig. 1.1 which summarises the results obtained in the present series of investigations. Under the feeding conditions employed, poorer liveweight gains on 45 - 50 % lactose diets were largely the result of depressed food intake. On this diet, pigs weighing 50 lb liveweight were receiving more than 1.0 lb lactose daily. If the "Maximum Tolerable Intake" hypothesis outlined earlier is valid, this would mean that similar food intake depressions (and hence probably poorer liveweight gains) could be expected from diets containing several different percentages of lactose, providing

they are fed at a level which also results in more than 1.0 lb of lactose being ingested daily, at 50 lb liveweight.

It was not possible to determine the extent to which other factors contributed to the poorer growth of the treatment 4 animals, but it is reasonable to assume that the effects to be discussed in relation to treatments 1, 2 and 3 were also affecting liveweight gains in treatment 4.

The results from urinary sugar analysis clearly showed that greater energy losses are experienced when the lactose level in the diet is increased. However, even the largest losses recorded in the present study would be unlikely to have a marked effect on liveweight gain.

With increasing levels of lactose, animals showed a progressively poorer ability to digest the energy and nitrogen components of the diet. These differences were small but significant in many cases in the collection study, but may have been larger in the main investigation, thereby accounting, at least in part, for some of the treatment differences observed in the latter experiment.

Very limited data suggests that increased sugar loss occurs in the faeces when pigs are scouring. The data obtained from one pig which voided almost 5 grams of sugar on one day, suggests that between 1 and 2 % of the lactose fed could be lost in this way. It was unfortunate that the preservative used during the collection of faeces, prevented the determination of faecal lactic acid concentrations, as it is likely that similar amounts of energy are lost in this form under diarrhoeal conditions.

The extent to which faecal energy losses from the pigs in the main investigation contributed to their poorer liveweight gains is difficult to assess. Treatment 2 and 3 pigs scoured for no more than 4 weeks and it is unlikely that during this time sufficiently large amounts of faecal sugar were lost to account for the poorer growth rates recorded.

High lactose intake had only two beneficial effects:-

(a) Nitrogen retention improved despite a decrease in nitrogen digestibility. For this reason results should be regarded as tentative only, until further work has been carried out on this point. However, the improvement in nitrogen retention recorded was small and probably unlikely to have any marked effect on liveweight gain.

(b) Caecum size increased with increased dietary levels of lactose. It was more likely that factors associated with this increase in caecum size, i.e. increased bacterial hydrolytic activity, had a larger beneficial effect upon liveweight gain than did (a). This increased enzyme activity in the caecum would result in enhanced sugar utilization so that much smaller energy losses would be expected in the faeces.

It is therefore unlikely that any single factor contributed to the progressively poorer liveweight gains recorded in the main investigation with increasing lactose levels.

The poorer growth rates probably resulted either from the additive effect of all the factors discussed, or from other factors not within the scope of the investigations.

> "At first he said he thought Gus●'s pigs reminded him of Arkansas Razor-backs, but it beat him how a foreign breed like that could suddenly show up in Taranaki. Then after a closer examination, and he got closer to them than I would have liked to, he thought they might be Large Blacks, crossed with Tamworths, with a Captain Cook strain thrown in between the third and fourth Generations."
>
> (Frank S. Anthony and Francis Jackson)

CHAPTER 2

POSSIBLE TOPICS FOR FUTURE RESEARCH

Several possible topics for research into lactose utilization in the growing pig arise from the results obtained in the present series of investigations:-

2.1 Determination of Metabolizable Energy Losses Resulting from high Lactose Diets

There is a definite need for a more accurate assessment of the urinary energy losses involved in feeding growing pigs high levels of lactose. The total reducing sugar method employed in the present investigations gave some indication of the energy losses involved but the 'balance' between lactose intake and total reducing sugar output could give only a very approximate assessment. More reliable results would be obtained from determinations of urinary calorie content, so that the energy balance would be solely on a caloric basis.

2.2 The Effect of Lactose Levels on Nitrogen Digestibility and Nitrogen Retention

In view of the doubts expressed concerning the results obtained in the collection study, it is felt that further experiments are necessary before conclusions are possible on the influence of lactose on protein utilization.

2.3 Establishment of a 'Maximum Tolerable Intake' Scale

This could be carried out, as suggested earlier, by chosing a range of dietary lactose levels and feeding them at perhaps two scales of feeding. In this way careful choice of diets would result in several different amounts of lactose being ingested at any particular liveweight, enabling a fairly accurate assessment of the amount of lactose an animal at that liveweight, will voluntarily consume.

2.4 Studies on the Development of Lactase Activity in the Growing Pig

An extension of the above experiment would be to remove the lactose diet as soon as each animal's individual 'M.T.I.' is determined and allow the animals to consume a non-lactose diet for at least 3 weeks. By removing the lactose diet, the possibility of enzymic adaptation to lactose is minimised. The various lactose diets could then be reimposed at a heavier liveweight and the 'M.T.I.' again determined. If lactose activity alters with increasing age, the changes should be reflected in similar changes in the 'M.T.I.'.

2.5 The Use of Artificial 'Whey' Diets

There is a need for greater use of artificial 'whey' diets to elucidate the relative contributions of individual whey components to factors typical of whey feeding such as diarrhoea. The value of working with each component individually was illustrated in the present investigations in which both diarrhoea and food refusals were attributable to lactose. Extension of this work would be to investigate the effect of bulk, uncomplicated by differences in lactose intake, on food consumption, or the effect of whey salts alone or in the presence of lactose, on diarrhoea. Lactose utilization may be improved by altering the protein level in the diet, or by changing the source of protein. Using artificial diets, such changes are relatively simple to make.

2.6 The Influence of Fat in Lactose Diets

Work with lactose-fed rats suggests that urinary sugar losses may be reduced by increasing dietary levels of fat. It would be interesting to see whether similar effects occur in pigs, but in view of the apparently small urinary sugar loss observed in the present studies, it is unlikely that any markedly beneficial effects on performance would be noted.

2.7 Further Caecum Studies

Considerably more information is required on the role of the caecum in the nutrition of the pig. Questions to be answered are:

(a) To what extent does the caecal digestion contribute to the total digestion process?

(b) Does this contribution change appreciably with changes in the diet, i.e. lactose diets, pasture diets, garbage, and if so, by how much?

(c) What enzyme systems exist in the caecum and to what extent are these altered by changes in the diet?

(d) What are the products of digestion?

These questions could be answered comparatively simply by catheterization of the caecum to permit sampling of the caecal contents. The fourth question would involve cannulation of the caecal vein so that blood samples could be collected and analysed.

2.8 Characterisation of Faecal Energy losses under Diarrhoeal Conditions

Limited evidence suggests that total reducing sugars and lactic acid are lost in greater amounts in the faeces when diarrhoeal conditions exist. The extent of this loss in lactose fed pigs which are scouring, has not been determined but may constitute an important avenue of energy loss.

2.9 The Extent of Heat Losses from the Body as a factor contributing to the Total Energy loss in Lactose-fed Pigs

Considerable energy expenditure may be involved in the removal of lactose and galactose from the circulatory system either by a conversion to glucose, or by elimination in the urine. The extent of this energy loss from the body as heat, could be determined by animal calorimetry.

2.10 Isotope Studies on the Fate of Ingested Lactose

By labelling the galactose - 1-C^{14} position of lactose, very interesting results could be obtained from the analysis of faecal and urinary samples, caecal contents and samples of blood from the veins associated with the small intestine and the caecum.

Determinations of the concentration of labelled sugar in the feed, minus labelled sugar in the caecal contents could indicate the extent to which lactose is hydrolysed and absorbed by the intestinal mucosa. Also, analysis of caecal contents and of the faeces could indicate by difference, the caecal contribution to lactose hydrolysis.

The magnitude and form to which the labelled sugar is absorbed from the two sites of hydrolysis could be determined by blood sample analysis.

In addition it may be possible to assess the amount of absorbed galactose which is converted to glucose-1-phosphate.

CHAPTER 3

SUMMARY OF RESULTS

1. Four experiments are described in which levels of lactose ranging from 0 to 50 % of the diet were fed to pigs over a liveweight range of 50 - 120 lb.

2. Results are presented for growth rate, food consumption and dung consistency from the two preliminary investigations involving 16 pigs.

3. Growth rate and food consumption data are presented for the main investigation and the collection study, in which diets containing 0, 15, 30 and 45 % lactose were fed to a total of 40 pigs. In the main investigation liveweight gains after 56 days for treatments 2, 3 and 4, relative to treatment 1, were 7.2 %, 10.0 % and 39.0 % poorer respectively. No food consumption data was available for treatment 4 pigs at 120 lb liveweight but the mean food consumptions of treatment 2, and 3 pigs were 5.5 % and 10.8 % greater than that of the treatment 1 animals.

No significant treatment differences in growth rate or food consumption were detected in the collection study.

4. In the main investigation, the depressed growth rate on the 45 % lactose diet was largely due to a reduced food intake. At the two lower levels of lactose (15 % and 30 %) poorer growth rates were still recorded (although only the 30 % level differed significantly from the controls) despite negligible food refusals.

5. The incidence and duration of diarrhoea increased with increasing levels of dietary lactose. Diets containing up to 30 % lactose caused a transient diarrhoea, but some of the animals on 45 % lactose continued to scour until the termination of the investigation.

6. Chronic diarrhoea did not appear to have a marked effect on live-weight gain.

7. Caecum size, as assessed by wet and dry weights, and tissue volume measurements, increased progressively with percentage lactose in the diet.

8. Level of dietary lactose appeared to have no consistent effect on carcass characteristics.

9. A positive relationship existed between the amount of reducing sugar lost in the urine of the collection study animals, and the level of dietary lactose.

10. Sugars voided in the urine were identified by chromatography. Lactose, galactose and glucose were all found in the urine samples of pigs fed lactose, whereas only traces of glucose were found in that of the control animals.

11. Levels of dietary lactose had little effect on the level of reducing sugar in the faeces.

12. The major sugar in faeces of 'normal' consistency was tentatively identified as the pentose sugar, xylose.

13. Samples from the only animal which scoured while on collection showed a very marked increase in faecal sugar loss. These samples contained large amounts of glucose and galactose in addition to the pentose, but no lactose was detected.

14. Increasing the dietary level of lactose appeared to decrease nitrogen digestibility but increased nitrogen retention.

15. A small but significant decrease in energy digestibility resulted when diets contained 30 or 45 % lactose.

16. The level of lactose in the diet was found to have no significant effect on ether extract digestibility.

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PART 1

PHYSIOLOGICAL AND ANATOMICAL ASPECTS

APPENDIX 2.2/1

COMPOSITION OF THE 'CARRY-ON' MEAL MIXTURE

Barley	25%
Buttermilk Powder	25%
Wheatmeal	15%
Maizemeal	15%
Fortified Meatmeal	10%
Rice Flour (Semolina)	10%
Bone Flour	1%

In addition the mixture contained (per 100 lb):

* 'Apac' Vitamin A and D Supplement	2.0 oz
+ 'Vetspen' (contains 1% Procaine Penicillin)	4.0 oz
Ferric Ammonium Citrate	1.0 oz
Manganese Sulphate	0.5 oz
Copper Sulphate	0.75 oz

and 0.15 ppm Selenium.

APPENDIX 2.2/2

COMPOSITION OF THE CEREAL/MEATMEAL MIXTURE

Barley	55%
Pollard	20%
Pea Meal	10%
Fortified Meatmeal	15%

In addition the mixture contained (per 100 lb):

* 'Apac' Vitamin A and D Supplement	0.7 oz
Manganese Sulphate	18.0 gm

Ferric Sulphate	22.5 gm
Zinc Sulphate	19.5 gm

* Proprietary Product containing (per gram): 10,000 i.u. Vitamin A

2,000 i.u. Vitamin D_3

\+ Proprietary Product

PART I

PHYSIOLOGICAL AND ANATOMICAL ASPECTS

APPENDIX 3. 2/1

MEANS OF WEEKLY AMBIENT TEMPERATURE RECORDINGS AND THE MAXIMUM RANGE ABOVE AND BELOW THE MEAN.

Week	Mean Ambient Temperature	Maximum Range
1.	70.5	± 6.75
2.	70.5	± 5.75
3.	70.5	± 5.5
4.	70.5	± 5.0
5.	73.0	± 4.75
6.	68.0	± 6.0
7.	70.0	± 6.0
8.	73.0	± 5.0
9.	73.0	± 3.0
10.	68.0	± 6.0
11.	69.0	± 3.0
12.	70.0	± 2.0
MEAN	70.5	± 5.0

APPENDIX 3. 3/1

MEAN TREATMENT LIVEWEIGHTS AFTER 60 DAYS
FOLLOWING TREATMENT INTRODUCTION

Analysis of Variance - (Dixon and Massey 1957 pp 123)

	Mean ± SE	N	Variance	$\frac{\text{Variance}}{N}$	$\left\{\frac{\text{Variance}}{N}\right\}^2$
T1	120.0 ± 1.73	3	9.00	3.00	9.00
T2	119.0 ± 1.64	3	8.10	2.70	7.29
T3	77.0 ± 7.61	3	173.56	57.86	3347.78
T4	84.5 ± 9.90	3	294.09	98.03	9609.88

	$\bar{X}i - \bar{X}j$	t	f. (d.f.)	Result
T1/T2	1.00	0.42	6.0	NS
T1/T3	43.00	5.51	2.4	*
T1/T4	35.50	3.55	2.3	*
T2/T3	42.00	5.39	2.4	*
T2/T4	34.50	3.45	2.2	*
T3/T4	7.50	0.60	5.5	NS

NS ($P > 0.05$)

* Denotes a significant difference at 5% ($P < 0.05$).

APPENDIX 4.2/1

RESULTS OF PROXIMATE ANALYSES ON THE 4 TREATMENT DIETS,
AND THEIR GROSS ENERGY (KCAL/KG) DETERMINED BY BOMB CALORIMETRY.

GROSS ENERGY		MOISTURE		CRUDE PROTEIN		CRUDE FIBRE		ASH		ETHER EXTRACT		N.F.E.	
KCAL/KG		'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'
4,368	TR.1 Diet (0% Lactose)	13.21	-	15.62	18.00	2.44	2.81	5.53	6.37	3.21	3.70	59.99	69.12
4,318	TR. 2 Diet (15% Lactose)	11.40	-	16.04	18.10	2.81	3.17	5.62	6.34	3.26	3.68	60.87	68.71
4,260	TR. 3 Diet (30% Lactose)	9.46	-	16.48	18.20	2.71	2.99	5.68	6.27	3.31	3.65	62.36	68.89
4,170	TR. 4 Diet (45% Lactose)	9.28	-	16.33	18.00	2.06	2.27	5.77	6.36	3.29	3.63	63.27	69.74

'A' Air Dry Basis

'O' Oven Dry Basis

APPENDIX 4.2/2

RESULTS OF PROXIMATE ANALYSES
AND MINERAL COMPOSITION OF DIETARY COMPONENTS, AND THE CALCULATED COMPOSITION OF THE TREATMENT DIETS

	MOISTURE		CRUDE PROTEIN		CRUDE FIBRE		ASH		ETHER EXTRACT		N.F.E.	
	'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'	'A'	'O'
Meatmeal	5.21	-	57.50	60.50	0.73	0.77	22.58	23.58	12.76	13.46	1.22	1.75
Pollard	10.81	-	15.75	17.44	7.07	7.93	3.78	4.19	4.22	4.74	58.37	65.70
Wheat Starch	11.15	-	0.56	0.63	-	-	0.06	0.07	0.05	0.06	88.18	99.24
Lactose	0.08	-	-	-	-	-	0.08	0.09	-	-	99.84	99.91
Calculated composition of												
Tr. 1 Diet (0% Lactose)	9.86	-	16.51	17.65	2.27	2.52	5.68	6.02	3.85	4.15	61.85	69.68
Tr. 2 Diet (15% Lactose)	8.29	-	16.42	17.55	2.27	2.52	5.68	6.02	3.84	4.14	63.61	69.79
Tr. 3 Diet (30% Lactose)	6.50	-	16.34	17.46	2.27	2.52	5.69	6.02	3.83	4.13	65.37	69.89
Tr. 4 Diet (45% Lactose)	4.83	-	16.25	17.36	2.27	2.52	5.69	6.02	3.82	4.12	67.14	70.00

'A' = Air Dry Basis

'O' = Oven Dry Basis

	PHOSPHORUS	POT-ASSIUM	SODIUM	MAG-NESIUM	CALCIUM	ZINC	COPPER	MANGANESE	IRON
	%	%	%	%	%	ppm	ppm	ppm	ppm
Meatmeal	3.80	0.78	0.99	0.21	6.18	90	11	28	382
Pollard	0.72	0.95	0.01	0.29	0.07	92	11	164	99
Wheat Starch	0.04	-	0.02	-	0.03	-	-	3	12
Calculated mineral levels, common to all treatment diets.	0.98	0.44	0.20	0.13	1.26	46.0	5.5	55.0	106.0

APPENDIX 4.3/1

LIVEWEIGHT GAIN (LB) IN 28, and 56 DAYS
FOLLOWING TREATMENT INTRODUCTION
- MAIN INVESTIGATION

Analysis of Variance - (Dixon and Massey 1957 pp 123)

		Mean ± SE	N	Variance	$\frac{\text{Variance}}{N}$	$\left\{\frac{\text{Variance}}{N}\right\}^2$
28 Days	T1	29.14 ± 0.37	7	0.98	0.14	0.0196
	T2	27.00 ± 1.32	7	12.25	1.75	3.0625
	T3	26.71 ± 0.60	7	2.49	0.36	0.1296
	T4	22.36 ± 1.34	7	12.48	1.78	3.1684
56 Days	T1	67.86 ± 0.73	7	3.73	0.53	0.2809
	T2	63.00 ± 3.26	7	74.33	10.62	112.7844
	T3	61.10 ± 0.41	5	1.20	0.17	0.0289
	T4	41.43 ± 5.11	7	182.54	26.08	680.1664

		$\bar{x}i - \bar{x}j$	t	f. (df)	Result
28 Days	T1/T2	2.14	1.55	7.27	NS
	T1/T3	2.43	3.43	11.37	* *
	T1/T4	6.78	4.88	7.25	* *
	T2/T3	0.29	0.20	9.16	NS
	T2/T4	4.64	2.47	14.00	*
	T3/T4	4.35	2.98	9.11	*
56 Days	T1/T2	4.86	1.46	6.80	NS
	T1/T3	6.76	8.05	10.28	* *
	T1/T4	26.43	5.12	6.33	* *
	T2/T3	1.90	0.58	6.26	NS
	T2/T4	21.57	3.56	11.59	* *
	T3/T4	19.67	3.84	6.10	* *

APPENDIX 4.3/2.(a)

FOOD CONSUMPTION (LB) FROM 50 - 80 LB. LIVEWEIGHT
- MAIN INVESTIGATION

Analysis of variance - (Dixon and Massey 1957 pp 123)

50-80	Mean ± SE	N	Variance	$\frac{\text{Variance}}{N}$	$\left(\frac{\text{Variance}}{N}\right)^2$
T1	85.81 ± 1.14	7	9.118	1.303	1.698
T2	90.71 ± 4.16	7	121.128	17.304	299.428
T3	93.84 ± 2.50	7	43.656	6.237	38.900
T4	121.16 ± 9.49	7	630.177	90.025	8104.500

50-80	$\bar{x}i - \bar{x}j$	t	f. (df)	Result
T1/T2	4.90	1.137	7.19	NS
T1/T3	8.03	2.920	9.20	*
T1/T4	35.35	3.698	6.23	* *
T2/T3	3.13	0.645	11.10	NS
T2/T4	30.45	2.942	8.97	*
T3/T4	27.32	2.785	7.10	*

* * ($P < 0.01$)

* ($P < 0.05$)

NS ($P > 0.05$)

APPENDIX 4.3/2.(b)

FOOD CONSUMPTION (LB) FROM 50 - 120 LB LIVEWEIGHT
- MAIN INVESTIGATION

Analysis of variance - (Dixon and Massey 1957 pp 123)

50-120	Mean ± SE	N	Variance	$\frac{\text{Variance}}{N}$	$\left\{\frac{\text{Variance}}{N}\right\}^2$
T1	194.74 ± 1.99	7	27.753	3.965	15.721
T2	205.44 ± 6.71	7	314.807	44.972	2022.481
T3	215.66 ± 3.83	5	73.268	14.654	214.740

50-120	$\bar{x}i - \bar{x}j$	t	f (df)	Result
T1/T2	10.70	1.530	7.40	NS
T1/T3	20.92	4.848	7.18	* *
T2/T3	10.22	1.324	10.39	NS

APPENDIX 4.3/3

LIVEWEIGHT GAIN (LB) FROM 0 - 28 DAYS - COLLECTION STUDY

Analysis of Variance

	s.s.	df	m.s.	F	Result
BL	24.05	2	12.025	3.48	NS
TR	13.09	3	4.363	1.26	NS
E1	17.28	5	3.456		
TG	54.42	10			

N.S. ($P > 0.05$)

APPENDIX 4.3/4

LIVEWEIGHT GAIN (LB) FROM 0 - 56 DAYS - COLLECTION STUDY

Analysis of Variance

	s.s.	df	m.s.	F	Result
BL	37.62	2	18.810	1.78	NS
TR	40.16	3	13.387	1.27	NS
E1	52.72	5	10.544		
TG	130.50	10			

N.S. ($P > 0.05$)

APPENDIX 4.3/5

FOOD CONSUMPTION (LB) FROM 50-80 LB. LIVEWEIGHT - COLLECTION STUDY

(Coded X - 80)

Analysis of Variance

	s.s.	df	m.s.	F	Result
BL	396.04	2	198.02	3.098	NS
TR	97.50	3	32.50	1.0	-
E1	319.60	5	63.92		
TG	813.14	10			

N.S. ($P > 0.05$)

APPENDIX 4.3/6

FOOD CONSUMPTION (LB) FROM 50-120 LB. LIVEWEIGHT - COLLECTION STUDY

Analysis of Variance (Coded X - 200)

	s.s.	df	m.s.	F.	Result
BL	414.39	2	207.195	3.24	N.S.
TR	522.63	3	174.210	2.72	N.S.
E1	320.15	5	64.030		
TG	1257.17	10			

N.S. ($P > 0.05$)

APPENDIX 4.3/7

CARCASS ANALYSIS

LIVEWEIGHT AT SLAUGHTER - (POUNDS) - MAIN INVESTIGATION

Analysis of Variance

	s.s.	df	m.s.	F.	Result
BL	43.74	6	7.290	< 1.0	-
TR	41.21	2	20.605	1.95	N.S.
E1	116.21	11	10.556		
TG	201.07	19			

N.S. ($P > 0.05$)

"COLD" CARCASS WEIGHT - (POUNDS) - MAIN INVESTIGATION

Analysis of Variance

	s.s.	df	m.s.	f	Result	d0.05	d0.01
BL	48	6	8.00	1.52	N.S.		
TR	186	2	93.00	15.74	**	2.9	4.1
E1	65	11	5.91				
T_G	299	19					

** Denotes a significant difference at the 1% ($P<0.01$) level of probability.

N.S. ($P>0.05$)

LIVEWEIGHT AT SLAUGHTER - (POUNDS) - COLLECTION STUDY

Analysis of Variance

	s.s.	df	m.s.	f	Result
BL	18.500	2	9.250	< 1.0	-
TR	25.229	3	8.410	< 1.0	-
E1	67.334	5	13.467		
T_G	111.063	10			

"KILLING-OUT" PERCENTAGE - COLLECTION STUDY

Analysis of Variance

	s.s.	d.f.	m.s.	f.	Result	d0.05	d0.01
BL	2.652	2	1.326	3.55	N.S.		
TR	25.009	3	8.336	22.29	**	1.28	2.01
E_1	1.868	5	0.374				
T_G	29.529	10					

N.S. ($P > 0.05$)

** Denotes a significant difference at the 1% ($P < 0.01$) level of probability.

MID LOIN FAT MEASUREMENT - (MM) - COLLECTION STUDY

Analysis of Variance

	s.s.	d.f.	m.s.	f	Result
BL	4.50	2	2.250	2.33	N.S.
TR	14.93	3	4.977	5.16	N.S.
E_1	4.82	5	0.964		
T_G	24.25	10			

N.S. ($P > 0.05$)

APPENDIX 4.3/8

CARCASS SPECIFIC GRAVITY MEASUREMENTS - MAIN INVESTIGATION

Analysis of Variance

	s.s.	d.f.	m.s.	f	Result
BL	140.91	6	23.49	2.35	N.S.
TR	3.03	2	1.52	< 1.0	-
E_1	109.85	11	9.99		
T_G	253.79	19 ≠			

≠ 1 Missing Plot Value

N.S. ($P > 0.05$)

SPECIFIC GRAVITY MEASURMENTS - COLLECTION STUDY

Analysis of Variance

	s.s.	d.f.	m.s.	f	Result	d0.05
BL	72.8467	2	36.4234	8.8150	*	0.0034
TR	92.2600	3	30.7533	7.4427	*	0.0029
E_1	20.6600	5	4.1320			
T_G	185.7667	10				

	Block Means ± S.E.	Significance of Difference
H	1.0565 ± 0.00102	H > J *
I	1.0544 ± 0.00102	
J	1.0520 ± 0.00117 ≠	

≠ Data contains 1 missing plot value.

* Denotes a significant difference at the 5 % ($P < 0.05$) level of Probability.

APPENDIX 4.3/9

EYE MUSCLE AREA (CM^2) MEASUREMENTS - MAIN INVESTIGATION

Analysis of Variance

	s.s.	d.f.	m.s.	f	Result
BL	21.9	6	3.650	2.558	NS
TR	62.5	2	31.250	21.900	**
E_1	15.7	11	1.427		
T_G	100.1	19 ≠			

≠ 1 Missing Plot Value

NS ($P > 0.05$)

** Denotes a significant difference at the 1% ($P < 0.01$) level of Probability.

EYE MUSCLE AREA (CM^2) MEASUREMENTS - MAIN INVESTIGATION

Analysis of Covariance (X = "Cold" Carcass Weight lb)

	d.f.	ssx	spxy	ssy	ssy'	d.f'	m.s.	f	Result
T_G	19	229	139.0	100.1					
BL	6	48	12.0	21.9					
TR	2	186	104.4	62.5					
E_1	11	65	22.6	15.7	7.842	10	0.7842		
TR+E	13	251	127.0	78.2	13.941	12			
Diff.					6.099	2	3.0495	3.889	NS

NS ($P > 0.05$)

EYE MUSCLE AREA (CM^2) MEASUREMENTS - COLLECTION STUDY

Analysis of Variance

	s.s.	d.f.	m.s.	f	Result
BL	14.05	2	7.025	5.34	NS
TR	17.99	3	5.997	4.56	NS
E_1	6.58	5	1.316		
T_G	38.62	10			

NS ($P > 0.05$)

APPENDIX 4.3/10

ANALYSIS OF VARIANCE OF CAECUM CHARACTERISTICS - COMBINED DATA

Analysis of Variance of Caecum Wet Weights

(Treatment Means from 10 Pigs)

	s.s.	d.f.	m.s.	f	Result
BL	1464	9	162.67	2.53	*
TR	7286	2	3643.00	56.76	**
E_1	1091	17	64.18		
T_G	9841	28			

** Denotes a significant difference at the 1% ($P < 0.01$) level of Probability.

* Denotes a significant difference at the 5% ($P < 0.05$) level of Probability

TR. Means	1	81.85	2	110.90	3	117.90 ≠
BL. Means	B	117.00	F	107.67	A	107.33
	H	107.00	E	103.83	D	103.67
	E	103.67 ≠	J	100.33	G	94.67
	I	90.33				

	SE	SE ≠	d0.05	d0.01
TR means	2.53	2.67	7.55	10.38
BL means	4.63	5.66	13.61	18.96

≠ 1 Missing Plot Value

Analysis of Regression of Caecum Wet Weight on Liveweight at Slaughter

Source	d.f.	m.s.	f.	Result
Error	16	67.3		
Regression	1	13.8	< 1.0	-

ANALYSIS OF VARIANCE OF CAECUM DRY WEIGHTS

(Treatment Means From 10 Pigs)

	ss	df	ms	F	Result
BL	412	9	45.78	6.43	**
FR	70	2	35.00	4.92	*
E	121	17	7.12		
TG	603	28			

** Denotes a significant difference at the 1 % ($P < 0.01$) level of Probability.

* Denotes a significant difference at the 5 % ($P < 0.05$) level of Probability.

TR Means	1	22.95	2	25.45	3	26.75 ≠
BL Means	F	30.83	B	28.17	A	27.67
	H	27.33	G	26.00	J	24.67
	E	24.67 ≠	C	22.17	D	21.67
	I	17.33				

	SE	SE≠	d 0.05	d 0.01
TR Mean	0.84	0.89	2.52	3.46
BL Mean	1.53	1.89	4.60	6.32

≠ one missing plot value

ANALYSIS OF REGRESSION OF CAECUM DRY WEIGHT ON LIVEWEIGHT AT SLAUGHTER

Source	df	ms	F	Result
Error	16	6.58		
Regression	1	15.7	2.39	

NS ($P > 0.05$)

ANALYSIS OF VARIANCE OF CAECUM WATER DISPLACEMENT

(Treatment Means From 10 Pigs)

	ss	df	ms	F	Result
BL	1437	9	159.67	1.36	NS
TR	10095	2	5047.50	43.08	**
E_1	1992	17	117.18		
T_G	13524	28			

** Denotes a significant difference at the 1 % ($P < 0.01$) Level of Probability.

NS ($P > 0.05$)

	TR Means	SE	d 0.05	d 0.01
T1	91.0	3.43	10.21	14.03
T2	122.50	3.43		
T3	134.50 ≠	3.61		

≠ one missing plot value

ANALYSIS OF REGRESSION OF CAECUM WATER DISPLACEMENT ON LIVEWEIGHT AT SLAUGHTER

Source	df	ms	F	Results
Error	16	122.2		
Regression	1	37.2	< 1.0	-

ANALYSIS OF VARIANCE OF CAECUM LENGTH

(Treatment Means from 10 Pigs)

	ss	df	ms	F	Result
BL	27.80	9	3.09	< 1.0	-
TR	70.30	2	35.15	2.96	NS
E_1	202.20	17	11.89		
T_G	300.30	28			

NS (P > 0.05)

TR	Means	S.E.
1	25.55	1.09
2	26.25	1.09
3	27.90 ≠	1.15 ≠

≠ one missing plot value

PART II

BIOCHEMICAL ASPECTS

APPENDIX 3.1/1

TOTAL REDUCING SUGAR RECOVERY EXPERIMENT. RESULTS OF 4 ANALYSES ON SAMPLES OF URINE AND FAECES TO WHICH KNOWN QUANTITIES OF GLUCOSE WERE ADDED.

DUNG	Concentration of TRS Determined	Concentration of TRS Calculated	$\frac{\text{Determined}}{\text{Calculated}} \times \frac{100}{1}$	
Sample	mg/100 ml	mg/100 ml		
1	16.0	-	-	
2	24.1	24.00	100.4	Mean 100.0 %
3	29.6	32.00	92.5	Range
4	43.2	40.00	108.0	92.5-108.0 %
5	55.2	56.00	99.1	
URINE				
1	100.6	-	-	
2	105.5	108.6	97.2	
3	116.6	116.6	100.0	Mean 100.3 %
4	130.0	124.6	104.3	Range
5	140.7	140.6	100.1	97.2-104.3 %

APPENDIX 3.1/2.

TOTAL REDUCING SUGAR REPEATABILITY EXPERIMENT

RESULTS OF 18 ANALYSES OF PROTEIN FREE FILTRATES PREPARED FROM ONE DUNG (A) AND ONE URINE (B) SAMPLE.

	T.R.S. Concentration		T.R.S. Concentration
Sample A. (Dung)	Mg/100 ml.	Sample B. (Urine)	mg/100 ml.
1	39.2	1	91.2
2	41.7	2	94.4
3	46.2	3	95.7
4	50.1	4	95.7
5	52.0	5	96.3
6	52.6	6	98.9
7	53.3	7	100.2
8	53.3	8	100.8
9	55.9	9	102.7
10	59.1	10	104.7
11	62.3	11	105.9
12	62.3	12	107.2
13	63.6	13	108.5
14	68.1	14	110.4
15	69.3	15	111.7
16	73.2	16	112.4
17	73.8	17	112.4
18	75.8	18	115.6
Total	1051.2	Total	1864.7
Mean	58.4 $\pm$ 2.58	Mean	103.6 $\pm$ 1.73
Coefficient of Variance	18.7 %	Coefficient of Variance	7.08 %

APPENDIX 3.1/3.

CHANGES IN THE SUGAR CONCENTRATION OF PROTEIN FREE FILTRATES STORED FOR ZERO (A) AND 56 DAYS (B) at -12°C

(a) RAW DATA (mg/100 ml)

	A	B
	46.3	45.6
	97.1	96.9
	52.0	53.1
	39.4	34.0
	75.9	78.5
	55.4	54.8
	70.2	73.9
	114.2	124.1
	44.0	41.6
	75.9	66.4
MEAN	67.04	66.89
± SE	7.73	8.74

(b) F TEST OF HETEROGENEITY OF REGRESSIONS FOR TWO DETERMINATIONS (A and B) OF TOTAL REDUCING SUGAR (mg / 100 ml.)

	df	SSX	SPXY	SSY	SSY'	df'	ms	F	Result
A	4	23680	41464	73491	887	3			
B	4	23680	41008	72357	1341	3			
					2228	6	371.33		
A+B	8	47360	82472	145848	2232	7			
					4	1	4	< 1.0	NS

APPENDIX 3.1/4.

CHANGES IN THE SUGAR CONCENTRATION (mg./100 ml.) OF URINE STORED INTACT FOR FOUR PERIODS OF TIME.

(a) RAW DATA

DAY A	URINATION	24 HOURS AT 68°F	120 HOURS AT -12°C	240 HOURS at -12°C
T1	66.7	54.9	100.9	77.9
T2	47.8	44.8	56.1	64.3
T3	101.5	60.2	78.5	123.9
T4	54.9	78.5	60.8	49.6
DAY B				
T1	29.9	33.4	40.6	40.6
T2	14.3	19.1	20.3	19.1
T3	5.4	9.6	11.3	1.0
T4	50.8	50.8	41.2	47.2
MEAN	46.4 ± 10.8	43.9 ± 7.9	51.2 ± 10.4	53.0 ± 13.3

(b) ANALYSIS OF VARIANCE

	ss	df	ms	F	Results
DAYS	14736.15	1	14736.15	8.34	NS
TR	1973.48	3	657.83	< 1.0	-
E_1	5300.04	3	1761.68		
HOURS	420.03	3	140.01	< 1.0	-
TR x HR	1732.72	9	192.52	1.03	NS
E_2	2234.38	12	186.20		
T_G	26396.80	31			

NS ($P > 0.05$)

(c) F TEST OF HETEROGENEITY OF THE TWO REGRESSIONS

DAY	df	SSX	SPXY	SSY	SSY'	df'	ms	F	Result
A	3	23600	39970	68385	690	2			
B	3	23600	39510	66637	491	2			
					1181	4	295.25		
A+B	6	57200	79480	135022	24584	5			
					23403	1	23403.00	79.26	**

** The two regressions differed significantly at 1 % ($P < 0.01$)

APPENDIX 3.1/5.

THE EFFECTS OF THE STORAGE OF FAECES AT $-12^{\circ}C$ FOR FIVE DAYS, ON THEIR TOTAL REDUCING SUGAR CONCENTRATIONS

(a) RAW DATA

A DEPROTEINIZED FRESH	B DEPROTEINIZED AFTER 5 DAYS
53.63 mg/100 ml	110.74 mg/100 ml.
28.08	60.81
10.05	70.89
10.00	22.07
52.78	114.70
13.37	57.35
53.70	50.73

(b) ANALYSIS OF VARIANCE

	ss	df	ms	F	Result
BL	7332	6	1222	3.78	NS
TR	5042	1	5042	15.61	**
E_1	1936	6	323		
TG	14310	13			

** Denotes a significant difference at 1 % ($P < 0.01$)
NS ($P > 0.05$)

	MEAN	± SE	d0.05	d0.01
TR A	31.66 (mg/100 ml)	± 6.79	23.50	35.61
TR B	69.61 "	± 6.79		

APPENDIX 3.1/6.

URINARY AND FAECAL LOSSES FROM ALL TREATMENT GROUPS ON COLLECTION 'C'.

			URINARY LOSSES		FAECAL LOSSES	
PIG NO.	TREATMENT	TOTAL SUGAR LOSS IN DUNG AND URINE (gms)	SUGAR LOSS (gms)	PERCENTAGE OF TOTAL SUGAR LOSS (%)	SUGAR LOSS (gms)	PERCENTAGE OF TOTAL SUGAR LOSS (%)
12	1	2.36	2.21	93.5	0.15	6.5
5	1	4.22	3.95	93.5	0.28	6.5
7	1	2.10	1.95	92.7	0.15	7.3
2	2	7.21	6.80	94.3	0.41	5.7
3	2	5.33	5.20	97.5	0.13	2.5
6	2	9.07	8.33	91.8	0.75	8.2
11	3	10.18	9.85	96.8	0.32	3.2
9	3	8.45	7.73	91.5	0.72	8.5
8	3	10.45	9.89	94.6	0.56	5.4
1	4	13.30	11.94	89.7	1.37	10.3
10	4	13.78	13.66	99.1	0.12	0.9
4	4 → 1	3.42	3.32	97.2	0.10	2.8
	MEAN			94.4		5.6
	± SE			± 0.9		± 0.9

APPENDIX 3.1/7

TOTAL REDUCING SUGAR OUTPUT IN THE URINE
FROM COLLECTIONS 'A' & 'C'

(a) RAW DATA

	COLLECTION 'A'			COLLECTION 'C'		
Pig No.	mg/100 ml.	Urine Output	Sugar Output	mg/100 ml.	Urine Output	Sugar Output
		ml.	mg.		ml.	mg.
1.	33.7	12740	4293.38	62.2	19195	11939.0
2.	21.7	13361	2899.34	32.0	21246	6799.0
3.	10.0	13802	1380.20	24.8	20956	5197.0
4.	10.8	15172	1638.58	17.5	22551	3946.0
5.	74.1	8900	6594.90	18.1	18362	3324.0
6.	34.9	12955	4521.30	36.8	22621	8325.0
7.	10.0	13392	1339.20	9.1	21401	1948.0
8.	52.4	14356	7522.54	44.1	22414	9885.0
9.	42.7	13646	5826.84	35.6	21714	7730. 0
10.	72.2	12900	9313.80	60.4	22607	13655.0
11.	27.7	12076	3345.05	45.3	21750	9853.0
12.	10.0	11991	1199.10	11.5	19219	2210.0

APPENDIX 3.1/7

(b) F TEST OF HETEROGENEITY OF REGRESSIONS FOR TWO DETERMINATIONS (COLLECTION 'A' AT 60 LB LIVEWEIGHT, COLLECTION 'C' AT 110 LB LIVEWEIGHT) OF TOTAL REDUCING SUGAR IN THE URINE.

	df	SSX	SPXY	SSY	SSY'	df'	ms	F	RESULT
A	4	23680	39360	65924	500	3			
C	4	23680	39200	65846	954	3			
					1454	6	242.3		
A+C	8	47360	78560	131770	1456	7			
					2	1	2.00	<1.0	NS

(c) ANALYSIS OF VARIANCE OF TOTAL REDUCING SUGAR OUTPUT IN THE URINE COLLECTED AT 60 LB. AND 110 LB. LIVEWEIGHT.

	SS	df	MS	F	RESULT	d0.05	d0.01
BL	7.823	2	3.912	1.141	NS		
TR	203.727	3	67.909	19.804	**	1.171	1.732
E1	20.573	6	3.429				
COL^n	85.059	1	85.059	57.982	**	2.266	3.433
CxTR	18.851	3	6.284	4.284	NS		
E_2	10.267	7	1.467				
TG	346.300	22					

** Denotes a significant difference at 1% ($P < 0.01$)

NS ($P > 0.05$)

APPENDIX 3.1/8

SUGAR 'BALANCE' FOR COLLECTION 'C!

		REDUCING SUGAR INTAKE (LACTOSE)	REDUCING SUGAR VOIDED IN DUNG + URINE	
	Tr.	gms	gms	% OF INTAKE
1	4	3753.15	13.305	0.36
2	2	1236.90	7.209	0.58
3	2	1221.82	5.328	0.44
4	1	-	4.224	-
5	1	-	3.420	-
6	2	1221.82	9.071	0.74
7	1	-	2.101	-
8	3	2558.80	10.446	0.41
9	3	2466.31	8.447	0.34
10	4	3160.06	13.778	0.44
11	3	2527.97	10.177	0.40
12	1	-	2.363	-

APPENDIX 3.2/1a

MEAN DAILY D.E. INTAKE (KCALS) - 85 lb LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED x + 200)

Source	ss	df	ms	F	Result	d 0.10
BL	0.662	2	0.311	3.11	NS	
TR	1.857	3	0.619	6.19	+	0.550
E_1	0.398	4	0.100			
T_G	2.877	9				

NS ($P > 0.10$)

\+ Denotes a significant difference at the 10 % ($P < 0.10$) level of Probability.

APPENDIX 3.2/1b

MEAN DAILY D.E. INTAKE (KCALS) - 110 lb LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED x + 200)

Source	ss	df	ms	F	Result
BL	0.036	2	0.018	< 1.0	-
TR	0.029	2	0.015	< 1.0	-
E_1	1.018	4	0.255		
T_G	1.083	8			

APPENDIX 3.2/2a

MEAN NITROGEN DIGESTIBILITY PERCENTAGE - 85 lb. LIVEWEIGHT

ANALYSIS OF COVARIANCE X = MEAN DAILY D.E. INTAKE

Source	df	SSX	SPXY	SSY	SSY'	MS	F	Result
T_G	9	2.877	1.674	120.52				
BL	2	0.622	-0.957	10.84				
TR	3	1.857	3.743	82.12	82.16			
E_1	4	0.398	-1.112	27.56	27.56			
Regression	1				3.11	3.11	< 1.00	-
Residual	3				24.45	8.15		

APPENDIX 3.2/2b

MEAN NITROGEN RETENTION (GMS) - 85 lb. LIVEWEIGHT

ANALYSIS OF COVARIANCE X = MEAN DAILY D.E. INTAKE

Source	df	SSX	SPXY	SSY	SSY'	MS	F	Result
T_G	9	2.877	0.798	15.957				
BL	2	0.622	-0.414	0.291				
TR	3	1.857	0.883	13.738	13.319			
E_1	4	0.398	0.329	1.928	1.928			
Regression	1				0.272	0.272	< 1.0	-
Residual	3				1.656	0.552		

APPENDIX 3.2/2c

MEAN NITROGEN RETENTION (PERCENT) - 85 lb. LIVEWEIGHT

ANALYSIS OF COVARIANCE X = MEAN DAILY D.E. INTAKE

Source	df	SSX	SPXY	SSY	SSY'	M.S.	F	Result
T_G	9	2.887	-2.470	343.383				
BL	2	0.622	-2.853	13.824				
TR	3	1.857	2.761	275.295	289.463			
E_1	4	0.398	-2.380	54.264	54.264			
Regression					14.232	14.232	1.07	NS
Residual					40.032	13.344		

N.S. Regression non significant at 5% ($P > 0.05$)

APPENDIX 3.2/3a

MEAN DAILY NITROGEN INTAKE (GMS) - 85 LB LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED X ÷ 2)

Source	ss	df	ms	F	Result	d 0.05
BL	0.429	2	0.215	2.99	NS	
TR	3.077	3	1.026	14.25	*	0.608
E_1	0.288	4	0.072			
T_G	3.794	9				

N.S. ($P > 0.05$)

* Denotes a significant difference at 5% ($P < 0.05$) level of Probability.

APPENDIX 3.2/3b

MEAN DAILY NITROGEN INTAKE (GMS) - 110 LB LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED X + 2)

Source	ss	df	ms	F	Result
BL	0.024	2	0.012	< 1.0	-
TR	1.504	2	0.752	4.53	NS
E	0.665	4	0.166		
T_G	2.193	8			

N.S. ($P > 0.05$)

APPENDIX 3.2/4

MEAN NITROGEN RETENTION (GMS) - 85 LB LIVEWEIGHT

ANALYSIS OF COVARIANCE X = MEAN DAILY NITROGEN INTAKE (GMS)

Source	df	SSY	SPXY	SSY	SSY^1	ms	F	Result
T_g	9	3.794	4.917	15.957				
BL	2	0.429	-0.348	0.291				
TR	3	3.077	5.617	13.738	10.254			
E_1	4	0.288	-0.352	1.928	1.928			
Regression	1				0.430	0.430	< 1.00	-
Residual	3				1.498	0.499		

APPENDIX 3.2/5

MEAN NITROGEN DIGESTIBILITY (PERCENT) 85 and 110 lb LIVEWEIGHT

ANALYSIS OF VARIANCE

Source	ss	df	ms	F	Result	d 0.10
BL	9.427	2	4.714	< 1.0	-	
TR	49.395	2	24.698	6.702	+	3.92
E_1	14.741	4	3.685			
Col^n	34.693	1	34.693	6.117	+	
Col^n x TR	45.560	2	22.780	4.016	+	
E_2	28.360	5	5.672			
T_G	182.176	16				

\+ Denotes a significant difference at 10 % ($P < 0.10$) level of Probability.

APPENDIX 3.2/6a

MEAN NITROGEN RETENTION (GMS) - 85 lb LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED x ÷ 2)

Source	ss	df	ms	F	Result	d 0.05
BL	0.291	2	0.146	< 1.0	-	
TR	13.738	3	4.579	9.50	*	1.574
E_1	1.928	4	0.482			
T_G	15.957	9				

* Denotes a significant difference at 5 % ($P < 0.05$) level of Probability.

APPENDIX 3.2/6b

MEAN NITROGEN RETENTION (PERCENT) - 85 lb LIVEWEIGHT

ANALYSIS OF VARIANCE

Source	ss	df	ms	F	Result	d 0.05
BL	13.824	2	6.912	< 1.0	-	
TR	275.295	3	91.765	6.764	*	8.35
E_1	54.264	4	13.566			
T_G	343.383	9				

* Denotes a significant difference at 5 % ($P < 0.05$) level of Probability.

APPENDIX 3.2/7a

MEAN NITROGEN RETENTION (GMS) - 110 lb LIVEWEIGHT

ANALYSIS OF VARIANCE (CODED X + 2)

Source	ss	df	ms	F	RESULT
BL	79.06	2	39.530	1.306	NS
TR	298.43	2	149.215	4.93	NS
E_1	121.04	4	30.260		
T_G	498.53	8			

NS ($P > 0.05$)

APPENDIX 3.2/7b

MEAN NITROGEN RETENTION (PERCENT) - 110 lb LIVEWEIGHT

ANALYSIS OF VARIANCE

Source	ss	df	ms	F	RESULT
BL	15.171	2	7.586	1.016	NS
TR	32.412	2	16.206	2.171	NS
E_1	29.856	4	7.464		
T_G	77.439	8			

NS ($P > 0.05$)

APPENDIX 3.3/1

APPARENT DIGESTIBLE ENERGY OF THE FOUR TREATMENT DIETS (KCALS/KG) - 60, 85 and 110 lb LIVEWEIGHT.

ANALYSIS OF VARIANCE (CODED x + 1000)

Source	ss	df	ms	F	Result
BL	0.064	2	0.0320	10.67	*
TR	0.241	3	0.0803	26.77	***
E_1	0.015	5	0.0030		
C	0.031	2	0.0155	2.39	NS
CxT	0.025	6	0.0041	<1.00	NS
E_2	0.085	13	0.0065		
T_G	0.461	31			

TR. $d_{0.001} = 0.177$; $d_{0.01} = 0.104$; $d_{0.05} = 0.066$

*** $P < 0.001$ * $P < 0.05$

APPENDIX 3.3/2

COEFFICIENTS OF APPARENT DIGESTIBLE ENERGY (PER CENT) - 60, 85, 110 lb LIVEWEIGHT

ANALYSIS OF VARIANCE

Source	ss	df	ms	F	Result	d 0.05	d 0.01
BL	28.95	2	14.48	19.05	**	0.93	1.45
TR	16.08	3	5.36	7.05	*	1.06	1.67
E_1	3.82	5	0.76				
C	17.92	2	8.96	5.64	*	1.11	1.55
CxT	11.26	6	1.88	1.18	NS		
E_2	20.72	13	1.59				
T_G	102.75	31					

** $P < 0.01$ * $P < 0.05$

APPENDIX 3.4/1

APPARENT DIGESTIBILITY OF ETHER EXTRACT (PERCENT)

ANALYSIS OF VARIANCE

	ss	df	ms	F	Result
BL	1084.64	2	542.32	13.595	**
TR	274.70	3	91.57	2.296	NS
E_1	239.33	6	39.89		
C	1312.90	2	656.45	28.988	**
CxTR	270.66	6	45.11	1.992	NS
CxBL	792.26	4	198.07	8.746	**
E_2	226.46	10	22.646		
TG	4200.95	33			

BLOCK MEANS	H	68.16 $\pm$ 1.82 %	I	74.11 $\pm$ 1.90 % ≠	J	60.69 $\pm$ 1.90% ≠
COL.^{n} MEANS	A	59.16 $\pm$ 1.37 %	B	72.67 $\pm$ 1.44 % ≠	C	71.13 $\pm$ 1.44% ≠

	d.0.05	d.0.01
BLOCK MEANS	6.31	9.56
COLLECTION MEANS	4.33	6.16
COLLECTION x BLOCK MEANS	7.50	10.66

≠ Data contains 1 missing plot value