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Yours faithfully,

P.D. Cranwell

P.D. CRANWELL
Lecturer in Agriculture.

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PDC.

STUDIES ON THE FAECAL MICRO-FLORA AND MICRO-FAUNA OF THE YOUNG PIG AS INFLUENCED BY DIET, AGE AND TIME OF WEANING

A thesis presented to the Massey University College of Manawatu in partial fulfilment of the requirements of the Degree of Master of Agricultural Science

---

P. D. CRANWELL
MASSEY UNIVERSITY COLLEGE OF MANAWATU

November, 1963.

The creatures outside looked from
pig to man, and from man to pig,
and from pig to man again; but
already it was impossible to say
which was which ......

..... George Orwell

ACKNOWLEDGMENTS

The author wishes to record his sincere appreciation and thanks for the guidance and continuous interest of Mr. B. A. Reynolds throughout this project.

Acknowledgment is due to Mr. A. C. Glenday and Mr. D. A. Evans for statistical advice; Dr. R. Clark and Miss Margaret Soulsby for preparation of the photographs; Mr. J. C. Newhook for helpful discussion; Miss M. G. Campbell and Miss Pamela Forsyth, for assistance in obtaining literature; Mr. T. Rogers of the Research Piggery (at the time of the trial) for his assistance and Mr. P. R. Hockey for help in preparation of the graphs and proof reading.

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TABLE OF CONTENTS

TABLE OF CONTENTS (contd.)

PART II

STUDIES ON THE FAECAL MICRO-FLORA AND MICRO-FAUNA OF THE YOUNG PIG AS INFLUENCED BY DIET, AGE AND TIME OF WEANING.

TABLE OF CONTENTS (Contd.)

LIST OF TABLES

PART I

PART II

LIST OF TABLES (contd.)

LIST OF TABLES (contd.)

LIST OF FIGURES

LIST OF FIGURES (contd.)

INTRODUCTION

The occurrence of chronic and acute digestive disorders of farm animals during the rearing period is an important problem. Not only do these disorders reduce the animal's food conversion efficiency, but they may result in other economic losses by lowering the quality and quantity of the animal product or by causing the death of the animal.

It has been established that early weaning of piglets increases the number of litters that a sow can produce during a year. However, the problem of providing the early weaned piglet with an adequate and digestible post-weaning diet is more complex than when weaning at a later age is practised.

Weaning at 3 weeks of age is commonly practised at the Massey University College of Manawatu Research Piggery. During the last 4 years scouring has been a recurrent problem in piglets just prior to weaning and up to 9 weeks of age. At times this scouring has resulted in the death of the animal. Post mortem examination of the piglets revealed a considerable amount of damage to the large intestine. Cultural and microscopic examination of the faeces of scouring piglets has failed to find a common single etiological agent which would have been responsible. In the majority of cases scouring has been accompanied by the presence in the faeces of large amounts of undigested starch and large numbers of Balantidium coli. There were indications that weaning at 6 weeks of age lowered the incidence of scours.

The trial reported in this thesis was conducted at the Research Piggery and the Veterinary Pathology and Animal Physiology Department of the

Massey University College of Manawatu. The principle objective was to study the changes which occurred in the flora and fauna of the large intestine during the early growth of the pig, comparing early and late weaning. Also, to see if these changes are related to the digestive disturbances which have been found to occur in piglets at the Research Piggery. For economic reasons it was not possible to sacrifice piglets of this age in order to examine the large intestine and its contents as was originally planned. Recourse had to be made to the collection and examination of faecal samples.

The investigations into the scouring problem prior to this work were carried out on post mortem material or faeces obtained from piglets which had been observed to scour. No trials had been carried out in which the flora, fauna and amounts of undigested material present in the faeces were regularly observed during the first 8 weeks of the piglets life.

The experiments reported in Part II involved 24 piglets, six from each of four litters, and their dams. The piglets from two litters were weaned at 3 weeks of age and the others at 6 weeks. Faeces samples were taken from each piglet weekly during the first eight weeks of life and from the sow while it was with the litter. Several groups and species of bacteria were enumerated by cultural methods. The samples were then preserved, stored and later examined by microscopy for undigested material, starch digesting bacteria, and various species of Protozoa.

From the literature it is evident that the cultural methods commonly used for the enumeration of bacteria in such material are suspect in their ability to provide reproducible results. Also they would be too cumbersome for the number of determinations necessary.

It was essential, therefore, to devise a technique by which these two limitations could be sufficiently overcome to make the experiments worthwhile.

Part I is a report on the work undertaken to develop and test a technique by which the enumeration of bacteria from faecal samples could be satisfactorily carried out.

PART I

ENUMERATION OF THE FAECAL MICRO-FLORA

OF THE PIG

BY THE DROP TECHNIQUE

CHAPTER I

REVIEW OF LITERATURE

The identification and enumeration of each species or group of bacteria present in the contents of the alimentary tract of animals is of considerable importance. The knowledge which is provided is essential before the role played by bacteria in the health and nutrition of the host animal can be elucidated.

The cultural procedures available for the enumeration of the bacteria present in gut contents and faeces are similar to those used in the examination of water, sewage, milk and milk by-products. They are:

(a) The Most Probable Number Technique (M.P.N.)

(b) The Agar Pour Plate or Standard Plate Count

(c) The Membrane Filter Technique

(d) The Drop Technique

Enumeration depends on either

(i) Ascertaining the highest dilutions at which the organism will grow in a liquid medium (M.P.N.)

or

(ii) Counting the number of colonies which develop in/on a solid medium from a known dilution.

From these results the number of organisms per g. of material can be calculated.

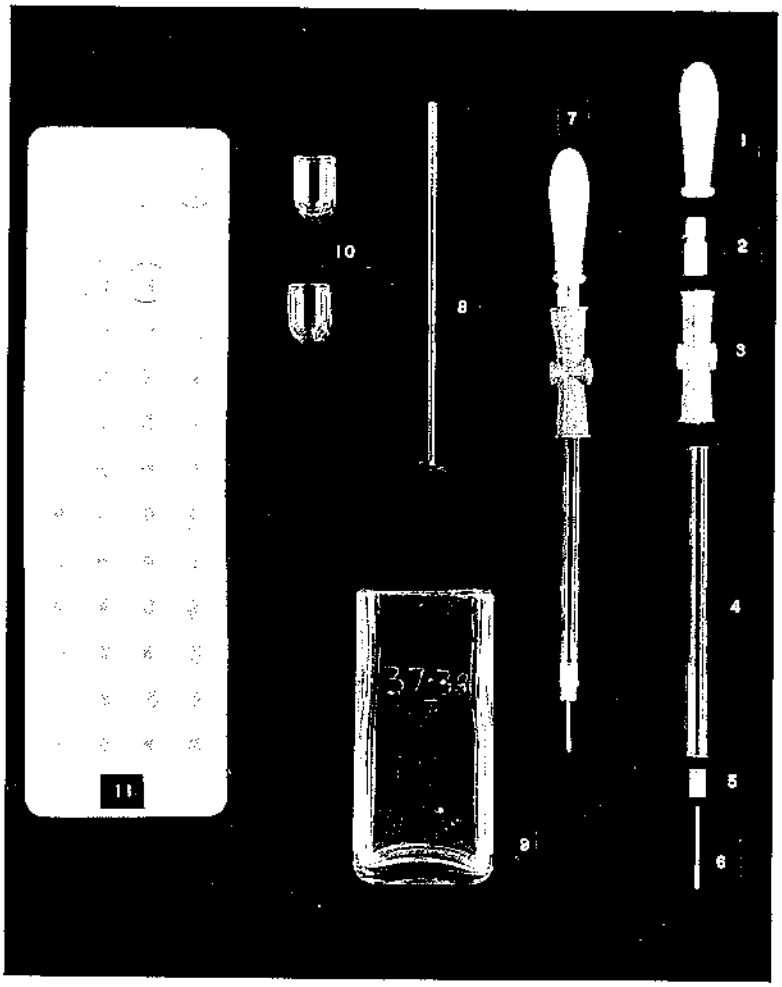
The inherent lack of precision of the M.P.N. technique has been stressed in publications by the Ministry of Health (1957), the American Public Health Association (1960) and Taylor (1962). This technique was not designed to ascertain the exact number of organisms present but the maximum number that may be present (Ministry of Health 1957).

Wilson (1935) carried out an extensive investigation into the sources and magnitude of the error in the Standard Plate Count. From the work of Wilson loc. cit. and Jennison and Wadsworth (1940) it is evident that although this method is more precise than the M.P.N. technique, it is subject to quite a large total error.

The Membrane Filter technique also has a higher degree of precision than the M.P.N. technique (American Public Health Association 1960). Its suitability for the enumeration of bacteria from faecal material would probably be limited because of the degree of dilution necessary to obtain an even spread over the membrane and also because the proteinaceous and fibrous nature of pig faeces would tend to block the filter pores.

These three methods of enumeration, i.e. a, b, and c., are also limited by the amount of time, labour, equipment, media and incubator space required when a large number of samples and groups or species of bacteria are being investigated.

Early work on the use of dropping pipettes for colony counts has been reviewed by Irvin and Hall (1955 and 1959). These authors also described three types of dropping pipettes and outlined the Drop technique they have developed. The advantages of this technique over other methods for the enumeration of bacteria were discussed.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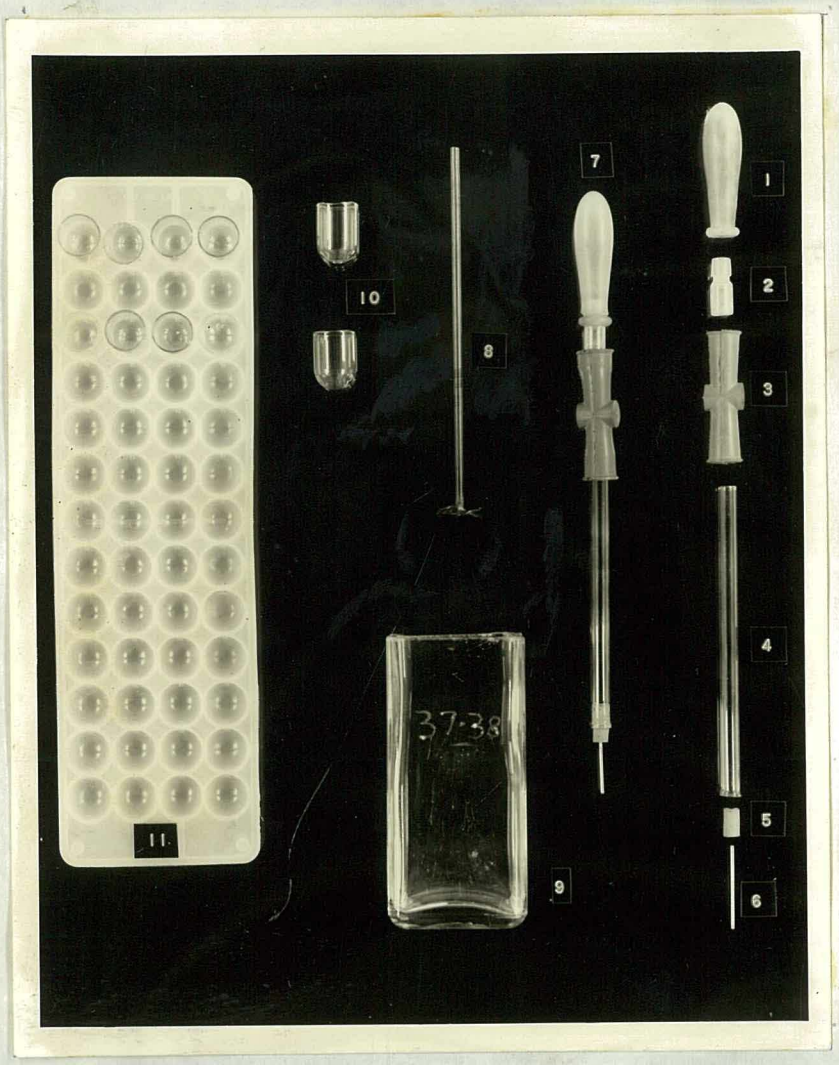

FIGURE 1: A P P A R A T U S (magnification 0.37X)

1. Rubber Teat
2. Glass connecting piece
3. 'Griffin' pinchvalve
4. Glass barrel
5. Rubber Bung
6. Quill
7. Complete dropping pipette
8. Stirrer
9. Mixing vessel
10. Dilution cups
11. Plastic rack for dilution cups.

CHAPTER II

MATERIALS AND METHODS

A. Animals and Sampling

The two faecal samples used in this experiment were collected from randomly selected large White X Berkshire piglets reared at the Massey University College of Advanced Research Piggery. On day (a) the sample was collected from an eight week old piglet and on day (b) a nine week old piglet. As soon as the piglet defaecated a sample (10g. approx) was placed in a sterile container and transported to the laboratory.

B. The Dry Technique

1. Apparatus Used

A photograph illustrating some of the apparatus is presented in Figure 1.

(i) Dropping Pipette

The quill of a dropping pipette consisted of a 1 in. length of the shank of an 18 S.W.G., stainless steel hypodermic needle. Both ends were cut off and finely ground at right angles to the shank. It was necessary to remove any burr formed on the inner or outer edge of the ends of the quill.

The rubber bung was pierced with a hot needle to allow insertion of the quill. The bung was then pushed into the barrel of the pipette. When in position it was important that the top of the quill was level with the upper surface of the bung and that both fitted firmly. This step was aided by moistening the inner surface of the barrel with water.

The pipette was charged and dilutions were mixed by squeezing the pinchvalve and manipulating the rubber teat. When a dilution series was prepared and plates or dilutions were inoculated, the pipette was charged, the teat was removed and the inoculum or diluent in the pipette was controlled by means of the pinchvalve. This allowed the drops to form by gravity.

The pipettes were calibrated by allowing 25 drops of quarter-strength Ringer solution to fall slowly into a watchglass of known weight and immediately reweighing. This was repeated five times for each of the 36 pipettes used. It was found that drops from all the pipettes used had an average weight of 0.021 g. with an error of $\pm$ 0.0005g. or $\pm$ 2%.

The pipettes were placed in test tubes closed with cotton wool bungs and sterilised by autoclaving for 30 minutes at 121°C. It was necessary to renew the rubber bungs after about ten autoclavings as they tended to shrink. This was neither time consuming nor costly.

(ii) Mixer

The stirrer consisted of a 5" long shaft to one end of which was attached a paddle having four sharp edged blades. The diameter of the paddle was 0.7in. The stirrer was driven by a 'Skil' ¼in. power drill (Model 503, Type 5, 2500 R.P.M.) which was clamped in the vertical position in a retort stand.

(iii) Mixing Vessel

The vessels were of glass, 2 in. square and 4 in. high. This type of vessel was found to be the most satisfactory in which to mix faeces and a diluent. No vortex was formed and particles were broken down by hitting the side of the vessel as well as by coming into contact with the blades of the stirrer.

Both the stirrers and the mixing vessels (one pair for each sample or sub-sample) were placed in a dressing drum and sterilised by autoclaving for 30 minutes at 121°C.

(iv) Dilution Cups and Rack

The cups were 5/8in. diameter, round bottom tubes, 3/4in. high which fitted firmly into a plastic ice cube tray. The cups were placed in cans and sterilised by dry heat at 180°C for 2 hours. They were placed in the rack with the aid of sterile, curved, fine pointed forceps.

2. Procedure

Bacterial counts were made on a solid differential medium by a modification of the Drop Technique of Davis and Bell (1958 and 1959). This technique makes two major assumptions and is subject to the following limitations:-

(a) that each colony develops from only one cell and

(b) that only the organisms desired produce the observed characteristic colonies.

(i) Diluent

The diluent used on all occasions was sterile quarter-strength Ringer solution. This solution was prepared by adding together the indicated amounts of the following stock solutions and making up to 1 litre with distilled water.

12.5 mls of 16% Sodium Chloride solution
1.25 " " 4% Sodium Bicarbonate "
1.25 " " 2% Magnesium Chloride "
1.25 " " 4% Calcium Chloride "
1.25 " " 4% Potassium Chloride "

The solution and the siphon were autoclaved at 121°C for 30 minutes prior to use.

(ii) Medium

The medium used was Phenol Red Lactose Agar (Difco).

(iii) Preparation of Plates

On both occasions plates were poured the day prior to sampling because considerable time was involved in preparation and pouring of the medium. The agar was cooled to about 48°C prior to pouring to reduce steaming and subsequent condensation. All plates were poured direct from the flask. This method not only saved labour and the use of test tubes, but avoided further heating of the medium.

To prevent confluent growth of colonies and to reduce the absorption time of the drops of inoculum, it was necessary to dry the plates before use. The standard procedure was:-

(a) allow the medium to solidify;

(b) invert each plate and tilt the bottom so that its edge rested on the inside edge of the lid;

(c) leave the plates in this position on the bench overnight; and

(d) on the following day incubate the plates for 2 to 3 hours at 37°C. in the upright position with lids slightly raised.

(iv) Initial Dilution

All weighings, dilutions and inoculations were made inside a culture hood.

Each sample was divided into a number of sub-samples, there being six sub-samples on day (a) and five on day (b). The sub-samples were weighed in sterile mixing vessels. They were initially diluted by weight, 1g. of sub-sample to 99g. of sterile quarter-strength Ringer solution

siphoned in from a 1 litre flask.

Sets of four dilution blanks per sub-sample were prepared, using sterile dilution cups and a sterile pipette. Each set comprised: one of 20 drops and three of 10 drops each, of sterile quarter-strength Ringer solution.

The initial dilution mixture was blended for two minutes at 2500 R.P.M. using the apparatus described earlier. One drop of the blended mixture was added to the first cup of a set, being that containing 20 drops of diluent, thus by giving a 10^{-1} dilution.

(v) Subsequent Dilutions and Inoculation of Plates

The plates were removed from the incubator and checked for dryness of both the lids and the surface of the medium. The bottom of each plate was divided into quadrants by marking with a grease pencil, there being four quadrants per dilution of inoculum. The top of each plate was marked with the appropriate code number.

Using a fresh, sterile pipette, the 10^{-1} dilution was mixed by aspirating and expelling five times. Two drops were then added to the next dilution cup (1:10 giving a 10^{-2} dilution) and the plates were inoculated, one drop being placed in each of four quadrants of a plate. This process was repeated for the higher dilutions, up to a 10^{-6} dilution.

The following rules were observed when inoculating a plate:-

(a) The quill of the pipette was kept vertical.

(b) The drop was allowed to form slowly, and

(c) The tip of the pipette was no higher than one inch above the medium.

Rules (a) and (b) were necessary to reduce drop size variation and Rule (c) to prevent the drop from splashing.

The drops were spread by moving the plate in an epicycloid motion, care being taken not to allow the drops to run together. The plates were placed on the bench to dry.

(vi) Incubation

When dry, the plates were incubated aerobically and in the inverted position at 37°C for 24 hours.

(vii) Counting

Counts were made in the four quadrants of the dilution which produced the greatest number of colonies without signs of confluence or of gross diminution in colony size due to overcrowding (Miles and Misra 1938) On the medium used, members of the Tribe Escherichease produce canary yellow colonies. It was the number of this type of colony which was recorded.

TABLE I

The log number of coliforms per g. of faeces on each of the two sampling days as determined in quadruplet from a number of sub-samples.

Day (a)

Sub-Sample	1	2	3	4	5	6
Drop						
1	8.40	8.51	8.45	8.42	8.52	8.49
2	8.32	8.46	8.42	8.38	8.60	8.56
3	8.20	8.46	8.48	8.34	8.58	8.57
4	8.48	8.45	8.43	8.15	8.52	8.43

Day (b)

Sub-Sample	1	2	3	4	5
Drop					
1	8.45	8.11	8.48	8.28	8.45
2	8.40	8.32	8.49	8.23	8.34
3	8.36	8.28	8.56	8.38	8.46
4	8.23	8.38	8.48	8.45	8.30

Analysis and components of variance

sis of variance						Components of variance		
e	d.f.	S.S.	M.S.	F				
TS (P)	1	0.0555	0.0555	1.79	N.S.	$\sigma^2 d$	$+4\sigma^2 s$	$+4k\sigma^2 p$
AMPLES (S) w P	9	0.2795	0.03106	4.49	**	$\sigma^2 d$	$+4\sigma^2 s$	
(D) w S w P	33	0.2281	0.00691			$\sigma^2 d$		
	43	0.5631						

N.S. Not significant at the 5% level ($P>0.05$)

** Significant at the 1% level ($P<0.01$)

CHAPTER III

RESULTS

The log number of coliforms per g. of faeces from the piglets on day (a) and day (b) and the analysis and components of variance are presented in Table I.

It can be seen from Table I that the difference in counts between the two piglets was not significant and that the difference in counts between Sub-Samples w Piglets was significant at the 1% level.

The mean log counts and their standard errors, of this organism for the sample from the two piglets were:

8.44 ± 0.036 (day a) and 8.37 ± 0.039 (day b) per g. of faeces. The standard errors for these mean counts were estimated from the formula presented in Table II.

The highest and lowest counts on day (a) were 8.15 and 8.60 (1.4×10^8 and 4.0×10^8) respectively and on day (b) 8.11 and 8.56 (1.3×10^8 and 3.6×10^8) respectively. The standard error for any individual count was ± 0.042 and was estimated from the formula presented in Table II.

Prior to estimating the components of variance it was necessary to determine the value of k (Table I) because there were an unequal number of sub-samples between the two sampling days.

TABLE II

Formulae used for estimating standard errors.

i) S.E. of the mean counts

$$S.E. = \pm\sqrt{\frac{(SwP)\ M.S.}{24 \text{ (day a) or } 20 \text{ (day b)}}}$$

(ii) S.E. of individual counts

$$S.E. = \pm\sqrt{\frac{(DwSwP)\ M.S.}{4}}$$

Estimates of components of variance and their percentage contribution

Component	σ^2_d	σ^2_s	σ^2_p
Estimate	0.00691	0.00604	0.00111
%	49.16	42.96	7.88

Estimated standard errors of a mean for D, drops per sub-sample, and S, sub-samples per pig.

Sub-Samples	1	2	3	4	5	6
Drops						
1	0.114	0.078	0.065	0.057	0.051	0.047
2	0.095	0.067	0.055	0.047	0.042	0.039
3	0.089	0.063	0.052	0.045	0.040	0.036
4	0.089	0.063	0.052	0.045	0.040	0.036

The formula used for estimating k was:

$$k = N - \frac{n_1^2 + n_2^2}{N}$$

Where n_1 = No. of sub-samples for day (a)

n_2 = No. of sub-samples for day (b)

$N = n_1 + n_2$

The estimates of the components of variance and their percentage contribution are presented in Table II. Standard errors of a mean for D, drops per sub-sample, and S, sub-samples, per piglet (Table II) were calculated from the formula:

$$S.E. = \pm \sqrt{\frac{\sigma^2 d + D\,(\sigma^2 s)}{D \times S}}$$

Where $\sigma^2 d$ = Estimates of variance between drops

$\sigma^2 s$ = Estimates of variance between sub-samples

D = Number of drops

S = Number of sub-samples.

Table II shows that as the number of drops per sub-sample increased up to three the standard errors decreased, after which they remained the same for each number of sub-samples. Similarly, as the number of sub-samples increased, the standard errors decreased, but in diminishing amounts.

CHAPTER IV

DISCUSSION

The non significance of the difference between the two piglets for the counts of coliforms was probably fortuitous. The piglets were randomly selected, were of different ages, came from different litters and the samples were collected on different days.

Examination of the estimates of the components of variance (Table II) shows that the contribution of the differences between Drops w Sub-samples w Piglets to the total variation was slightly higher than the differences between Sub-samples w Piglets. This would indicate that the variation in distribution of coliforms between the various sub-samples of either of the samples collected was slightly lower, than the variation in distribution within any one sub-sample after dilution and mixing. It must be assumed however that each organism had an equal opportunity to grow and develop into a colony. The total contribution of the differences between each of these two sources to the observed variation was 92.12%. Although these differences were statistically significant they may not be of practical importance because of the large numbers involved, the small relative differences between the highest and lowest counts on each of the two days, and the probable variation in the distribution of bacteria in the faeces of pigs.

In the experiment reported here the variance of an individual observation, i.e. one drop from one sub-sample, was: $\sigma^2 s + \sigma^2 d = 0.01295$ (Table II). The standard deviation of the method including all drops

and sub samples was: $\sqrt{0.01295} = 0.114$. The Coefficient of Variation of the total method was: $\frac{0.114}{8.40} \times \frac{100}{1} = 1.4\%$, where 8.40 = overall mean count for the experiment. The degree of precision of this method was considered to be satisfactory and the method was used in the experiments described in Part II.

For the cultural enumeration experiments in Part II the combination of 1 sub-sample per piglet and 2 drops per sub-sample was chosen. From Table II it can be seen that the expected standard error of a mean for this combination is $\pm$ 0.095. In this experiment the overall mean log count for coliforms was 8.40 per g. of faeces. The expected total error of this combination would be 1% approx. By adding and subtracting one standard error($\pm$ 0.095) from the above mean and reconverting the resulting figures to natural numbers, the new standard error expressed as a percentage was $\pm$ 22%.

Provided that there is no bias because of any inherent fault/s in technique the values obtained by this combination may be too high by 24% or too low by 20%.

In this experiment the Drop Technique only was tested so that a direct comparison of the accuracy of this method with that of other methods as determined by other workers for different sources of material would be invalid. However, the accuracy of these methods of enumeration is of interest.

In determining the accuracy of the M.P.N. technique, Halvorson and Ziegler (1933) found that when 5 tubes are used for each of 3 tenfold dilutions, the M.P.N. from the tables may be too high by 260% of the true

value, or too low by 70%.

From an investigation into the accuracy of the Standard Plate Count, Wilson (1935) concluded that:

> ....' in routine milk analysis a margin of at least ± 90 per cent. should be allowed on any count of raw milk based on a single plate. An allowance of ± 64 per cent. should be made for counts based on 2 plates, and of ± 52 per cent. for counts based on 3 plates. These figures are calculated on the assumption that a standard technique is used ......'

Thus it is concluded that, provided there is available a suitable medium and a satisfactory method for suspension without destruction of bacteria, the numbers of each group or species of bacteria present in piglet's faeces can, for comparative purposes, be estimated with sufficient accuracy by the Drop Technique outlined and the combination chosen. Also, because of its known degree of precision and that it is far more economical in terms of physical outlay and components, it is preferable to the other methods mentioned.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. A modification of the Drop Technique for the enumeration of bacteria from piglet faecal samples was developed and tested.

2. A dropping pipette was developed, tested and found to be satisfactory for use in making colony counts.

3. Other apparatus used in the Drop Technique is described.

4. Results showed that there were significant differences in counts of coliforms between both Drops v Sub-samples and Sub-samples v Piglets. It was suggested that the magnitude of these differences may not be of practical importance.

5. The Drop Technique was considered to be sufficiently accurate and more economical than the other methods available for the enumeration of bacteria from piglet faeces.

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PART II

STUDIES ON THE FAECAL MICRO-FLORA AND MICRO-FAUNA OF THE YOUNG PIG AS INFLUENCED BY DIET, AGE AND TIME OF WEANING.

CHAPTER I

REVIEW OF LITERATURE

A. Introduction

There are two main methods available with which to study the intestinal flora and fauna of the pig. These are:-

(i) Microscopy - Direct microscopic examination of fresh or preserved samples from various parts of the alimentary tract.

and

(ii) Cultural methods - The isolation and enumeration on suitable culture media of various microbial or protozoan species from the mixed population found in the alimentary tract.

To date, the source of samples examined by these techniques has been restricted to either fresh faeces or material removed from various parts of the alimentary tract immediately after slaughter.

A method of obtaining samples for digestion studies from various parts of the alimentary tract of the living pig by a re-entrant fistula has been investigated (Cunningham, Friend and Nicholson 1962). However, this method is restricted to weaned pigs fed on finely ground rations. Ludvigsen and Thorbek (1961) mentioned that they used an 'artificial' canula to obtain samples from the caecum of pigs for pH, volatile fatty acid and lactic acid determinations but give no details. No reports concerning the bacteriological examination of material obtained from the living pig by cannulae or fistulae have been found in the literature.

The problems involved and the limitations of the techniques mentioned previously in fundamental work on the normal flora of the pig alimentary tract have been discussed by Baker (1946), Baker and Nasr (1947),

Baker and Harriss (1947-48), Larson and Hill (1955) and Fuller and Briggs (1962).

The principal views concerning these problems and limitations are:-

(i) It is not possible, at the present time, to grow in vitro many of the representative microbial species found in the alimentary tract of the pig.

(ii) Microscopic examination of intestinal contents (fresh or stained smear) can be confusing due to the heterogeneity of the material, the occurrence of atypical forms of bacteria and the presence of dead organisms which may not give characteristic staining reactions.

(iii) Management, diet, breed and age of the animal together with the collection and handling of samples may cause as much variation in the composition of the bacterial population as that produced by any experimental condition imposed.

(iv) Different culture media vary in their ability to support the growth of any one species or group of organisms, even though the media employed are selective for or can differentiate between the organisms in question. There is no conformity between groups of workers with regard to the types of media employed. Consequently, comparison of the results obtained by one group of workers with that of another group may be misleading and unwarranted.

(v) Cultural or microscopic studies using faecal samples may be performed over a period of time, but only give information applicable to one region of the alimentary tract. Similar studies on material collected after slaughter can give information on all regions of the alimentary tract but can only be made at one time for any particular pig.

B. The Faecal Micro-flora of the Pig

1. The Influence of Age

Samples of meconium from baby pigs farrowed by hysterectomy were found to be free of bacteria (Larson and Hill 1955). Also, it has been reported that the intestinal tract of the naturally born pigs is devoid of microbial growth, (Wilbur 1959; Wilbur, Catron, Quinn, Speer and Hays 1960; Smith and Crabb 1961 and Smith 1961). All the above workers have found that within 48 hours of birth bacterial populations of the pig intestinal tract reach their greatest density in terms of viable cells (10^{10} organisms per g. faeces approx.).

Komarev (1940) cited by Fuller and Briggs (1962) detected the presence of Escherichia coli, streptococci and lactobacilli in the faeces of one day old pigs; spore bearing anaerobes at three days and spirochaetes at ten days of age.

Wilbur (1959) and Wilbur et al (1960) found that faecal counts of total aerobes, total anaerobes, lactobacilli, streptococci, staphylococci, moulds and yeasts declined slowly from one to 14 days of age when piglets were weaned onto dry diets. Log counts for the first three organisms mentioned approached 10.0 per g. faeces at one day of age and 8.5 at 2 weeks of age. Counts for the other organisms were slightly lower. Counts of all organisms rose sharply during the first few weeks after weaning to values just below those attained at one day of age and then dropped gradually until the pigs reached market weight (200 lbs approx.). Coliforms showed an over decreasing count from one day of age to market weight except for a 'brief' increase between 3 and 5 weeks of age.

Smith and Crabb (1961) and Smith (1961) observed that faecal counts of Escherichia coli, Clostridium welchii, streptococci and bacteroides decreased with age up to 24 weeks when the experiment was terminated. There was no apparent upward trend after weaning at eight weeks. However, lactobacilli counts did not decrease as the piglets grew older but followed a steady high count throughout (10^9 organisms per g. faeces). These findings were based on observations made on seven pigs which came from three litters, two from one farm, and one from another. No indication was given of the post weaning diet or if pre-weaned piglets were supplemented with a creep feed.

In an experiment designed to show the effect of antibiotics and copper sulphate dietary supplements on the pig gut flora, Fuller, Newland, Briggs, Braude and Mitchell (1960) found that in the 5 unsupplemented pigs (8 to 24 weeks of age) used as controls, streptococci, lactobacilli and coliforms were the main constituents of the faecal flora. Counts of these organisms were within the range 10^5 - 10^9 (streptococci and lactobacilli) and 10^4 - 10^7 (coliforms) per g. faeces. Their results showed that counts of these three organisms fluctuated between the sampling time intervals, but there was no discernible trend with the age of the pigs. Similar results were obtained by Willingale and Briggs (1955) with pigs of the same age.

Fuller and Briggs (1962) reported, without giving references, that the occurrence of Clostridium welchii in faeces is correlated with the age of the pig.

They state that:-

> "Large numbers are detectable at three days of age but from then on the numbers decline and after weaning at eight weeks it is difficult to isolate any of these organisms".

The only support given to this statement was that of Horvath, Seeley, Warner and Loosli (1958) and Andrla and Briggs (unpublished) who were unable to isolate clostridia from older pigs.

Subsequently, Andrla and Briggs (1962) in an experiment involving 18 pigs (9 from each of two litters) have found that from 12 to 15 weeks of age faecal counts of Clostridium welchii decreased steadily until at 15 weeks none were isolated. This organism reappeared at 31 weeks and steadily increased in number until 33 weeks when the number of pigs was reduced to 12, 9 of which were fed diets supplemented with antibiotics. In the three unsupplemented pigs used as controls, counts of Clostridium welchii fluctuated within the range 10^3 - 10^7 per g. of faeces until the pigs were 42 weeks old.

Smith and Crabb (1961) have reported high counts of Clostridium welchii in the faeces of young pigs and that these counts decreased rapidly after 2 weeks of age. However, these workers were able to isolate small numbers of this organism from pigs 2 weeks to 6 months of age. Similar observations were made by Larson and Hill (1955).

Wahlstrom, Merril and Johnson (1952) found no evidence of clostridia type organisms in pigs removed from their dams at 48 hours of age and reared under laboratory conditions in wire bottom cages.

SUMMARY

From the literature examined it is evident that:-

(i) The alimentary tract of the newborn pig is sterile.

(ii) Most of the groups of organisms usually found in the faeces of pigs are present during the first week of life and that the numbers of organisms within each group are relatively higher during this period than at any other time.

(iii) There is a decrease in the numbers of organisms in these groups during the first 6 - 10 weeks of the piglet's life, but weaning at 2 weeks of age causes a temporary upsurge in microbial density.

(iv) After 6 - 10 weeks there is conflicting evidence for the influence of age. However, results from experiments specifically designed to examine age effects suggest that there is a gradual decline in the density of most groups of organisms as the pigs become older.

(v) There is a possibility that age has no effect on the numbers of some of the groups of organisms, e.g. lactobacilli, and that others may be absent from the faecal flora for a period of time, e.g. Clostridium welchii.

2. The Influence of Diet

Until recently, little has been done under well defined and controlled conditions concerning an intensive investigation of how the faecal bacterial flora varies with diet.

Steps were made in this direction by Quinn et al (1953 a, b.), Willingale and Briggs (1955) and Horvath et al (1958). However in these experiments the highly complex diets used would provide numerous sources of variation; also relatively small numbers of animals were studied bacteriologically.

The first of these three groups of workers concluded that there was no "normal flora" in the pig gut and suggested that the existing flora was a reflection of the quality and quantity of the ration consumed. Conversely the other two groups considered that the intestinal flora was "buffered" and not readily subject to change.

In a series of four experiments involving 53 piglets sampled weekly from one day to 12 weeks of age Larson and Hill(1955) found that faecal counts of total anaerobes, total aerobes, Escherichia coli, enterococci, clostridia and yeasts fluctuated within each experiment independent of whether the piglets were fed milk or a solid diet. Also, differences between experimental groups on different diets and at different ages were no greater than differences within experiments. An exception was lactobacilli which varied from very low counts to being the predominating organism dependent on the particular experiment reported. Although each of these experiments represented a somewhat different dietary regime, the change in diet cannot be directly implicated as other variables were also present.

The influence of diet on the faecal flora of young pigs has been studied by Wilbur (1959) and Wilbur et al (1960). Purified diets were used, with casein as the source of protein and either beta-lactose or raw corn starch as the source of carbohydrate. The piglets (48) were weaned at 2 weeks and fed on the above diets to 6 weeks of age. All faecal organisms (see page 20) with the exception of the total anaerobes and lactobacilli were lower in numbers when lactose was the carbohydrate fed as compared with starch.

These authors postulated that diet and age have an interelated effect on the flora of young pigs. They suggested that the similarities in counts of the organisms studied during the pre- and post-weaning period for the lactose fed pigs reflected the similarity of the diet at those times. Conversely the starch diet provided a sharp change in substrate for the faecal flora.

It has been established that the pig's ability to utilise lactose is high initially and decreases with increasing age (Bailey, Kitts and Wood 1956 and Walker, 1959). Walker (1959) notes that although lactose activity per unit weight of intestine decreases with age, the total weight of the intestine increases and the total lactose activity stays about constant. In contrast, raw cereal starch is not digested rapidly until the pigs are 23 to 25 days of age or even older, and it is possible that the digestion of raw starch by young pigs is restricted by inability to rupture the starch granule (Hudman, Friend, Hartman, Ashton and Catron, 1957., Braude, Dollar, Mitchell and Porter, 1958., Cunningham, 1959., Lucas and Lodge, 1961.).

In the discussion, Wilbur (1959) stated that:-

> 'When considering only substrate concentration in the large intestine, the recent studies on digestive enzymes of the baby pig provide the basis for some of the effects observed. For example, the rather poor utilisation by the pigs of starch in the initial period, provided relatively large quantities of this undigested carbohydrate as substrate in the large intestine, but as the experiment progressed, feed consumption increased and the pig's ability to utilise the starch was improved resulting in a tendency of these counts to plateau at about five weeks of age.
>
> Conversely, the pig's ability to utilise lactose is rather high initially and is generally considered to decrease with increasing age. This would provide more available carbohydrate for microbial growth in the posterior area of the digestive tract as time elapses, yet not to the extent as with starch feeding. With organisms which do not respond in a manner indicated by the above patterns of carbohydrate availability, other factors such as protein utilisation, pH, gastrointestinal secretions, etc. may be more intimately involved.'

Mansson and Olsson (1961 a,b,c. and 1962 a, b, c.) performed a series of experiments using diets which were not designed to meet conventional feeding standards, but were expected to induce changes in the intestinal flora.

In the first two of these experiments (Mansson and Olsson 1961 a, b.), 24 pigs were used, 7 in the control groups and 17 in the experimental groups. These workers found that in the pigs which were fed the experimental diet (digestible protein 14.29%, calcium 1.75% and phosphorous 0.97%) there was a marked change in the faecal counts of Clostridium perfringens (welchii) compared with those fed the control diet (digestible protein 7.8%, calcium 0.66% and phosphorous 0.4%).

The ration for the control group was based upon cereals and wheat bran. For the experimental group fish meal replaced some of the cereal in order to increase the protein and calcium levels.

For the first 3 weeks of the experiment there were no marked differences in faecal counts of enterococci, coliforms or Clostridium perfringens between the control and experimental groups. After 3 weeks on experiment the number of Clostridium perfringens in the experimental group rose from $<10^3$ to 10^6 per g. faeces and remained at the higher level, whereas counts of this organism for the control group remained at $<10^3$ per g. faeces. Enterococci counts in both groups were similar (10^8 per g. faeces approx.) and coliform counts were significantly higher for the experimental than for the control group, 10^6 and 10^5 approx., respectively.

Concomitant with the change in flora the pigs of the experimental group developed parakeratosis and their appetite and rate of gain decreased. No clinical abnormalities were observed among the control animals. About 5 or 6 weeks following the occurrence of parakeratosis zinc sulphate was added to the drinking water of the experimental group. From this point onward there was an improvement in weight gain and skin lesions but the increase in counts of Clostridium perfringens and coliforms persisted.

A later experiment (Hansson and Olsson 1961, c.) showed that with the same experimental diet just described plus zinc (50 p.p.m.) as zinc carbonate, similar changes occurred in the faecal flora of 6 pigs but no skin lesions were observed and weight gains were satisfactory.

The addition of 1% citric acid to this experimental diet fed to 12 pigs (Mansson and Olsson 1962, c) resulted in lower faecal counts of Clostridium perfringens ($<10^4$ per g. faeces) and enterococci ($<10^7$), and similar counts for coliforms to those found in earlier experiments by these authors. The weight gains of the pigs were satisfactory throughout the experiment even though some pigs developed slight parakeratosis.

Results similar to those of earlier experiments were found by these authors (Mansson and Olsson 1962, b) when they fed an experimental diet high in vegetable protein (soybean meal) to 5 pigs. No faecal Clostridium perfringens were detected in the first 2 weeks, but after that time they increased rapidly to 10^4 per g. and the pigs developed skin lesions typical of parakeratosis.

Finally Mansson and Olsson (1962c) fed diets similar to those described in earlier experiments (Mansson and Olsson 1961 a, b.), to 13 pigs divided into 3 experimental groups. They found that there was a clear difference in faecal counts of Clostridium perfringens between the 3 groups. In group 1 (control diet) the mean log count was 1.10, in group 2 (experimental diet fed dry) 4.70 and in group 3 (experimental diet fed wet) 6.00 organisms per g. Only the pigs given the high protein diet dry developed parakeratosis and the numbers of Clostridium perfringens in the faeces of these pigs increased rapidly after 2 weeks on experiment. Numbers of this organism in those pigs given wet feed were not high until the seventh week. The addition of 30 p.p.m. zinc to the diet of pigs with parakeratosis improved the skin lesions but did not alter the intestinal flora.

In these later experiments (Mansson and Olsson 1962 b,c,) histamine activity of blood and histaminase activity of blood serum of the 17 pigs were evaluated every 2 weeks. Regardless of diet or flora counts, no demonstrable changes of the values took place during the experiments, although individual variations of histaminase activity were found.

SUMMARY

(a) All the experiments of authors reviewed in this section, apart from those of Wilbur (1959) and Wilbur et al (1960),

either

(i) were not specifically designed to examine the effect of diet on the faecal population of micro-organisms but had other objects in view,

or

(ii) did not employ a sufficient number of experimental animals on which to base valid conclusions.

(b) There are three other factors which make it difficult to compare the results obtained by different workers. These are:-

(i) Different diets were used in all the experiments mentioned here.

(ii) In the majority of cases workers used diets which would provide numerous sources of variation.

(iii) The starch diet used by Wilbur (1959) and Wilbur et al (1960) and the high protein diet of Mansson and Olsson (1961 a, b, c, and 1962 a, b, c.) did not conform to conventional feeding standards.

(d) With the above limitations in mind it was apparent from the work reviewed that:-

(i) In young pigs the faecal flora can be influenced by the type of diet the pigs receive.

and

(ii) Compared with standard diets, high protein-high calcium diets can cause a marked increase in the number of Clostridium perfringens(welchii) found in the faeces.

3. The Influence of Management

Experiments have been designed to examine the effect on the faecal flora of rearing pigs singly or in groups. These experiments have been confined to the following. Determination of the differences in flora between:-

(i) pigs individually housed in wire bottom crates without access to their own faeces and pigs individually or group housed on concrete with access to their own faeces (Quinn et al 1953 a, b, and Larson and Hill 1955).

and

(ii) Individually and group fed pigs housed on concrete with access to their own faeces. (Willingale and Briggs 1955, Wilbur 1959 and Wilbur et al 1960).

The last two groups of workers found that individually fed pigs had better weight gains and slightly lower counts of faecal bacteria. This was noted by Willingale and Briggs (1955).

Wilbur (1959) suggested that the better weight gains of the individually fed pigs might be related to less competition between pigs as compared with group feeding. Also, that the lower coefficients of variation with group feeding indicated that there was some influence of one pig on another on their faecal flora. No reliable conclusions can be drawn from this experiment in this respect because other variables were also present.

The other two groups of workers (Quinn et al 1953 a, b, and Larson and Hill 1955) found that individual or group rearing of pigs with or without access to their own faeces had little effect on the faecal flora.

There are no reports in the literature of experiments designed to find out what effect the sow has on the development of the faecal flora of her litter. However, Larson and Hill (1955) in an experiment with pigs obtained by hysterectomy and kept in individual isolation units until they were 8 weeks old found that they developed a 'typical' flora independent of the presence of other pigs. These pigs did not receive colostrum and were never in contact with their dam or litter mates.

Wilbur (1959) observed that there was a significant litter influence on the density of various groups of organisms. He suggested that litter effects must be directly related to physiological characteristics of the individual pig as influenced by genetics and the environment immediately following birth. This litter influence has been observed to a limited extent in pigs as old as 5 months of age.

Papers published regarding the effects of inadequate nutrition and poor management on the faecal flora of pigs and the presence of potential pathogens in the faeces of apparently healthy pigs have recently been reviewed by Fuller and Briggs (1962).

SUMMARY

(a) From the work reviewed here it appears that group or single rearing of pigs with or without access to their own faeces has little qualitative or quantitative effect on the faecal flora. However, two factors complicated the interpretation of the results of these experiments. These are:-

(i) With the exception of the pigs used by Larson and Hill (1955), all pigs were reared by their sow for at least 2 weeks before the experimental conditions were imposed.

and

(ii) In the experiment of Larson and Hill (1955), the pigs reared in groups received different diets from those reared singly.

(b) The statement of Larson and Hill (1955) that pigs reared in isolation developed a 'typical' flora is a little misleading, because, this term has yet to be clearly defined.

(c) The observation made by Wilbur (.959) that there was a significant litter influence on the faecal flora is of considerable interest. It suggests the sow may have a marked influence on the development of the faecal flora of her litter.

4. The Influence of Chemotherapeutics as Dietary Supplements.

It has been established by a number of workers that the addition of certain antibiotics to the diet of pigs increases their growth rate and/or food conversion efficiency. In an attempt to ascertain the reason for these effects, some workers have made bacteriological examinations of the intestinal contents and faeces of pigs receiving diets with and without an antibiotic supplement. Complete references to these experiments can be found in the reviews by Braude, Kon and Porter (1953), Stockstad (1954), Jukes (1955), Finland (1956), Quinn (1956), Merck (1957), Cunha (1958) and Luckey (1959). These reviewers indicate that the total microbial population is generally not reduced, disease producing strains do not generally emerge and no agreement between papers relating to specific changes in flora is seen.

Similar conclusions were reached in later work by Fuller et al, Op.cit., page 195 with penicillin and chlortetracycline. These authors noted that dietary supplements of copper sulphate (250 p.p.m. Cu) caused a reduction in the number of streptococci and a change in the type of streptococci and lactobacilli found in the faeces of pigs, compared with those receiving unsupplemented diets. These changes could not be correlated with the growth stimulating effect of this compound.

Bacteriological examination of the faeces of pigs kept under ordinary commercial conditions on many different premises revealed that lower numbers of lecithinase-producing (α-toxin) Clostridium welchii were found in the faeces of those animals fed on diets containing either tetracyclines or penicillin, than those fed diets that did not contain antibiotics (Smith 1959). There was no difference in the total number

of these organisms present between the pigs fed on chlortetracycline supplemented diets and those fed on diets without antibiotic, but there were smaller numbers in the pigs fed on diets supplemented with penicillin. Interpretation of the results from this experiment was complicated by the fact that there were dietary differences between the pigs fed on antibiotic supplemented diets and those on unsupplemented diets.

Andrla and Briggs, Op cit, page 42 studied in vitro the lecithinase activity of Clostridium welchii isolated from the faeces of pigs 9 - 15 weeks of age. They found that as the faecal population of Clostridium welchii declined in number so also did their lecithinase activity. The reverse process occurred after 30 weeks of age when as the numbers of Clostridium welchii increased the lecithinase activity was restored. These authors suggested that the absence of this organism from the faeces of pigs 15 to 30 weeks of age was due to natural antibiosis which may be functional during that period. In the same experiment it was found that antibiotic supplementation of the diet caused a sharp decline in the numbers of Clostridium welchii in comparison with the control group. Also, as the numbers of this organism declined so did their lecithinase activity though this could not be correlated with any significant weight gains. The decrease in the number of Clostridium welchii due to chlortetracycline supplementation of the diet is in contrast with the findings of Smith (1959).

Michel (1961) in in vitro experiments showed that the micro-organisms of the stomach and small intestine of the pig can cause the breakdown of sugar with the formation of lactic acid which can in turn

be reduced to volatile fatty acids in the small intestine. It was found that the addition of chlortetracycline to the cultural medium, in amounts which corresponded to those used in the diet, inhibited the breakdown of glucose without affecting the amounts of protein and nucleic substances formed by the micro-organisms. This author suggested that this is a possible reason for the more efficient use of energy of the feed when antibiotics are given.

Larson and Hill (1960) observed that there was a lower metabolic activity of the micro-organism and lesser amount and variety of amines present in the ileum contents of young pigs which received low levels of chlortetracycline compared with pigs which received no antibiotic. They suggested that these effects may contribute to the sparing of nutrients for the host animal and in this way contribute to its thriftiness.

SUMMARY

(a) For earlier work in this subject the reader is referred to the summary by Luckey (1959) of the proposed modes of action of antibiotic growth stimulation.

(b) The later work reviewed here is in agreement with some of the proposals set out by the above reviewer.

(c) It is apparent from the last four authors mentioned in this section that one antibiotic, chlortetracycline, affects the gut flora in a number of ways, namely:-

(i) Reduction of the numbers of Clostridium welchii

(ii) Reduction in the lecithinase activity of Clostridium welchii

(iii) Inhibition of the breakdown of glucose by the intestinal micro-organisms.

(iv) Depression of the metabolic activity and inhibition of protein breakdown by the intestinal micro-organisms.

C. The Micro-flora of the Alimentary Tract of the Pig as estimated immediately after slaughter.

1. The Stomach

The stomach, once thought to be sterile, has been shown to have a bacterial flora similar to that of the feed (Horvath et al 1958). They found that lactobacilli were consistently present in the stomach at levels more than 10^3 organisms per g. greater than those found in the feed. The numbers of the other organisms studied, except moulds, were found to be about 10^2 organisms per g. higher in the stomach than in the feed.

Alexander and Davies (1963) were able to isolate lactobacilli from the stomach contents of 8 in 10 pigs and found that these organisms were present within the range 10^6 - 10^8 per g. which is in agreement with Horvath et al (1958).

Dickinson and Mocquot (1961) found that the mean log count of coliforms from observations made on the gastric contents of 125 pigs was 4.63 organisms per g. (range 1.00 - 6.70). Raibaud, Caulet, Galpin and Mocquot (1961) examined 27 samples of gastric contents and found that the mean log count of streptococci was 4.98 (range 2.00 - 6.30). The last two groups of workers did not make a bacteriological examination of the feed. Their figures are between 10^2 - 10^3 lower than those found by Horvath et al (1958) and the differences may reflect the differences in the bacterial populations of the feed and/or the methods used for the enumeration of these organisms.

2. The Small Intestine

Wilbur (1959) and Wilbur et al (1960) observed that regardless of diet the ileum contained very much larger numbers of micro-organisms than the duodenum. Approximate log counts of the various organisms found in the duodenum were: lactobacilli, total aerobes and total anaerobes 6.0 organisms per g., coliforms and staphylococci 4.0 and streptococci, moulds and yeasts 3.0. It was also found that the pH values of ilial contents were about 1 unit higher than those of the duodenal contents. These results are in agreement with those of Hansson and Olsson (1961 a,b,c. 1962 a,b,c.) and Larson and Hill (1955) although counts of all organisms obtained by the latter group of workers were higher for both positions and showed greater differences. Hansson and Olsson (1961 a,b,c.1962 a,b,c) observed that throughout their experiment there were no significant differences between ilial and duodenal counts of Clostridium perfringens.

Other workers (Horvath et al 1958 and Dickinson and Mocquot 1961) have enumerated various groups of bacteria found in the contents of the small intestine, but do not state from which part of this organ the samples were removed.

3. The Large Intestine

Throughout their experiments Larson and Hill (1955) observed that counts of lactobacilli and enterococci were consistently higher in the caecum than in the ileum. Also in 75% of the observations made, counts of total anaerobes, total aerobes and Escherichia coli showed a similar trend. The differences in counts of the above organisms between these two sections of the alimentary tract were not as marked as the differences between

duodenal and ilial counts. There were no consistent differences in the number of clostridia found in the ileum and the caecum; also the counts of yeasts were always higher in the ileum.

The above results of Larson and Hill (1955) were in accordance with those of Wilbur (1959) and Wilbur et al (1960). The last two groups of authors found that the number of coliforms, streptococci, staphylococci, moulds and yeasts in the ileum and caecum of pigs fed on the lactose diet (see page 25)., were respectively lower than those obtained for the pigs fed on the starch diet. However, within each dietary treatment the counts of these organisms were higher in the caecum than in the ileum. The counts of total anaerobes, total aerobes and lactobacilli showed only slight variations due to dietary differences. The difference in pH levels between these two sections of the gut was greater in the pigs fed the lactose diet, viz 6.8 (ileum) and 5.6 (caecum) compared with 6.3 (ileum) and 6.1 (caecum) for the pigs fed the starch diet.

Dickinson and Hocquot (1961) are in agreement with the above as regards differences in coliform counts between the caecum and ileum.

Horvath et al (1958) found that there were no consistent differences in counts of lactobacilli, enterococci, coliforms, moulds and yeasts between the small and large intestines. However, no mention was made of which part of the two gut sections was sampled.

Mansson and Olsson (1961 a,b,c. 1962 a,b,c.) were unable to find consistent differences between counts of enterococci or coliforms in the ileum and mid-colon. In the pigs which had been fed a high protein diet counts of Clostridium perfringens (welchii) were greater in the mid-colon than in the ileum.

Other species or groups of organisms, apart from those already mentioned, are known to be present in the caecum. It appears from the literature reviewed below that the type of substrate present can affect the density of each group or species.

Willingale and Briggs (1955) state that:-

> 'Vartiovaara & Roine (1942) incubated caecal contents with cellulose in vitro and showed that decomposition of the cellulose occurred. They isolated two cultures but neither was pure; one, an anaerobic, short-chain, Gram-positive coccus was able to decompose cellulose. In further experiments Vartiovaara, Roine and Poijarvi (1944) fed cultures of these organisms to two pigs on a diet containing spruce sulphite pulp; eleven days after administration of the cultures the proportions of the cellulose digested had increased from 30.6 and 49.6% respectively to 69.2%. Later, when aspen sulphate pulp was fed, the authors claimed that the pigs were digesting 87 and 96.8% respectively of the cellulose in the diet.'

The dietary and physiological circumstances which affect the breakdown of starch have been discussed by Baker and Nasr (1947). Experimental work by Baker, Nasr, Morrice and Bruce (1950) has shown that, in pigs, maize starch is digested in the small gut and relatively few granules reach the caecum. Conversely potato starch granules accumulate in the caecum in large numbers where they are attacked by bacteria. They suggested that the differences in digestibility between different sources of starch are due to the presence or degree of development of and resistance to enzymes action of the surface membranes of the starch granules.

Baker et al (1950) also performed a series of in vitro experiments on the caecal contents of pigs collected after slaughter.

The pigs had been fed on diets containing large amounts of untreated potato and untreated maize starch. They state that:-

> 'From these experiments it became clear that the mixed microbial population of the pig's caecum is able to ferment starch and a wide variety of soluble carbohydrates. Breakdown of starch and soluble carbohydrates is accompanied by the deposition of iodophil polysaccharide within many of the bacterial species and by the production of acid and gas. Soluble starches are more rapidly fermented than structural starches and structural maize than structural potatoe starch. .....
>
> The results suggest that in the caecum of the pig lactobacilli, enterococci, yeasts and coliforms do not play a leading part in the breakdown of starch. On the other hand, sporing rods are predominant in caecal samples which, on incubation, attack sugars as well as starch.
>
> Lactobacilli, enterococci, yeasts and coliforms are also capable of attacking a wide range of soluble carbohydrates; this means that the number of species in the pig's caecum capable of metabolising the carbohydrate products of starch decomposition is greater than the number capable of decomposing starch itself. ..... The demonstration of free amylase in the fresh caecal contents confirms the finding that soluble products of starch decomposition are actually present in the pig's caecum in life. ..... The phenomena which we observed in the pig's caecum show the influence on the mixed bacteria already present of the arrival of a new substrate - starch. The general population shows an increase in fermentative activity and a special group of spore-forming rods assumes sufficient dominance to become recognisable as a special facies.'

Baker et al (1950) also found that:-

(i) The predominant sporing rod was an iodophil strain of Clostridium butyricum which was motile and gram positive.

(ii) This organism was exclusively responsible for the production of the α-amylase found in these experiments.

(iii) Clostridium butyricum in pure culture is a far more powerful agent of breakdown for untreated starches, e.g. raw potato , than culture filtrates of amylase. They state that:-

> 'It may be significant that the bacteria of the special facies exercise their action in situ in close apposition to the surface of the granule, since Petrie (private communication) has shown that in plant tissues the corrosion of starch by diastase depends upon actual contact of the cell mitochondria with the surface of the granule. The available evidence emphasises, therefore, the importance of supermolecular organisation in the relative rates of breakdown of different starches and starch products by micro-organisms, cell-free bacterial enzymes and digestive secretions.'

(iv) Clostridium butyricum is capable of synthesising some members of the vitamin B-complex in significant amounts namely, riboflavin, nicotinic acid, panthothenic acid, pyridoxine and folic acid.

(v) Pigs can live satisfactorily on a diet deficient in B vitamins which contains raw potato starch. This phenomena is termed refection.

Nasr (1950) cited by Fuller and Briggs (1962) suggested that the stimulatory effect of the B vitamins produced by Clostridium butyricum on the lactobacilli and streptococci in the intestine would lead to an increase in production of lactic acid and a reduction in pH favouring absorption of the synthesised vitamins by the host animal.

Alexander and Davies (1963) were unable to isolate lactobacilli from the colon contents of 10 pigs. The caecal contents of these animals contained only small numbers of lactobacilli, $<10^3$ organisms per g. These authors found that the large intestine contained large numbers of lactate producing streptococci, 10^8 per g. approx. Lactate fermenting bacteria, namely Veillonella gazogenes, Peptostreptococcus elsdenii and a lactate fermenting strain of Escherichia coli, were also isolated from the large

intestine but in smaller numbers. In their paper, Alexander and Davies (1963) gave no indication of the diet the pigs received prior to slaughter.

An examination of the data published by Wilbur (1959) revealed that there was close agreement in microbial counts between the caecum and rectum. The ratio of rectal counts to caecal counts for the 24 pigs slaughtered were as follows:-

Total aerobes	1.07
Total anaerobes	1.05
Lactobacilli	1.05
Streptococci	1.08
Coliforms	1.02
Staphylococci	1.10
Moulds and Yeasts	0.98

The enumeration of coliforms, lactobacilli and streptococci in the faeces and caecal contents of 22 pigs slaughtered at bacon weight was carried out by Willingale and Briggs (1955). They found that, within each pig, the counts from both sources were similar, although there was a tendency for the caecal counts to be lower than the corresponding faecal counts. The ratio of faecal to caecal counts was estimated from their results (converted to logs) and are as follows:-

Coliforms	1.04
Lactobacilli	1.03
Streptococci	1.05

They suggested that faecal counts of the organism studied would provide a numerical index of those present in the caecum, but similarities in all types of organisms from these sources could not be inferred.

From the data of Dickinson and Mocquot (1961), who made observations on 103 caecal samples and 266 faecal samples, it was found that the ratio of faecal to caecal counts of coliforms was 1.06. Also, the average counts of this organism for both sites were in close agreement with those of Wilbur (1959).

A similar examination of the data published by Raibaud et al (1961) revealed that the ratio of faecal to caecal counts of enterococci was 1.04. This data came from 110 faecal samples and 42 caecal samples. The average faecal and caecal enterococci counts were similar to those obtained by Wilbur (1959).

The results of Larson and Hill (1955) were in agreement with the last 4 groups of workers, though it was not clear from their paper if the caecal counts of the slaughtered animals were made at the same time as the faecal counts.

SUMMARY

(a) From the literature reviewed in this section it is evident that:-

(i) Counts of most species or groups of organisms follow a pattern of increasing numbers from the anterior to the posterior end of the intestinal tract.

(ii) The counts of each group or species found in the stomach, except lactobacilli, may be a reflection of the numbers in the feed.

(iii) There is a greater increase in microbial density between the duodenum and the ileum than between the ileum and caecum.

(iv) The difference between the faecal and caecal populations of at least five groups of organisms is very small and that the faecal count of each of these groups would provide a numerical index of those present in the caecum.

(v) The presence of large quantities of starch or cellulose in the caecum can have a marked effect on the relative proportions of the species or groups of organisms that make up the total microbial population in this region of the alimentary tract.

(b) A number of authors have suggested that the differences in counts of the various intestinal organisms throughout the alimentary tract may be related to:-

(i) The increase in pH from the anterior to the posterior end of the intestinal tract.

(ii) The presence of intestinal secretions, bile-salts, and digestive enzymes in the duodenum.

(iii) A differential rate of passage throughout the various sections, probably being greater in the duodenum and ileum than the caecum and rectum.

D. The Faecal and Intestinal Micro-fauna of the Pig

Throughout this work, micro-fauna are regarded as being species of Protozoa, generally described as the first, and the lowest, phylum of the animal kingdom.

Pigs have been found to harbour about 40 species of Protozoa (Frye and Meleney 1932, Dunlap 1958, Moore 1959, and Simitch, Chibalitch, Petrovitch and Heneberg 1959) and, with the exception of the sporozoan species, few are known to be pathogenic. In most cases emphasis has been placed on the investigation of the possibility that pigs may act as carriers of some species pathogenic to man, e.g. Balantidium coli and Entamoeba histolytica, rather than the pathogenicity of these species to pigs.

A description of the various species harboured by pigs is presented in books by Craig (1942), Morgan and Hawkins (1948), Kudo (1954), Dunne (1958) and Hagan and Bruner (1961).

As mentioned in the Introduction, the most common species found in the large intestine and faeces of young pigs suffering from scours at the Massey University College of Manawatu Research Piggery was Balantidium coli (B. A. Reynolds pers. comm.).

Balantidium coli has been found to be a common inhabitant of the large intestine, but it rarely causes any visible lesions (Ray 1937, Arean and Koppisch 1956). Simithh et al (1959) found Balantidium coli in the faeces of 61.9% of the 1,800 pigs they examined.

Schumaker (1951) reviewed the work of early authors who studied infections of Balantidium coli in the pig. He concluded that the numbers of this organism present in the large intestine were correlated to the

amount of undigested food residue, including starch granules. The last author also examined the caecal contents of 79 pigs. It was found that, compared with light infections, heavy infections of Balantidium coli in the caecum were accompanied by larger amounts of starch, both microscopic and macroscopic, and an intestinal flora which contained greater numbers of aciduric organisms and lesser numbers of lactose fermenters and proteolytic anaerobes. The observation that grain diets favour infection by this organism was in agreement with the findings of Arean and Koppisch (1956).

Beck, Boucher and Poppensiek (1943) reported an outbreak of scouring accompanied by melena in young pigs. Faecal examination revealed a heavy infestation of Balantidium coli. Post mortem examination disclosed a severe haemorrhagic colitis and generalised enteritis. Histopathological study of sections of the small intestine and colon determined a marked penetration of the swollen enteric mucosa by Balantidium coli. No bacteriological examination of the faeces or intestinal contents was mentioned.

In an investigation of swine dysentery on five farms, Enchev, Genev, Mincheva and Mateev (1961) were unable to detect a causal agent other than Balantidium coli. The results of a histological examination of sections of the large intestine were in agreement with those reported by Beck et al (1943). Enchev et al (1961) found no evidence of leptospira, vibrio or bacterial toxins.

Tempelis and Lysenko (1957) have shown that Balantidium coli can produce an enzyme which has hyaluronidase activity. They suggest that an initial lytic factor or mechanical damage is necessary before the parasite can pass through the surface of the intestine. Also, that

hyaluronidase may then cause an enlargement of the lesion by attacking the ground substance between the host cells.

SUMMARY

(a) The pig is known to harbour numerous species of Protozoa any one of which may occur in large numbers. However, there is considerable variation between pigs as regards the species present.

(b) It has been established that Balantidium coli is a common inhabitant of the large intestine of the pig.

(c) The presence of large amounts of starch in the caecum has been shown to favour infection by this organism.

(d) There is conflicting evidence as to whether Balantidium coli is truly pathogenic to the pig or is only a secondary invader.

(e) It appears that young animals are more susceptible to balantidiasis. However, it is not clear whether the decrease in weight gain which accompanies the appearance of Balantidium coli lesions is due to the parasite or if the parasite attacks the host when it is debilitated by other factors.

CHAPTER II

THE EXPERIMENTAL DESIGN

The primary aim of this trial was to study the changes which occur in the flora, fauna and amounts of undigested food material present in the large intestine (faeces) during the early growth of the pig, comparing early and late weaning.

To eliminate the possible influence of sex differences on the counts of organisms, it was decided as far as possible to use only male piglets. However to measure the effect of littermates on each other it was considered that six piglets in any one litter were necessary.

The experiments involved 24 piglets six from each of four litters, and their dams. The piglets from two litters were weaned at 3 weeks of age and the others at 6 weeks. Dates of farrowing and weaning are presented in Table II. Faecal samples were collected from each piglet and its dam at one week after farrowing and each week thereafter until the piglets reached 8 weeks of age. Although this experimental design was considered the most suitable some apparent limitations justify comment.

Since each group of six piglets came from different litters any litter influence could confound the management effect. However, the earlier work by B. A. Reynolds (Pers. comm.) suggested that differences between litters weaned at 3 weeks and 6 weeks were considerable. If half of a litter were weaned at 3 weeks and the other half at 6 weeks the following variables would be introduced.

(i) The piglets weaned at 6 weeks would get more milk than under normal conditions.

(ii) Removing half the litter could have an effect on the sow's milk production.

(iii) By weaning half the litter at 3 weeks the effect the earlier weaned piglets would have had on their littermates is also removed.

(iv) At weaning time it is usual to leave the piglets in familiar surroundings and remove the sow. If half of the litter were weaned at 3 weeks they would have to have been housed elsewhere - a change in environment.

In addition the following problems would arise.

(i) The number of samples that would be examined on on any one day would reduce the accuracy of cultural enumeration because of the differences in time interval between sampling and plating.

(ii) Accommodation for litters was limited because of the number of sows farrowing during the period covered by these experiments.

CHAPTER III

MATERIALS AND METHODS

A. Animals and Sampling

1. Animals Selected for the Trial

The two experiments involved 24 piglets, 6 from each of four litters, and their dams. The piglets were from Large White sows mated to Berkshire boars. The piglets of the two sows in Experiment I were sired by the same boar and the piglets of the two sows in Experiment II were sired by other boars.

Where possible, only male piglets were selected for the Trial. In Experiment I, the litter selected for early weaning consisted of 6 gilts and 6 boars therefore no piglet selection was necessary. The litter selected for late weaning consisted of 5 gilts and 7 boars. The one boar piglet to be excluded from the trial was selected at random. In Experiment II, the litter selected for early weaning consisted of 5 gilts and 5 boars and the litter selected for late weaning, 7 gilts and 5 boars. In each case one gilt piglet was selected at random and included in the Trial.

2. Management and Housing

The piglets were farrowed and reared in New Zealand round houses. All the piglets of a litter were kept together during the course of each experiment. They had access to the sow's feed, water and faeces during the nursing period but were kept in the round house when the sows were allowed out to graze for one hour each day. The male piglets were castrated at ten days of age. All the piglets received (orally at three and ten days of age) a dose of a mixture of saccharated ferrous carbonate

(about 50% Fe CO_3) in Vetemul, a proprietary preparation containing 5,000 I.U. Vitamin A and 1,000 I.U. Vitamin D_3 per gram and 35% free fat. In each of the two experiments, one litter was weaned at three weeks of age and one at six weeks of age. After weaning the piglets were kept in their respective round houses until they were eight weeks old when the experiment was terminated.

The protected zone of each round house was heated by a 250 watt mercury coated heating lamp. This provided a warmer area for the piglets without distressing the sow. The heating lamps were used during the first eight weeks of the piglets' life.

Each round house was cleaned by scrubbing with a solution of chlorate of lime and left unoccupied for two weeks prior to use. While in use, the round houses were cleaned daily and any dirty straw bedding was replaced.

A self-feeder was provided in the creep area for the piglets. An open feeding trough was provided for each sow and an open water trough for the sow and her piglets.

All animals were observed at least twice daily for any abnormal symptoms as well as for consistency of the faeces. Each piglet was weighed at birth and again at three, six and eight weeks of age. At birth each piglet was ear marked, detusked and the number of nipples on each side of the body recorded. The standard procedure for ear marking was for the left ear to receive the marks representing the litter number and the right ear the individual piglet number.

3. DIET

The feed mixtures used for the piglets in these experiments are presented in Table I . In addition, the piglets were fed skim milk from three or six weeks of age depending on when they were weaned. If weaned at three weeks, skim milk was fed at the rate of 2 gallons per litter per day in three feeds increasing slowly to 4 gallons per litter per day at eight weeks. If weaned at six weeks, skim milk was fed at the rate of 3 gallons per litter per day increasing slowly to 4 gallons per litter per day, at eight weeks. Fresh soil (a supplementary source of iron) was provided for the piglets from two weeks of age and was replenished every other day until the piglets were eight weeks of age.

The farrowing and weaning dates of the sows in these experiments are presented in Table II. The daily rations fed to the sows are presented in Tables III - V. Meal mixtures were fed once a day and skim milk or whey and fodder beet three times a day. In addition, the sows were grazed on pasture for one hour each day.

The reasons for the differences between the daily rations fed to the sows are as follows:-

(a) whey was available for the sows until 17th June 1962. After this time a limited amount of skim milk was available for feeding the lactating sows.

(b) In 1962 there was a poor crop of fodder beet which was consumed by 20th July. The fodder beet was fed preferentially to sows with large litters and a restricted amount was fed to sows with small litters.

TABLE I

FEED MIXTURES USED FOR PIGLETS

	STARTER	CARRY-ON 1	CARRY-ON 2
Period Fed	10 to 31 days	32 - 48 days	49 - 63 days
Ingredients	**Parts/100**	**Parts/100**	**Parts/100**
Semolina	15		
Sugar	15		
Buttermilk Powder	60	25	12.5
Malt Meal	10	10	5
Barley Meal		25	62.5
Maize Meal		15	7.5
Wheat Meal		15	7.5
Pollard		10	5
Additives	**Per 100lb mix**	**Per 100lb mix**	**Per 100lb mix**
Mineral Mixture: Boneflour	1lb	1lb	8oz
Mineral Mixture: Ferrous Ammonium Citrate	40 g	40 g	40 g
Mineral Mixture: Copper Sulphate $5H_2O$	25 g	25 g	25 g
Mineral Mixture: Manganese Sulphate $5H_2O$	12 g	12 g	12 g
Aureo (A proprietary mixture containing - 10,000 I.U. Vitamin A/g 2,000 I.U. Vitamin D3/g)	2 oz	2 oz	1 oz
Vetsulfon Ration (A proprietary mixture containing 4% Procaine Penicillin	8 oz	4 oz	2 oz

TABLE II

Dates of farrowing and weaning of the sows in these Experiments

Sow	Litter Code	Date Farrowed	Date Weaned
Experiment I			
Rosie 2nd	L. 1.1	4 May, 1962	25 May, 1962
Ambrosia	L. 2.1	6 May, 1962	17 June, 1962
Experiment II			
Denise 2nd	L. 1.2	29 June, 1962	20 July 1962
Heather	L. 2.2	4 July, 1962	15 August, 1962

TABLE III

Daily Ration of each Sow in Experiment I

Period fed	3 days post-farrowing to weaning
Meat Meal	2lbs
Barley Meal	6lbs
Whey	8 gallons

TABLE IV

Daily Ration of the sow with the three week nursing period in Experiment II

Period fed	5 to 10 days post farrowing	11 to 16 days post farrowing	17 to 21 days post farrowing
Bran	1lb		
Meat Meal	2lbs		
Barley Meal	5lbs	5lbs	7lbs
Cheese		2lbs	2lbs
Fodder Beet		14lbs (2F.U. approx)	
Skim Milk	4gallons	4gallons	4gallons

TABLE V

Daily Ration of the Sow with the six week nursing period in Experiment II

Period fed	3 to 10 days post farrowing	11 to 42 days post farrowing
Barley Meal	5lbs	7lbs
Cheese	2lbs	2lbs
Fodder Beet	14lbs	
Skim Milk	4 gallons	4 gallons

4. Sampling Procedure

Fresh faecal samples were collected from the six piglets and from their dam when the litter was one week old and each week thereafter until the experiment was terminated. The samples were collected at 7a.m. (approx.) on each sampling day. This was found to be the best time because the piglets were rarely disturbed by the attendants prior to this time and seemed to be ready to defaecate when moved from the round house. When the sow was put out to graze, a faecal sample was collected and placed in a labelled, sterile container. Usually the sow moved about the paddock, defaecated, urinated and then took an interest in the pasture. To ensure that there was no confusion, each piglet was placed in a separate wire cage on clean concrete prior to collection of the samples. As soon as each piglet defaecated a sample of faeces was placed in a labelled, sterile container.

Occasionally, when the piglets were very young, it was necessary to induce defaecation by inserting in the rectum a sterile cotton wool swab dipped in glycerine. No observable irritation was produced by this procedure, Larson & Hill (1955).

B. LABORATORY TECHNIQUES

1. Fresh Material

1.1 Enumeration

Bacterial counts were made on solid selective and/or differential media using the technique described in Part I. The use of selective media makes a further assumption necessary and subjects the technique to the limitations thereof. The assumption is that all species of organisms

other than the ones desired fail to multiply at a rate fast enough to produce visible colonial growth in the allotted incubation time.

(i) Diluent

The same diluent and delivery apparatus were used as described in Part I.

(ii) Media Used and Organisms Studied

The groups of organisms studied and the selective and/or differential media used were as follows:

(a) Total anaerobes The medium employed was that used in the Veterinary Pathology and Animal Physiology Department, Massey University College of Manawatu. The composition of the medium was:

Bacto-Yeast Extract	0.5%
Bacto-Proteose Peptone	2 %
Sodium Chloride	0.5%
Bacto-Agar	2 %

The pH of the medium was adjusted to 7.4 with $\frac{N}{1}$ sodium hydroxide prior to autoclaving. Defibrinated rabbit blood (5%) was added aseptically to the above medium at 50°C prior to pouring the plates.

(b) Clostridium welchii (perfringens). As in (a) with the addition of 0.02% Sodium Azide (Lichstein and Soule 1944).

(c) Enterococci M-Enterococcus Agar (Difco Supplement Literature) and Mitis-Salivarius Agar (Difco) plus 10mls of a 0.1% solution of potassium tellurite per litre.[a]

(d) Coliforms Levine E.M.B. Agar (Difco)

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[a] Reasons for using two media are discussed on page 96.

(e) Lactobacilli Rogosa S L Agar (Difco Supplement Literature).

Each week sufficient media was prepared for the two collection days. The media employed for total anaerobes and Clostridium welchii were sterilised by autoclaving in flasks for 15 minutes at 121°C. If not used on the day of preparation, Mitis Salivarius Agar was sterilised by autoclaving in a flask for 15 minutes at 121°C. In Experiment I, the portion of Levine E.M.B.Agar not used on the day of preparation was autoclaved as above, but this lowered its growth promoting ability. The number of colonies produced was found to be lower than that on medium not autoclaved. Therefore in Experiment II it was prepared just prior to pouring the plates.

Rogosa S L agar and M-Enterococcus agar were either used on the day of preparation or were stored in flasks in the refrigerator. At no time were they autoclaved.

The potassium tellurite, tryphan blue, T.T.C., eosin Y and methylene blue were added aseptically to the respective media at 50°C prior to pouring the plates. The sucrose was added aseptically to Mitis Salivarius agar at 80°C prior to pouring the plates.

(iii) Preparation of Plates

Plates were prepared as described in Part I.

(iv) Initial Dilution

The same procedure was followed as in Part I except that one, 1g. sample was used from each pig. Each sample was handled individually in order to measure pig-to-pig variation. Occasionally it was not possible to obtain a full 1g. sample from the very young animals. In such cases the amount of diluting fluid was reduced to that required to give a one percent (W/w) faecal suspension.

(v) Subsequent Dilutions and Inoculation of Plates

The same procedure was followed as in Part I except that two, not four, quadrants of a plate were inoculated per dilution.

(vi) Incubation

All plates were incubated in the inverted position. The blood agar and blood agar plus azide plates were placed in McIntosh and Fildes' jars which were sealed with plasticene. The jars were evacuated with a water vacuum pump and refilled with hydrogen from a cylinder. This process was repeated four times. A vacuum, equivalent to 10cm. mercury, was left in the jars to compensate for the expansion of hydrogen at incubation temperature and to hold the lids of the jars in place. A tube of Methylene Blue Indicator (Society of American Bacteriologists Committee, 1937) was included in each jar. A dish, half filled with calcium chloride, was placed below the inoculated plates to absorb free moisture in the jars.

The Levine E.M.B., Mitis-Salivarius and anaerobic plates were incubated for 24 hours, the M-Enterococcus Agar plates for 48 hours and the Rogosa S.L. Agar plates for 72 hours at 37°C.

(vii) Counting

The same general procedure was followed as is described in Part I. The type of colony observed and the group of organism recorded on the various media used are described below.

(a) Total anaerobes were estimated from the number of colonies appearing on blood agar plates.

(b) Clostridium welchii were estimated from the number of colonies showing double zone haemolysis on blood agar plus azide plates. These estimates were carried out after the plates had been left for 12 hours on the bench at air temperature subsequent to incubation.

(c) Enterococci were estimated from the numbers of pink to dark maroon colonies of 0.5 - 3 mm. diameter appearing on the plates of M-Enterococcus Agar and from the number of blue or brown colonies with a white periphery appearing on the plates of Mitis-Salivarius Agar.

(d) Escherichia coli were estimated from the number of small, dark, colonies with a greenish metallic sheen appearing on the plates of Levine E.M.B. Agar.

(e) Lactobacilli were estimated by counts on the plates of Rogosa S. L. Agar. Several different colonial types were noted, the commonest having a very rough appearance. Other colonial types were smooth and varied in size from little more than pin point to several mm in diameter.

1.2 Characterisation

Individual colonial isolates were taken from various plates throughout the experiments and were characterised by their cultural, physiological and staining properties.

(a) Total anaerobes Isolates from the large, greyish colonies, both haemolytic and non-haemolytic, appearing on Blood Agar plates were inoculated heavily by smearing over the surface of a slant, and stabbing the butt of tubes of Triple Sugar Iron Agar (Difco). The tubes were observed after 24 and 48 hours incubation at 37°C. Tubes were judged as positive for Escherichia coli if a yellow slant and butt with gas formation was observed.

Smears were made from isolates of the main types of colonies appearing on Blood Agar plates. They were stained by the Hucker Modification of the Gram Stain (Society of American Bacteriologists Committee, 1957) hereinafter referred to as Gram Stain.

(b) Clostridium welchii Smears made from isolates of the colonies showing double zone haemolysis on Blood Agar plus Azide Plates were stained with Gram Stain.

(c) Enterococci Isolates from the various colony types found on plates of Mitis-Salivarius Agar and M-Enterococcus Agar were stroked on freshly prepared plates of both Mitis-Salivarius Agar and M-Enterococcus Agar. The type of growth and the colour of the streak were recorded after 24 and 48 hours incubation at 37°C. Smears made from both the colonial isolates and the streaks were stained with Gram Stain.

Further characterisation of the above was carried out by the physiological tolerance tests enumerated below. For the first two of these tests, a meat extract colloid, which ensures optimal growth of the organisms to be studied was chosen as a medium. The composition of this medium is as follows:

Bacto-Meat Extract	0.3%
Sodium Chloride	0.5%
Proteose Peptone	0.1%
Bacto-Tryptose	0.1%
Bacto-Dextrose	0.01%
Bacto-Agar	0.15%

(i) Resistance to an Inhibitory Substance

The above medium containing an additional 6% of Sodium Chloride was brought to pH7.2 with $\frac{N}{1}$ Sodium Hydroxide and was dispensed in 10ml amounts in screwcapped tubes. The tubed medium was autoclaved at 121°C for 15 minutes prior to use. The tubes were inoculated and were observed after 18, 36, and 54 hours incubation at 37°C. Growth was judged by turbidity.

(ii) Growth at pH9.6

Sufficient $\frac{N}{1}$ NaOH was added to the above medium to bring it to pH9.8 prior to autoclaving. The medium was dispensed, autoclaved, inoculated and observed as in (i) above. Growth was judged by turbidity.

(iii) Growth in milk containing 0.1% Methylene Blue

Pasteurised milk was autoclaved in bulk. It was dispensed in 6ml amounts in 6 x $\frac{5}{8}$ in. test tubes, the requisite amount of dye was added and the tubes were stoppered with cotton wool plugs. The tubes were inoculated and observed as in (i) above. Growth was judged by the degree of oxidation (decolourisation) of the Methylene Blue.

(d) Escherichia coli Isolates from the small dark colonies with a greenish metallic sheen appearing on Levine E.M.B. Agar were inoculated on to Triple Sugar Iron Agar. The cultural reactions were ascertained as for Escherichia coli in (a) above.

Smears made from isolates of these colonies were stained with Gram Stain.

(e) Lactobacilli Smears made from isolates of the two main colony types found on plates of Rogosa S.L. Agar were stained with Gram Stain.

Representative isolates of these two colony types were cultured in screwcapped tubes of Rogosa S. L. Broth (Difco Supplement Literature). The tubes were incubated for 72 hours at 37°C. Growth was judged by turbidity. Smears made from the tubes were stained with Loeffler's Alkaline Methylene Blue. Gram Stain was found to be unsatisfactory because of the low pH of the medium.

Representative isolates of the two main colony types found on plates of Rogosa S. L. Agar were stroked on freshly prepared plates of the same medium. The plates were incubated for 72 hours at 37°C and the types of colony produced were recorded.

The media used in these experiments were tested for their selectivity and differential ability by cross-sowing isolates of typical colonies from plates of one medium on to freshly prepared plates of all the media used and observing any growth after 24, 48 and 72 hours incubation at 37°C.

1.3 pH Measurements

All pH measurements were made with a Beckman Model G pH meter according to standard procedure. The instrument was standardised before and checked after daily readings against Beckman Buffer Solution, pH7.0. Approximately ½g. amounts of each fresh faecal sample were thoroughly mixed with 2ml. of sterile, distilled water in chemically clean, flint glass, 1 drachm phials. pH measurements were completed within 1 hour of the arrival

of the samples at the laboratory.

2. PRESERVED MATERIAL

2.1 Preservation

After the first dilution cup in each set had been inoculated as in 1.1, iv, Enumeration, 10mls. of formaldehyde solution 40% w/v were added to each jar containing the initial dilution mixture. The contents of each jar were stirred, centrifuged and all but 25mls. of supernatant and the sediment removed by suction pump. The sediment was suspended in the remaining supernatant and transferred into a plastic stoppered 1oz. phial, labelled and stored.

2.2 Mixing and staining

About 10 mls. of supernatant were removed from each stored phial by suction pump. The remaining supernatant and the sediment was transferred into a weighed, clean, plastic stoppered 1 oz. phial. Then 1.2 mls. of Lugols Iodine (5%) was added and the suspension made up to 20 g. with 10% formalin-saline solution. The phials were stoppered, labelled and attached to the horizontal spindle of a 60 RPM synchronous motor. By this method a thorough end-over-end mixing was obtained in 5 minutes. The final iodine concentration for each suspension was 0.3% (Baker & Nasr, 1947).

2.3 Enumeration

Two techniques were employed in the enumeration of organisms from this material.

(i) The enumeration of small protozoa, undigested starch granules and clones of iodophilic bacteria.

Using a clean dropping pipette, one drop (0.02g) of each mixed sample was placed on a microscope slide and covered with a No.2, 22mm^2 cover glass. The edges of the cover glass were sealed to the slide with immersion oil (Shillaber's Non-Drying, Grade B, High Viscosity) to prevent streaming and drying out while counting. This method of sealing had the added advantage that the whole drop could be counted as it was possible to view the total area under the cover glass.

Counts were made by means of a Watson Bactil binocular microscope, using a 4mm objective, x10 oculars, x1.7 inclining unit and a mechanical stage. An opaque diaphragm with a central rectangular aperture placed in each eyepiece gave the following advantages.

(a) Ease of counting, by restricting the field size and shape.

(b) Increased accuracy, by reducing the possibility of overlapping fields.

To increase contrast, particularly with iodophilic material and organisms, a dark-ground stop was inserted in the filter carrier below the sub-stage condenser.

The total area under the cover glass was examined and the counts were recorded on three separate Veeder tally counters. The total count multiplied by a factor of 1000 afforded an estimate of the number of organisms or undigested starch granules present per gram of faeces.

(ii) The enumeration of Balantidium coli

Balantidium coli were counted by a modification of the method of Schumaker (1950,1951). After thorough mixing of the 1 in 20 suspension, 0.2g. was weighed on to a microscope slide and covered with a 22 x 40mm No: 2 cover glass. The organisms were counted by the same method as in (i) above except that a 16mm objective and x7 eyepieces were used. When more than 10 organisms were counted per slide, a duplicate count was made. An average of the two counts multiplied by the factor 100 afforded an estimate of the number of organisms present per gram of faeces.

2.4 Photomicrography

All the photomicrographs presented in this thesis were taken with a Leica 35mm camera in combination with a Nikon micro-attachment and a Leitz 'Ortholux' microscope.

CHAPTER IV

RESULTS

1. Animal performance

In Experiment I two piglets from litter EWI died at the age of 6½ weeks, one (pig 1.) on the 18th June and the other (pig 2.) on the 19th June. Both piglets appeared to be quite normal on 17th June.

On post morten examination of both piglets, excess peritoneal and pleural fluid was found. Culture of heart blood and liver from both piglets revealed large numbers of haemolytic Escherichia coli in pure culture. From this evidence it was considered that death was due to a coli septicaemia. A predisposing factor could have been chillin due to a power failure on the night prior to the death of the first piglet.

Apart from the two deaths, both general condition and performance of the animals in these experiments were considered to be good. The average piglet weights at various ages; the corresponding piglet average for the litter groups to which the experimental litters belonged, and the average for piglets for the 1962 season at the Massey University College of Manawatu Research Piggery are presented in Table VI .

Table VI shows that there were no obvious treatment differences in liveweight. Except for birthweight, between litter averages varied considerably at corresponding ages. The average three and eight week piglet weights for the litters in Experiment I were respectively 1lb. less and 7lbs. greater than those in Experiment II. The corresponding differences between the litter groups were 0.5lbs. and +.5lbs. which indicates that the litters used were representative of their group with regard to liveweight.

TABLE VI

Liveweight Records (lbs.) for piglets
(Litter, Group and 1962 Season averages).

AGE	BIRTH	3 weeks	6 weeks	8 weeks
LITTER				
EW1	2.8	12.0	35.7	48.3
LW1	2.7	10.3	29.8	47.5
Average For Experiment I	2.75	11.15	32.75	47.9
EW2	2.8	12.3	28.3	41.0
LW2	3.0	11.8	29.7	40.8
Average For Experiment II	2.9	12.15	29.0	40.9
Average for Four Litters	2.8	11.7	30.9	44.4
Average for Litters in the April/May Farrowing Group	2.4	11.0	No Figures	44.6
Average for Litters in the June/July Farrowing Group	2.3	11.5	No Figures	40.1
Average for Litters in 1962 Season	2.7	11.8	No Figures	43.3

Observations on the conditions of the faeces revealed that up to about five weeks of age all piglets had well formed or pelleted faeces ranging in colour from yellow to black. From five to eight weeks of age the faeces were notably softer, being semi-formed, moist, granular and ranging in colour from brown to black. The changes in the appearance of the faeces corresponded to the changes in the creep diet; at 4½ weeks of age the piglets were presented with a feed mixture containing 65% grain meal.

Liquid faeces were only observed twice throughout the course of the two experiments.

2. Characterisation

Two factors limited the number of isolates from selective and/or differential media which were characterised. These are as follows:-

(i) The primary object of the experiments described here was the enumeration of bacteria and this occupied a considerable portion of the author's time.

(ii) The characterisation of organisms should be performed as soon as possible after isolation.

(a) Total anaerobes Isolates of the large, greyish colonies, both haemolytic and non-haemolytic, grown on Blood Agar, all produced a yellow slant and butt with gas formation within 24 hours at 37°C. in tubes of Triple Sugar Iron Agar. All organisms from these colonies were found to be gram negative, non sporing rods. As a confirmatory test, an agar stroke from each of the above tubes was made on plates of Levine E.M.B. Agar. Each of the strokes gave abundant growth and produced a dark streak with a greenish metallic sheen. These organisms were regarded as being Escherichia coli (Levine 1921 cited by Difco).

The three main colony types encountered on plates of Blood Agar are described below:-

(i) Large, greyish colonies both haemolytic and non haemolytic (see above).

(ii) Colonies characteristic of Clostridium welchii (see b.)

(iii) Small colonies which produced pronounced green zones of varying intensity (alpha haemolytic) typical of the viridans group of streptococci. Organisms from these colonies were found to be gram positive cocci which occurred in chains.

A number of other colony types were observed on this medium during these experiments. They did not occur in sufficient numbers or consistently enough to warrant description.

(b) Clostridium welchii In Experiment I Clostridium welchii were estimated from plates of Blood Agar. Because of the large numbers of haemolytic Escherichia coli colonies found on some plates, it was often impossible to count the number of Clostridium welchii present. In Experiment II Blood Agar and Blood Agar plus sodium azide were used and Clostridium welchii were estimated from plates of the latter. The inclusion of sodium azide (0.02%) in the medium restricted the growth of gram negative bacteria without affecting the growth of Clostridium welchii. (Lichstein and Soule 1944).

A description of the colony type regarded as being that of Clostridium welchii and the zones of haemolysis it produced on plates of Blood Agar and Blood Agar plus sodium azide is as follows:-

Results of the physiological tolerance tests performed on isolates from some of the colony types found on Mitis Salivarius Agar and M-Enterococcus Agar

		*Growth in Meat Extract Colloid. (Control)			*Growth in presence of 6.5% NaCl.			*Growth at pH 9.6			** Oxidation of 0.1% Methylene Blue in milk.		
Incubation time in hrs.		18	36	54	18	36	54	18	36	54	18	36	54
Colony Type (From plates of Mitis-Salivarius Agar)	Number of Isolates												
Circular, brown with white periphery	4	+	+	+	+	+	+	+	+	+	-	o	o
Circular, blue with white periphery	3	+	+	+	+	+	+	+	+	+	-	-	o
White, punctiform	2	-	+	+	-	-	-	-	-	-	-	-	-
Blue, circular	2	+	+	+	-	-	-	-	-	-	-	-	-
Brown, circular	2	+	+	+	-	-	-	-	-	-	-	-	-
(From plates of M-Enterococcus Agar)													
Maroon, circular	3	+	+	+	+	+	+	+	+	+	-	o	o
Red, circular	3	+	+	+	+	+	+	+	+	+	-	o	o
Pink, circular	3	+	+	+	+	+	+	+	+	+	-	o	o

*Growth indicated by +, no growth by -

**Oxidation indicated by o, no oxidation by -

Colony types found on plates of Mitis Salivarius Agar, growth on agar stroke of isolates of these colony types on two media, and reaction to Gram Stain of smears from the isolates or streaks

Colony Type	Number of Isolates	Mitis Salivarius 1st Agar stroke	Mitis Salivarius 2nd Agar stroke *	S-Enterococcus 1st Agar stroke	Gram Stain
Circular blue with white periphery. [a]	9	Blue	Blue	Pink to Red	Gram +ve diplococcus
Circular, brown with white periphery. [a]	5	Brown	Brown	Maroon	Gram +ve diplococcus
Large, irregular mucoid	2	Brown mucoid	Brown mucoid	No growth	Gram - ve coccobacillus
White Punctiform	5	White	White	White, poor growth	Gram +ve diplococcus
Blue, circular	13	10 Brown 3 Blue	10 Brown 3 Blue	No growth	Gram - ve coccobacillus
Brown circular, convex	13	Brown	Brown	No growth	Gram - ve coccobacillus
Small, black, circular pulvinate	4	Blue-Black	Blue-Black	No growth	Gram variable rods
Large, brown, granular undulate	9	No growth	-	No growth	Gram - ve, non sporing rods.

* The second stroke is made from an isolate of the first stroke on Mitis Salivarius

a These descriptions differ from those of Chapman (1944, 1946 and 1947) and are discussed on page .

(i) Colony type: Round, entire, convex, 2 to 3 mm in diameter.

(ii) Haemolysis: A primary clear zone surrounded by a wide diffuse zone, which is in turn bordered by a zone of darker red than the surrounding medium.

Organisms from these colonies were found to be gram positive, short, thick rods.

These observations are in agreement with Thompson (1936), Hood and Carr (1941) and Mann (1945).

(c) Enterococci: At least eight different colony types were distinguished on plates of Mitis-Salivarius Agar. A description of these colony types as seen by reflected light together with some of the cultural and staining properties of colonial isolates are presented in Table VII.

Isolates from the large, brown, granular, undulate colonies were also streaked on plates of Phenol Red Lactose Agar. Each of the nine isolates gave abundant growth and produced a canary yellow streak within 24 hours at 37°C. Organisms from the above colonies were found to be gram negative, non sporing rods. These organisms were regarded as belonging to the Coli-Aerogenes Group (Wilson and Miles 1936).

Results of the physiological tolerance tests performed on isolates from some of the colony types found on Mitis-Salivarius Agar are presented in TableVIII.

Four different colony types could be distinguished on plates of M-Enterococcus Agar in Experiment II. A description of these colony types, together with some of the cultural and staining properties of colonial isolates are presented in Table IX.

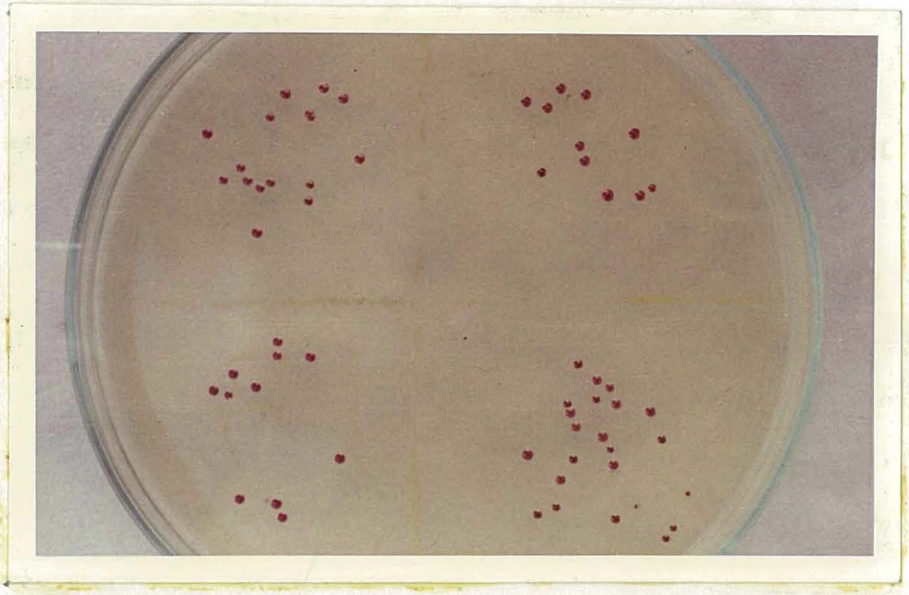


FIGURE 2: Typical red and maroon surface colonies of enterococci on M-Enterococcus Agar (actual size).

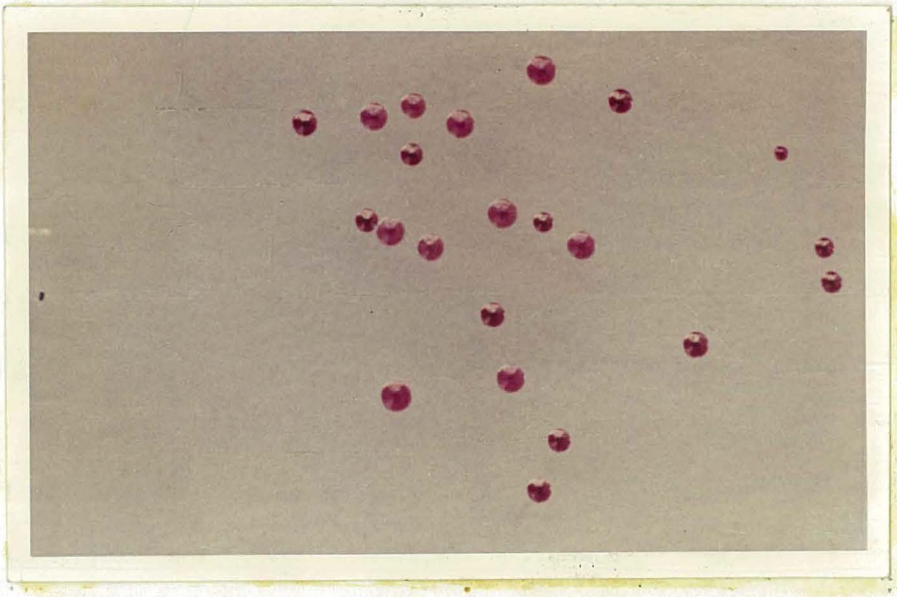


FIGURE 3: The lower right group of the above at a magnification of 2.5X.

The white, punctiform colonies were found on few occasions, and at no time did they overcrowd the other colonies.

Photographs illustrating the morphology of red and maroon colonies are presented in Figures 2 and 3. Figure 2 represents four groups of colonies grown on the surface of M-Enterococcus Agar. These groups grew from four 0.021 g. drops of a 10^{-6} dilution of faeces from a 3 week old pig. Figure 3 illustrates one of these groups of colonies at a magnification of 2.5x.

Results of the physiological tolerance tests performed on isolates from the three main colony types found on M-Enterococcus Agar are presented in Table VIII.

(d) Escherichia coli Isolates of the small, dark colonies with a greenish metallic sheen grown on Levine E.M.B. Agar all produced a yellow slant and butt with gas formation within 24 hours at 37°C in tubes of Triple Sugar Iron Agar. All organisms from these colonies were found to be gram negative, non sporing rods. These organisms were regarded as being Escherichia coli (Levine 1921 cited by Difco).

(e) Lactobacilli A description of the colony types grown on plates of Rogosa S. L. Agar is presented in Chapter III. These colony types are regarded as being characteristic of the Genus Lactobacillus on this medium.

Photographs illustrating the differences in colonial morphology are presented in Figures 4 and 5. Figure 4 represents four groups of colonies, containing both colony types, grown on the surface of Rogosa S. L. Agar. These groups grew from four 0.021 g. drops of the same inoculum as was used in (c). Figure 5 illustrates one of the groups of colonies at a

TABLE IX

Colony types found on plates of M-Enterococcus Agar, growth on agar stroke of isolates of these colony types on two media, and reaction to Gram Stain of smears from the isolates or streaks.

Colony Type	Number of Isolates	M-Enterococcus Agar stroke	Mitis Salivarius Agar Stroke	Gram Stain
Maroon, circular	9	Maroon	Brown	Gram + ve. diplococcus
Red, circular	7	Red	Blue	Gram + ve diplococcus
Pink, circular	8	Pink	Blue	Gram + ve diplococcus
White, punctiform	2	White	White	Gram + ve diplococcus

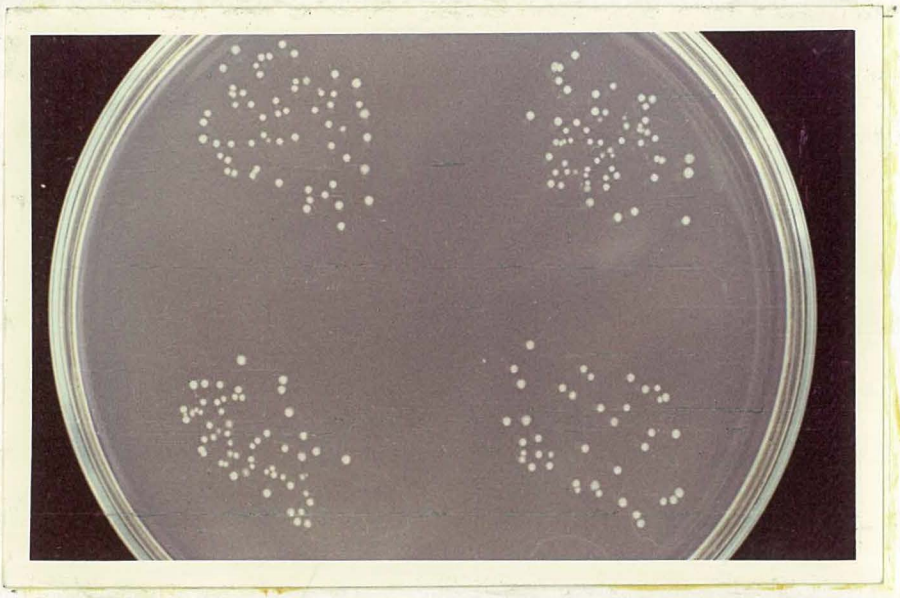


FIGURE 4: Typical rough and smooth surface colonies of lactobacilli on Rogosa S.L. Agar (actual size).

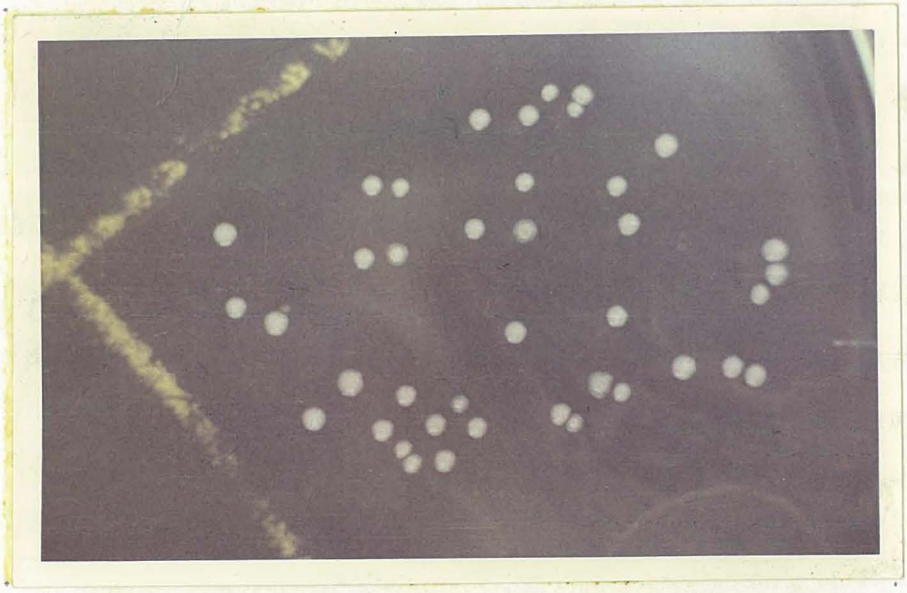


FIGURE 5: The lower right group of the above at a magnification of 2.5X

magnification of 2.5x. Since the smooth colonies were raised and compact, difficulties were encountered in focusing and lighting, and the colonies appear more granular than they are in reality. The rough colonies were characteristically sparse, flat and irregular.

All organisms from both colony types were found to be long, slender (some filamentous) gram positive rods.

In order to determine whether the two colonial types were stable on the medium used, isolates from each type were inoculated over the whole surface of plates of Rogosa S. L. Agar. Isolates from rough colonies produced predominately rough colonies with a few smooth colonies. Isolates from the smooth colonies produced more smooth than rough colonies.

Twenty isolates of the two colony types (8 smooth and 12 rough) were cultured in tubes of Rogosa S. L. Broth. After 72 hours incubation at 37°C, all the tubes were turbid, those from the rough colonies showing flocculation and some deposit on the bottom of the tube. All organisms from these broth cultures were long, slender rods with a tendency to form chains.

Results of the tests made on the selective and/or differential media employed in these experiments are presented in Table X . All media except Mitis-Salivarius Agar performed satisfactorily with the test organisms used.

TABLE X

Growth on agar stroke of colonial isolates from the selective and/or differential media used on four media

Incubation time in hrs.		*Growth on Levine E.M.B. Agar			*Growth on M-Enterococcus Agar			*Growth on Mitis-Salivarius Agar			*Growth on Rogosa S.L. Agar			
		24	48	Colour of Streak	24	48	Colour of Streak	24	48	Colour of Streak	24	48	72	Colour of Streak
Colony Type and Source	Number of Isolates													
(Rogosa SL Agar)														
Rough, white	6	-	-		-	-		-	-		±	+	++	White
Smooth, white	2	-	-		-	-		-	±	White	+	++	+++	White
(Levine E.M.B. Agar)														
Small, dark with greenish metallic sheen	8	+++	+++	Dark with metallic sheen	-	-		5- 3+	5- 3++	Brown	-	-	-	
(M-Enterococcus Agar)														
Maroon, circular	3	-	-		+	+++	Maroon	++	+++	Brown	-	-	-	
Red, circular	3	-	-		+	+++	Red	++	+++	Blue	-	-	-	
Pink, circular	3	-	-		+	+++	Pink	++	+++	Blue	-	-	-	
(Mitis-Salivarius Agar)														
Circular, brown with white periphery	4	-	-		+	+++	Maroon	++	+++	Brown	-	-	-	
Circular, blue with white periphery	4	-	-		+	+++	Pink to Red	++	+++	Brown	-	-	-	

b. Enumeration of faecal bacteria by cultural methods

The upper and lower limits of the method used in these experiments to enumerate the various organisms studied was 10^{10} and 10^{0} organisms per g. of faeces respectively. Occasionally counts were lower than 10^{0} organisms per g. of faeces and these were recorded as 0. No counts were higher than 10^{10} organisms per g. of faeces.

b.a. Faecal examinations of the pigeons

(a) Total anaerobes, bacteroides, enterococci[1] and escherichia coli

These organisms are reported together because the same method of statistical analysis was employed in examining the data of each.

The log number per g. of faeces of these organisms for all pigeons are presented in Appendices I - IV. The statistical analyses of these data and the estimates of the components of variance are presented in Tables XI - XV. Data for each type of organism studied were pooled for analysis.

As mentioned earlier in this chapter two pigeons from litter III died at 6½ weeks of age. Consequently there are no values for these two pigeons for weeks 7 and 8. For each missing item, average values of the remaining pigeons of litter III for the particular organism and week were inserted so that the data were complete for analysis.

The analysis of variance employed was in two parts:-

(i) A 3-way hierarchal classification involving the three nested effects treatment (T), litters (L) and pigeons (P)

and

(ii) A 2-way classification involving weeks (A) and the various interactions with the above effects.

---

1 Streptococci or faecal streptococci

Components of variance and their estimates for the pooled data for the four organisms studied

Components of variance

Component	σ^2_D	$\sigma^2_{AxD:LwM}$	$\sigma^2_{AxL:M}$	σ^2_{AxM}	σ^2_A	$\sigma^2_{P:LwM}$	$\sigma^2_{L:M}$	σ^2_M
Source								
M	1	2	12	24		16	96	192
LwM	1	2	12	24		16	96	
P:LwM	1	2	12	24		16		
A	1	2	12	24	48			
AxM	1	2	12	24				
Ax(LwM)	1	2	12					
Ax(PwLwM)	1	2						
DvS	1							

Estimates of the components of variance and their percentage contribution

Component	σ^2_D	$\sigma^2_{AxP:LwM}$	$\sigma^2_{AxL:M}$	σ^2_{AxM}	σ^2_A	$\sigma^2_{P:LwM}$	$\sigma^2_{L:M}$	σ^2_M	Total
Organism									
Total Anaerobes	0.0087	0.2727	0.0572	0.0075	0.2690	-0.0290	0.0515	-0.0285	0.6025
%	1.27	39.96	7.65	1.10	32.46		7.56		100.00
Escherichia coli	0.0080	0.3707	0.0917	0.0092	0.2498	-0.1044	0.0560	-0.0334	0.8154
%	0.98	45.46	11.25	4.80	30.64		6.87		100.00
Enterococci	0.0177	0.4248	0.6677	-0.2996	0.1894	-0.0308	0.3422	-0.1434	2.3618
%	0.76	18.14	28.51		07.90		14.61		100.00
Lactobacilli	0.0009	0.3655	0.1672	-0.0492	0.1738	-0.0425	0.0399	-0.0195	0.7525
%	1.18	48.28	22.19		23.10		5.25		100.00

TABLE XIV

Analysis of variance for lactobacilli.

Source	df	SS	MS	F	
MANAGEMENT (M)	1	0.94	0.4	<1	N.S.
LITTERS (L) w M	2	9.34	4.67	5.30	*
PIGLETS (P) wLwM	20	17.61	0.8805		
AGE (A)	7	69.27	9.90	6.35	*
A x M	7	10.90	1.56	<1	N.S.
A x (LwM)	14	38.42	2.74	3.73	**
A x (PwLwM)	135	100.03	0.7355	82.64	**
Duplicates w. samples	188	1.68	0.0089		
Total	375	248.19			

N.S. Not significant at the 5% level (P>0.05)

* Significant at the 5% level (P<0.05)

** Significant at the 1% level (P<0.01)

TABLE XIII

Analysis of variance for enterococci

Source	df	S	MS	F	
MANAGEMENT (M)	1	6.04	6.04	<1	N.S.
LITTERS (L) w M	2	67.14	33.57	46.75	**
PIGLETS (P) wLwM	20	14.36	0.7180		
AGE (A)	7	310.68	44.38	26.26	**
A x M	7	11.86	1.69	<1	N.S.
A x (LwM)	14	124.26	8.88	10.24	**
A x (PwLwM)	136	117.95	0.8674	49.00	**
Duplicates w. samples	188	3.32	0.0177		
Total	375	655.61			

N.S. Not significant at the 5% level ($P>0.05$)

* Significant at the 5% level ($P<0.05$)

** Significant at the 1% level ($P<0.01$)

TABLE XII

Analysis of variance for Escherichia coli

Source	df	SS	MS	F	
[illegible] (A)	1	0.03	0.03	<1	N.S.
[illegible] (B) w/ M	2	13.09	6.10	5.80	*
[illegible] w/M	20	22.43	1.12		
AGE (A)	7	103.48	14.78	5.53	**
A x B	7	19.02	2.72	1.01	N.S.
A x (w/M)	14	25.05	1.85	2.47	**
A x (w/M)	136	101.91	0.749	99.36	**
Duplicates vs. samples	168	1.01	0.0080		
Total	375	287.01			

N.S. Not significant at the 5% level ($P>0.05$)

* Significant at the 5% level ($P<0.05$)

** Significant at the 1% level ($P<0.01$)

TABLE VI

Analysis of variance for total anaerobes

Source	df	SS	MS	F	
MANAGEMENT (M)	1	0.42	0.42	<1	N.S.
LITTERS (L) w M	2	11.70	5.85	6.53	**
PIGLETS (P) wLwM	20	17.91	0.8955		
AGE (A)	7	106.91	15.27	11.23	**
A x M	7	9.52	1.36	1.15	N.S.
A x (LwM)	14	16.46	1.18	2.13	*
A x (PwLwM)	136	75.35	0.5540	63.60	**
Duplicates w. samples	188	1.64	0.0087		
Total	375	239.91			

N.S. Not significant at the 5% level (P>0.05)

* Significant at the 5% level (P<0.05)

** Significant at the 1% level (P<0.01)

The analyses were performed and the components of variance derived as outlined by Kempthorne (1952). The degrees of freedom were adjusted to allow for the missing items. The negative estimates of the components of variance have no real meaning (except that they were very small) and were omitted from the calculation of percentage contribution.

The non significance of the main effect, Management, and its interaction for each of the four organisms studied was interpreted as meaning that:-

(i) If there were differences between the two Managements an insufficient number of litters were tested to show this effect.

or

(ii) The real effect of Management was small. Reference to the specific standard errors of the management means for each organism studied (Table VI) indicated that these means were determined accurately.

The differences between Litters w. Management and the interaction with Age (A x L/M) were significant for the four organisms tested (levels of significance shown in Tables V - VIII. The differences between Litters w Management may be inflated because the combining of the two experiments for analysis injects a seasonal effect also.

Age exhibited a significant influence on the four organisms studied.

Examination of estimates of the components of variance showed that for the four types of organisms studied Age plus Age x Litters w Management plus Age x Piglets w Litters w Management contributed between 8% to 9% of the total variation.

Laboratory error (Duplicates w Samples) contributed approximately 1% to the experimental error for each of the organisms studied.

The negative values for the estimates of the effect of Piglets w Litter w Management indicated that although weekly variation between Piglets w Litters w Management (A x (PwLwM) significant at the 1% level for the four organisms) was considerable, the sum of each type of organism for each piglet during the experimental period was not significantly different.

The negative values for the estimates of the components Age x Management and Management indicated that, although Age exhibited a significant influence for the four organisms studied, the difference between Managements from week to week tend to cancel each other out over the experimental period.

It should be recognised that the components of variance were calculated using the missing items inserted. By this process, the Duplicates w Samples variance and Age x Piglets w Litters w Management variance components are unchanged but the other components may be slightly biassed.

The management and litter means and the standard errors for the four organisms studied are presented in Table XVI. The general standard errors applied to the litters of a population and provided a measure of the variation to be taken into account when comparing these litters. The specific standard errors applied to the piglets used and provided a measure of the accuracy of these experiments.

The general standard error of the Litter and Management means for each organism were calculated from the following formula.

$$S.E. = \pm \sqrt{\frac{(LwM)M.S.}{191}}$$

The corresponding specific standard errors were calculated from the following formula.

$$S.E. = \pm \sqrt{\frac{(1+1+M)\ M.S.}{191}}$$

TABLE XVI

Means and their Standard Errors for these experiments.

Management or Litter	MI Weaned at 3 weeks	EWI	EW2	M2 Weaned at 6 weeks	LWI	LW2	Specific standard error a	General Standard error a
Organism								
Total Anaerobes	8.22	8.58	8.07	8.16	7.97	8.35	±0.07	± 0.18
Escherichia coli	8.02	8.15	7.88	7.99	7.77	8.21	±0.08	±0.19
Enterococci	6.94	7.53	6.35	6.69	6.67	6.71	±0.06	±0.42
Lactobacilli	7.23	7.39	7.08	7.33	7.49	7.18	±0.07	±0.15

a Standard errors for MI and EWI were calculated using the adjusted degrees of freedom.

Table XVI shows that there is little difference between the Management or Litter means for each of the four organisms studied. Also the specific standard errors for the piglets used were small and were interpreted as meaning that if these experiments were repeated a number of times, in 66% of them the means would be determined within 1/ of their true values.

Fig.9 LACTOBACILLI

S.E. ±0·25

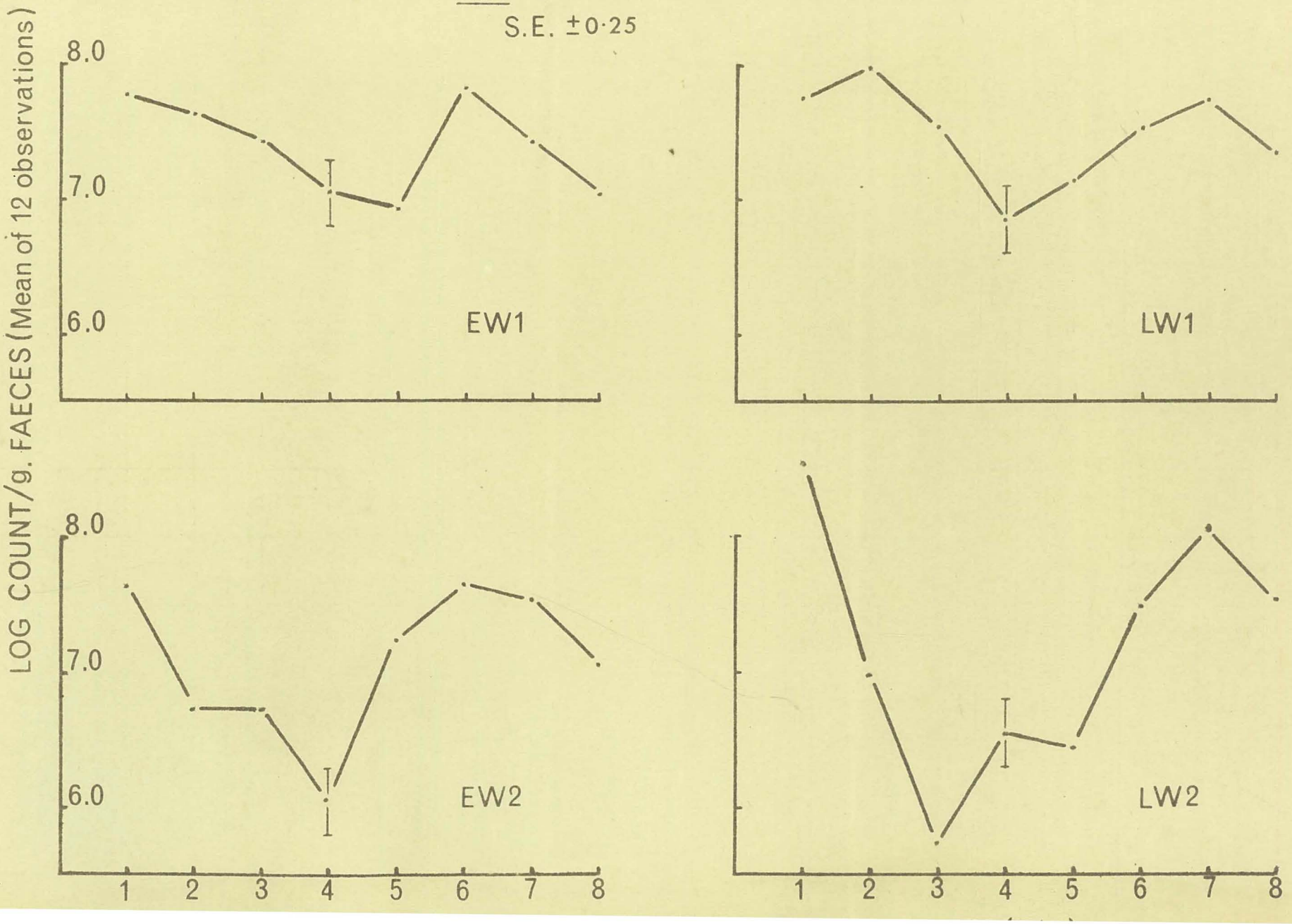


Fig.8

ENTEROCOCCI

Mitis-Salivarius Agar

S.E. ± 0·27

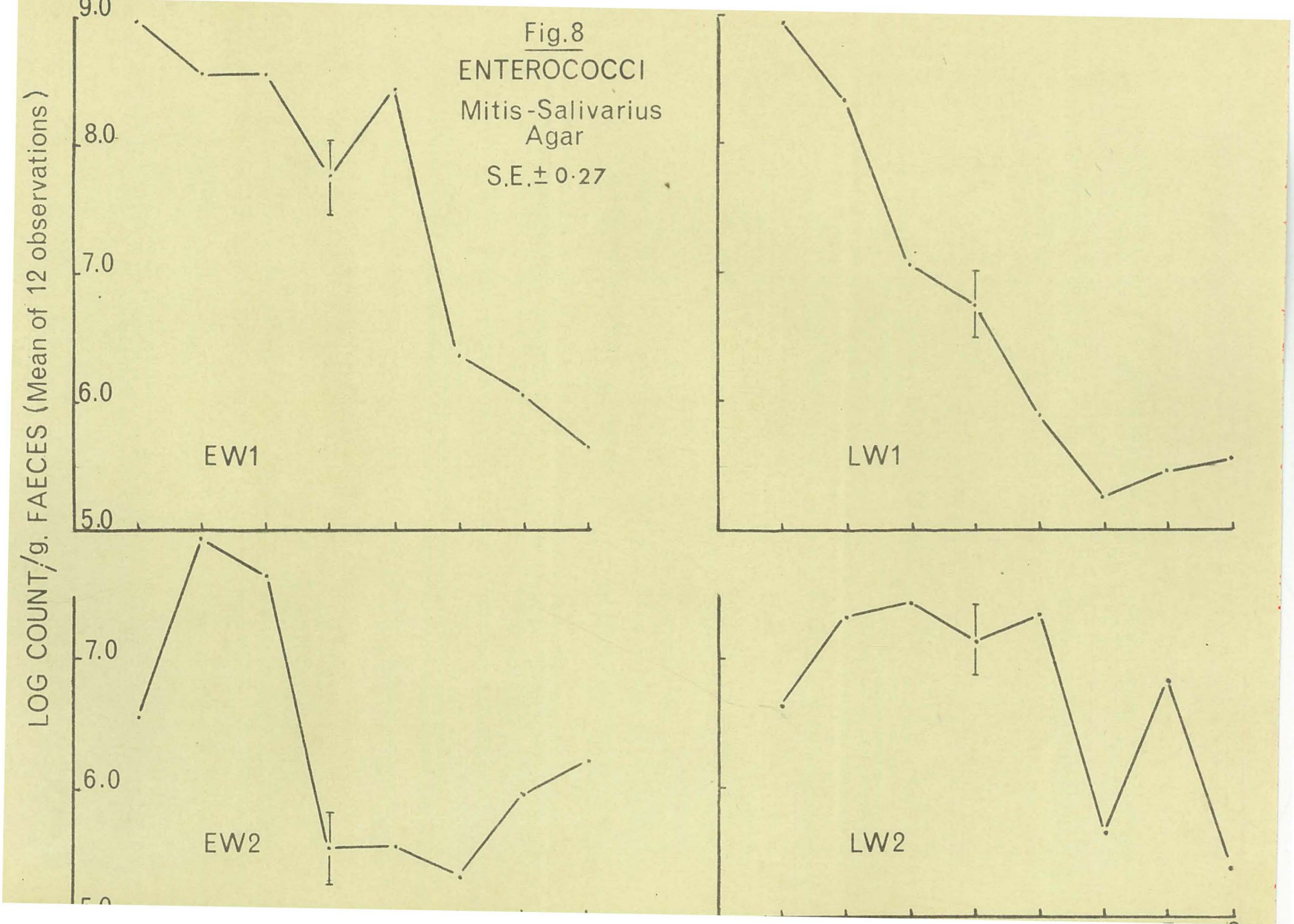


Fig.7 Escherichia coli

S.E. ± 0·25

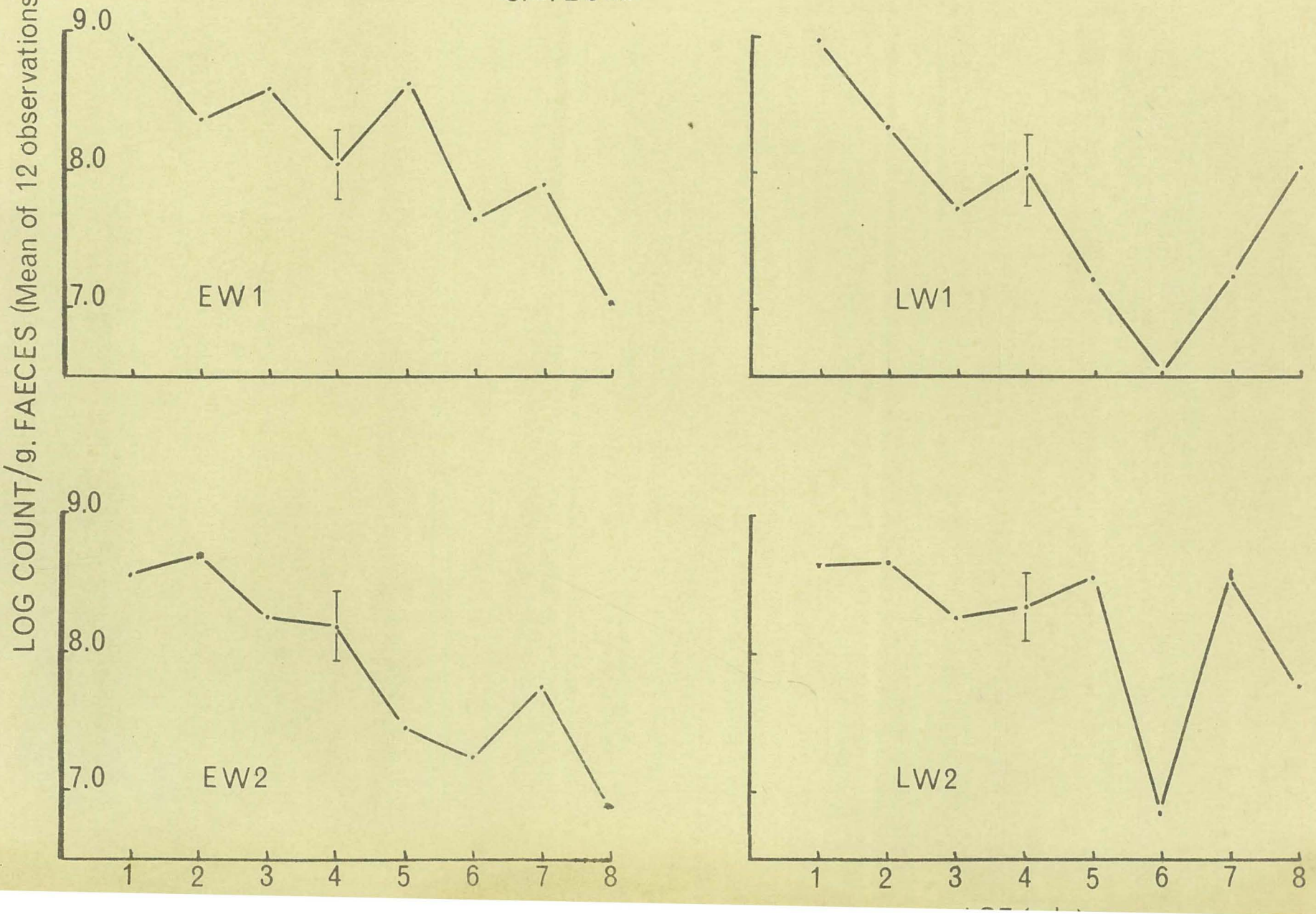
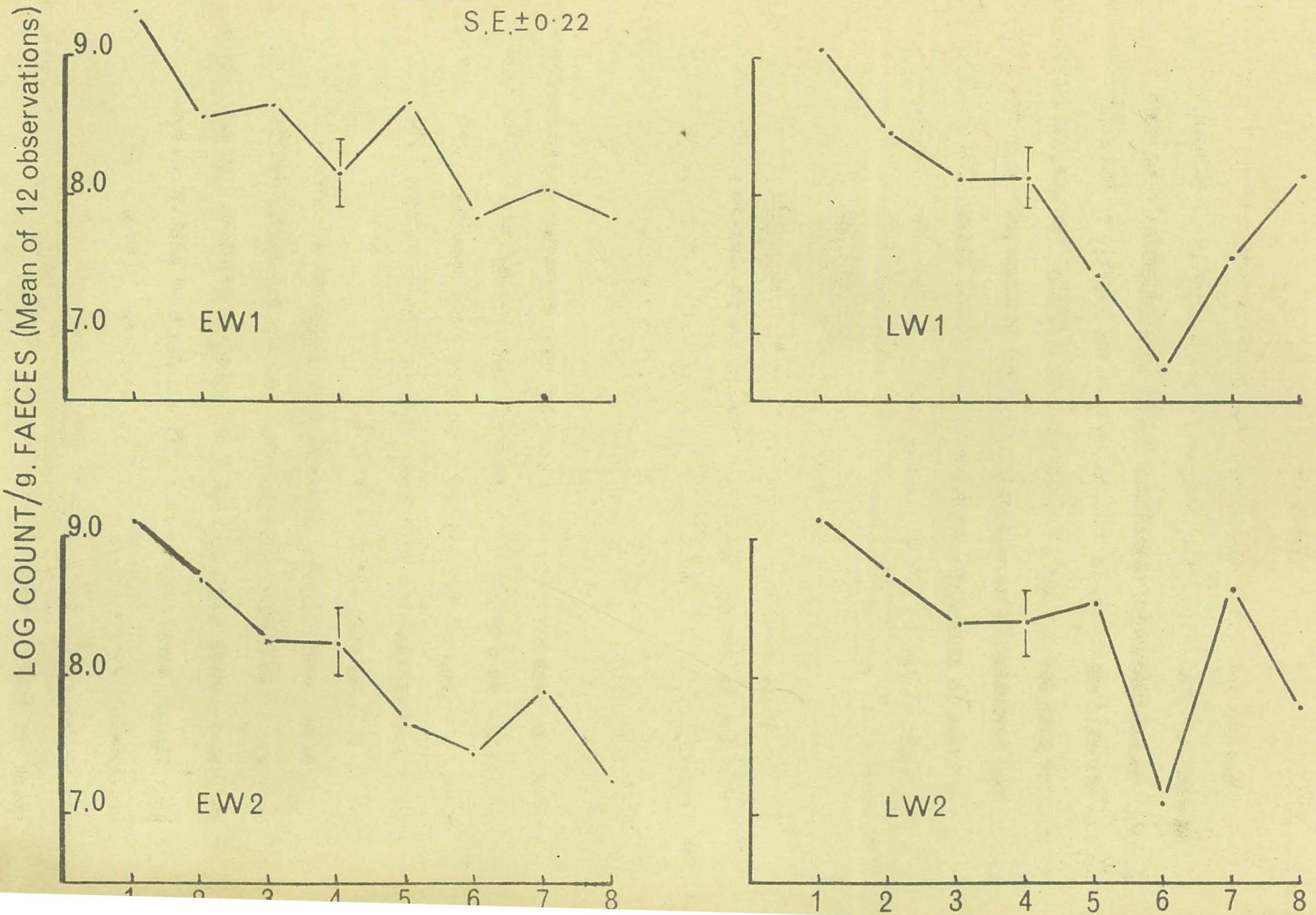


Fig.6 TOTAL ANAEROBES

S.E.±0·22



The log count of each organism (mean of 12 observations) plotted against age for each litter are presented graphically in Figures 6 - 9.

On examination of Appendixes I - IV it is apparent that there was considerable variation in the counts from the piglets of the same litter at any one week for any one of the organisms studied. To give an indication of this variation it was decided to include standard errors in the graphs. These standard errors are represented by vertical lines. The standard error of individual Age x Litters w Management means for each organism were calculated from the following formula

$$S.E. = \pm \sqrt{\frac{(AxPwLwM)\ M.S.}{12}}$$

The total anaerobes (Figure 6) and Escherichia coli (Figure 7) have similar graphs for the respective litters except that total anaerobes have slightly higher values. The initial levels of total anaerobes and Escherichia coli are similar for all litters. Both organisms for the two EW litters fluctuated from week to week but showed an overall downward trend from week 1 to 8. In litter LWI both organisms showed a definite fall from 1 to 6 weeks of age followed by a steady rise for the next two weeks. Counts of both organisms in litter LW2 remained steady for the first 5 weeks followed by a sharp fall at week 6, an equally sharp rise at week 7 falling away again at week 8.

Enterococci counts (Figure 8) for litter EWI followed a steady fall throughout the experimental period. Counts for litter LWI followed a sharper downward trend to week 6 and remained steady to week 8. In the same figure, counts for litters EW2 and LW2 were similar at week I, being considerably lower than those of the other two litters. The count for EW2 showed a very sharp initial rise followed by a steep fall to week 4 where the count remained stationary until week 6 and then rose to week 8.

The graph for litter LW2 illustrates a slight initial rise in enterococci to a level that remained stationary until week 5 and which fluctuated somewhat for the latter 3 weeks of the experiment.

Except for a higher count for litter LW2, the lactobacilli counts (Figure 9) at one week of age were similar for all litters. Counts for all litters then fell to about 3 - 5 weeks of age, rose and finally dropped gradually after 6 weeks of age for the EW litters and after 7 weeks of age for the LW litters.

(b) Clostridium welchii

The difficulties encounted in enumerating Clostridium welchii in Experiment I are reported earlier in this chapter. The data for this organism are incomplete for Experiment I and are not presented.

The log number (duplicate averages) per g. of faeces of Clostridium welchii for the piglets in Experiment II are presented in Appendix VI. An examination of Appendix VI shows that only an occasional piglet had counts of Clostridium welchii above 10^5 per g. of faeces after three weeks of age. The mean of counts of this organism for piglets in litters EW2 and LW2 up to three weeks of age are presented in Table XVII.

TABLE XVII

Log count of Clostridium welchii for the piglets in Experiment II (means of 12 observations)

Age (Weeks)	1	2	3
Litter			
EW2	8.15	7.53	6.25
LW2	8.25	6.97	5.33

Fig.10.

ENTEROCOCCI

M-Enterococcus Agar

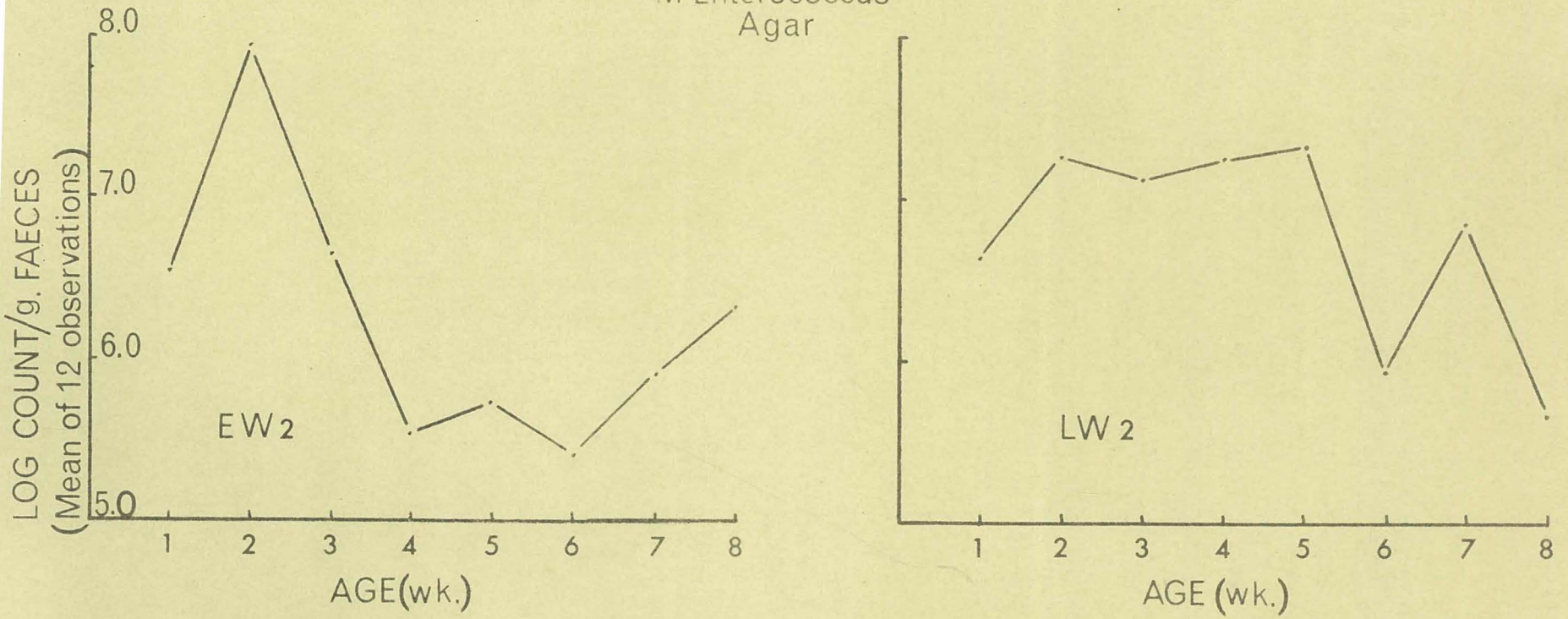


TABLE XVIII

Analysis of variance for the log count per g. of faeces (Duplicates) of enterococci on two media for litter LW2.

Source	df	SS	MS	F	
AGE (A)	7	136.79	19.54	27.91	**
MEDIA (M)	1	0.59	0.59	<1	N.S.
A x M	7	4.90	0.70	1.21	N.S.
ERROR	176	102.46	0.58		
Total	191	244.74			

TABLE XIX

Analysis of variance for the log count per g. of faeces (Duplicates) of enterococci on two media for litter LW2.

Source	df	SS	MS	F	
AGE (A)	7	86.70	12.39	36.32	**
MEDIA (M)	1	0.03	0.03	<1	N.S.
A x M	7	1.55	0.22	<1	N.S.
Error	176	69.54	0.40		
Total	191	157.80			

N.S. Not significant at the 5% level (P>0.05)

** Significant at the 1% level (P<0.01)

Table VII shows that the numbers of Clostridium welchii were similar for the piglets of both litters and that they showed a steady decrease with increasing age.

(c) Comparison of two media for the enumeration of Enterococci in ExperimentII

As mentioned in Chapter III the two media employed for the enumeration of enterococci in Experiment II were - Mitis-Salivarius Agar and M-Enterococcus Agar. Reasons for comparing these two media are discussed on page 96 .

The log number per g. of faeces (duplicates) of enterococci enumerated on M-Enterococcus Agar for all piglets in Experiment II are presented in Appendix V. These data are presented graphically in Figure 10.

The analyses of variance for litters EM2 and LM2 are presented in Tables VIII and IX respectively. Similar conclusions were drawn from the results of both analyses of variance. The differences due to Age were significant in both analyses which was consistent with the findings presented earlier in the Chapter. The non significance of the differences between the two media and the interaction with Age indicated that:-

(i) There was little overall difference in counts on the two media.

and

(ii) Differences from week to week were small. Comparison of Figures 8 and 10 illustrates these observations graphically.

3.2 Faecal Organisms of the Sows

The log number per g. of faeces (duplicate averages) of all the organisms studied for the four sows are presented in Appendix VII.

The means of the log count of each organism for the four sows during their nursing period are presented in Table X .

TABLE [illegible]

Analysis of variance for pH data

Source	df	SS	MS	F	
MANAGEMENT (M)	1	0.72	0.72	5.14	N.S.
LITTERS (L) w M	2	0.28	0.14	1.00	N.S.
PIGLETS (P) wLwM	20	2.71	0.14		
AGE	6	4.36	0.73	1.22	N.S.
A x M	6	3.60	3.60	<1	N.S.
A x (LwM)	12	11.04	0.92	9.20	**
A x (PwLwM)	116	11.14	0.10		
Total	163	33.85			

N.S. Not significant at the 5% level ($P>0.05$)

** Significant at the 1% level ($P<0.01$)

TABLE XX

The means of the log count of each organism studied for the four sows in these experiments

LITTER	EW1	EW2	LW1[c]	LW2
ORGANISM				
Total anaerobes	7.66	6.11[d]	6.31	7.82[d]
Lactobacilli	7.54	6.15	6.77	6.85
Enterococci (M-SA)[a]	6.53	0	6.23	5.81
Enterococci (M-EA)[b]	-	5.00[d]	-	5.94
Escherichia coli	5.68	5.48	6.38	7.31
Clostridium welchii	-	0	-	5.98

[a] Mitis - Salivarius Agar

[b] M-Enterococcus Agar

[c] Means of counts recorded or those above 10^5.

[d] Mean of 5 values because the piglets of this litter were weaned on the day prior to the collection day on the sixth week.

The above Table shows that the sow of litter EW2 had the lowest counts of the organisms studied. All the sows showed some variation in counts. The sows with 0 for some organisms may have harboured these organisms in their faeces but they were less than 10^5 per g.

4. pH

4.1 pH levels of the faecal samples from the piglets.

The analysis of variance of the pH data (single observations - Appendix VIII) is presented in Table XXI. The missing items were inserted

Fig.12 pH and LACTOBACILLI

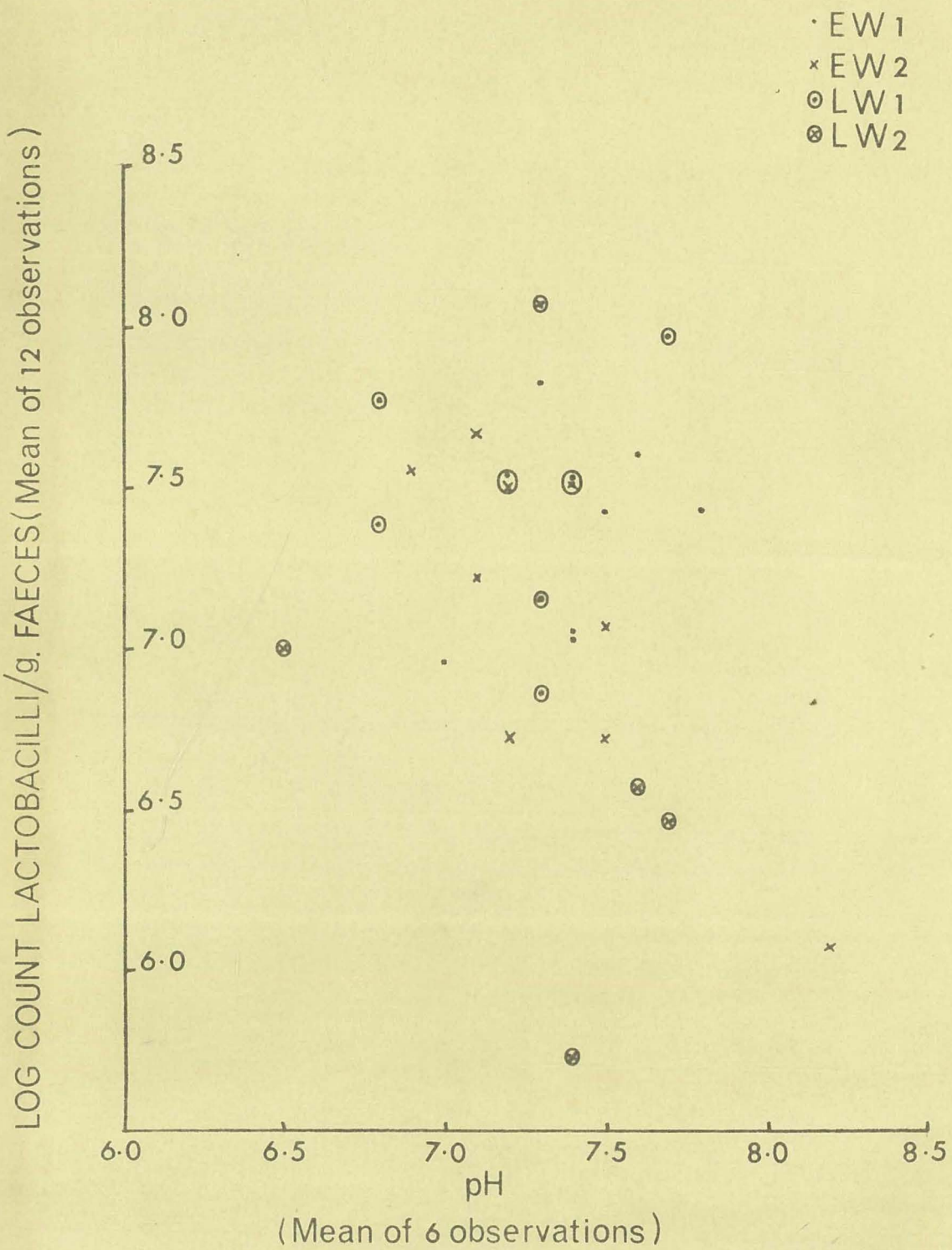
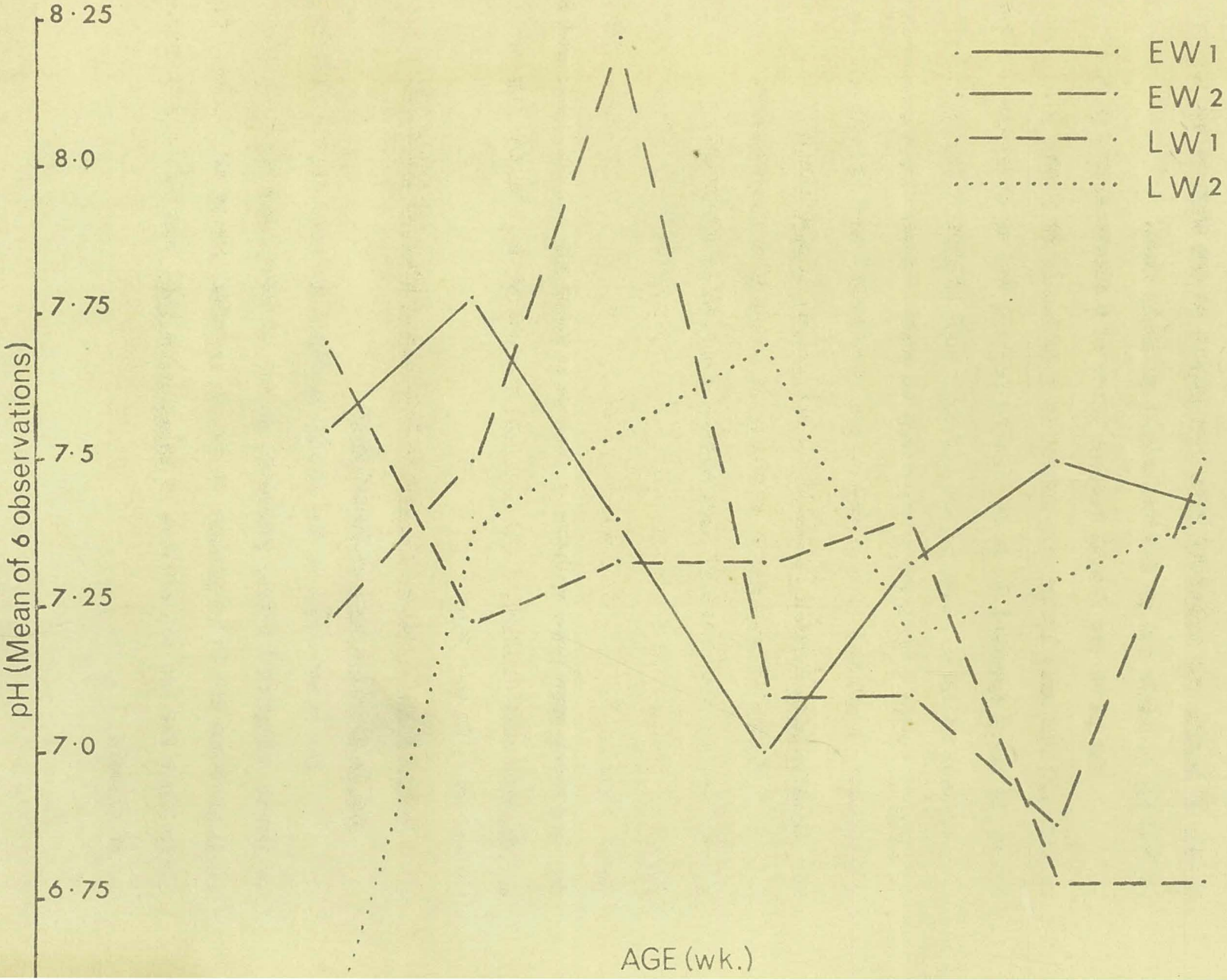


Fig.11 pH


8·25
8·0
7·75
7·5
7·25
7·0
6·75
pH (Mean of 6 observations)
AGE (wk.)
EW1
EW2
LW1
LW2

and the tests for significant effects were performed with a method similar to that used in the analyses for faecal organisms. Because an insufficient amount of sample was collected from each piglet at one week of age it was impossible to carry out pH determinations on these days.

The pH of the faecal samples (mean of 6 observations) plotted against age for each litter are presented graphically in Figure II. From Figure II there appeared to be no obvious effects due to Management, Litters w Management or A e. Also it was apparent that litters within either management varied considerably from week to week. These observations were subsequently confirmed as a result of the statistical analysis (TableVI).

4.2 Relationship between levels of pH and counts of organisms.

Figure 12 represents levels of pH (mean of 6 observations) plotted against log count of lactobacilli (mean of 12 observations) for all litters. Except for one point (pH8.2 and lactobacilli 6.05) there was no clear relationship between level of pH and numbers of lactobacilli. Graphs for the other organisms followed a similar pattern and are not presented here. Regressions were considered and rejected because of the obvious lack of relationship in the data.

5. Enumeration of undigested starch granules, clones of iodophilic and Balantidium coli by microscopy.

The lower limit of the method employed in these experiments to enumerate undigested starch granules, clones of iodophilic bacteria and small protozoa was 10^3 organisms or starch granules per g. of faeces. The lower limit for the enumeration of Balantidium coli was 10^2 organisms per g. of faeces.

Various species of small protozoa were encountered and these included Eimeria dobliechi, Endamoeba coli and Iodamoeba butschlii. These organisms did not occur consistently or in sufficient number to warrant counting.

5.1 Undigested starch granules

Starch granules were observed in the faeces of all piglets from five to eight weeks of age. Their presence at five weeks of age corresponded to the changes in the creep diet and in the appearance of the faeces (see 1, Chapter IV).

The log number per g. of faeces of undigested starch granules (single observations) for all piglets are presented in Appendix IX.[a] The statistical analysis of this data is presented in Table XVII. The missing items were inserted and the tests for significant effects were performed with a method similar to that used in the analyses for faecal organisms.

TABLE XVII

Analysis of variance for undigested starch

Source	df	SS	MS	F	
MANAGEMENT (M)	1	0	0	0	N.S.
LITTERS (L) w M	2	3.72	1.86	8.57	**
PIGLETS (P) w L w M	20	4.34	0.217		
AGE (A)	3	2.85	0.95	1.22	N.S.
A x M	3	2.35	0.78	2.79	N.S.
A x (L x M)	6	1.69	0.28	2.22	*
A x (P w L w M)	56	7.05	0.1259		
Total	91	22.00			

N.S. Not significant at the 5% level (P>0.05)
* Significant at the 10% level (P<0.10)
** Significant at the 1% level (P<0.01)

[a] Together with means and their standard errors

The significance at the 1% level of the differences between Litters w Management indicated that the cumulative amount of starch for the four weeks varied from litter to litter. However Management had no significant effect on the amount of undigested starch in these experiments. The interaction Age x Litters w Management was significant at the 10% level which indicated that the differences in undigested starch between Litters w Management varied from week to week. There were no significant Age differences in the levels of undigested starch during these four weeks.

5.2 Clones of iodophilic bacteria

For the purpose of these experiments iodophilic bacteria were defined as those micro-organisms which gave a blue colour reaction with Iodine (final concentration 0.3%).

Iodophilic bacteria were found to appear in the faeces of most piglets at 5 to 6 weeks of age. They occurred as clones either freely or in association with starch granules or other plant material.

Photographs illustrating the various forms of iodophilic bacteria encountered and their association with plant material are presented in Figures 13 - 18. All photographs were made from iodine stained, wet preparations of piglet faeces as used for enumeration (2. Chapter III). The size of the clones varied from small chains (Figure 15 and 17) to large groups, either free (Figure 16) or on the surface of starch granules or other plant cells (Figures 13—15, and 18).

The log number of clones of iodophilic bacteria per g. of faeces for all piglets are presented in Appendix X. These data were not statistically analysed because some of the piglets recorded values lower than 10^3 clones per g. of faeces. Also, because of the large variation in the size of the clones these counts provided no real measure of the number of

of organisms present. The number of piglets per litter with iodophilic bacteria present in their faeces and the range of counts are presented in Table XXIII

TABLE XXIII

Number of piglets of each litter with iodophilic bacteria in their faeces and the range of counts for the four weeks.

Age (Weeks)	5	6	7	8	Range in Log No. of clones of iodophiles per g. of faeces
LITTER					
W1	5	6	4	4	3.60 - 6.15
B2	2	5	6	5	3.30 - 5.54
L1	4	6	6	6	4.20 - 5.85
L2	0	5	6	4	3.30 - 5.44

Table XXIII shows that only at seven weeks of age did all the piglets have clones of iodophilic bacteria in their faeces. The occurence of iodophilic bacteria in the faeces of the piglets varied within and between litters. Once present, these organisms did not recur consistently in the faeces of some piglets during the following weeks.

Only one sow, (litter L2) had faecal counts of clones of iodophilic bacteria above 10^3 per g. These are presented in Table XXIV

TABLE XXIV

The log number of clones of iodophilic bacteria per g. of faeces for the sow of litter L2.

Weeks (Post Farrowing)	1	2	3	4	5	6
Sow of Litter L2	0	3.70	0	3.95	5.09	0

TABLE XXV

The number of piglets per litter with Balantidium coli in their faeces and the range of counts over the experimental period

AGE (Weeks) / LITTER	4	5	6	7	8	Range in Log No. of B. coli per g. of faeces
EW1	0	0	3	3	3	2.00-4.20
EW2	1	5	4	3	6	2.00-4.18
LW1	0	4	3	3	3	2.00-4.25
LW2	0	0	4	4	4	2.00-4.24

TABLE XXVI

The log number per g. of faeces of Balantidium coli for the four sows and the means during the nursing period.

WEEKS (Post Farrowing) / LITTER	1	2	3	4	5	6	Mean for the Nursing Period
EW1	2.78	2.95	3.04				2.92
EW2	2.90	3.45	3.49				3.28
LW1	3.93	3.04	0	3.57	4.06		2.92
LW2	3.32	3.51	2.85	3.95	2.48	3.23	3.26

5.3 Balantidium coli

Balantidium coli were found to appear in the faeces of some of the piglets of each litter at about 4 - 6 weeks of age. All Balantidium coli observed in these experiments occurred as cysts.

Photographs illustrating a cyst and various forms of trophozoites are presented in Figures 19 - 22. The photograph of the cyst was made from an iodine stained, wet preparation of piglet faeces as was used for enumeration (2.Chapter III). As the starch granules within the cysts were deeply stained with iodine they were decolourised with sodium thiosulphate in order to show the morphological details of the cysts.

The photographs of the three trophozoites were made from wet preparations of formalin fixed material from the caecum of a nine week old piglet from the same piggery.

The log number per g. of faeces of Balantidium coli for all piglets are presented in Appendix XI. Examination of Appendix XI reveals that three of the piglets were either not infected or had counts lower than 10^2 per g. of faeces during the experimental period. Also, Balantidium coli did not occur consistently in the faeces of some piglets during this time.

The number of piglets per litter with Balantidium coli in their faeces and the range of counts during the experimental period are presented in Table XV. The log number per g. of faeces of Balantidium coli for the four sows are presented in Table XVI. Table XV shows that there is considerable litter and piglet variation in the age at which Balantidium coli occurred in the faeces of the piglets. Only at eight weeks of age did all the piglets of a litter (B72) have Balantidium coli in their faeces.

The range of counts were similar for each litter. Table XVI shows that with the exception of the sow of litter L.I at week 3, all sows had counts of Balantidium coli above 10^2 per g. of faeces during the nursing period. As can be seen from the means, the counts for all sows were similar.

Figures 13 - 18

All photographs were made from iodine stained, wet preparations of piglet faeces.

Figure 13 — A starch granule surrounded by iodophilic bacteria; early phase of breakdown.

Figure 14 — A starch granule covered with iodophilic bacteria (rods), showing dissolution of the blue reacting component (amylose) with temporary persistence of the non-reacting (amylopectin) residue.

Figure 15 — Centre top: Chain of small iodophilic bacteria.
Left centre: Isolated rods of large iodophilic bacteria.
Centre bottom: Starch granule surrounded by iodophilic bacteria.

Figure 16 — Part of a large clone of iodophilic bacteria.

Figure 17 — A chain of large iodophilic bacteria on the surface of a plant cell.

Figure 18 — A plant cell covered with large iodophilic bacteria.

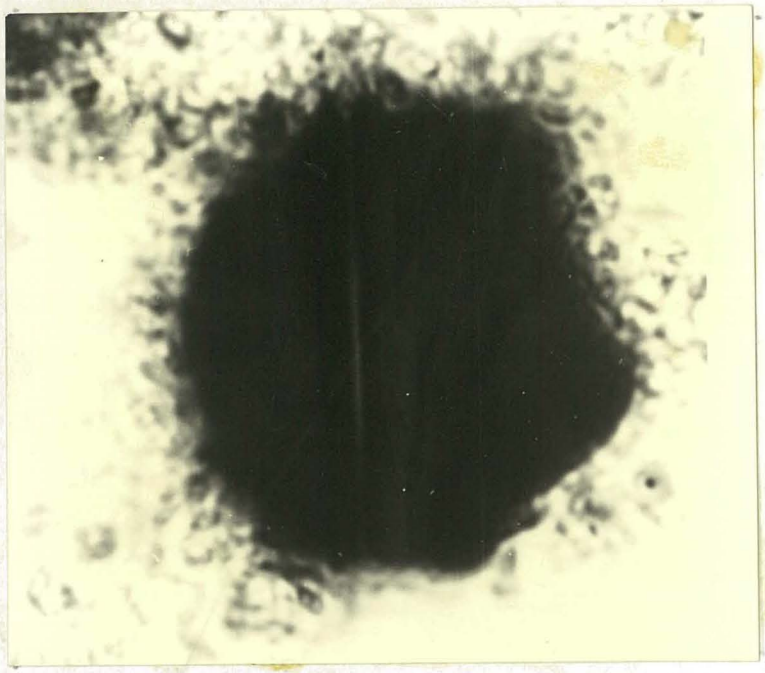


Figure 13 x 2875

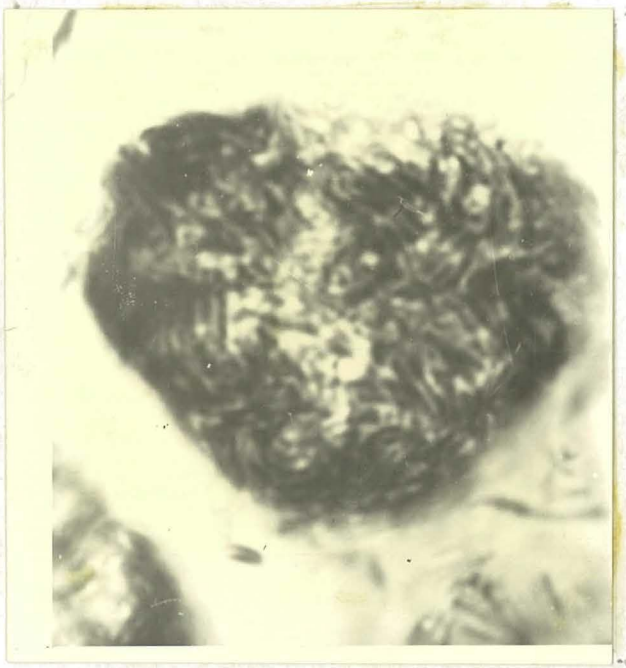


Figure 14 x 2875

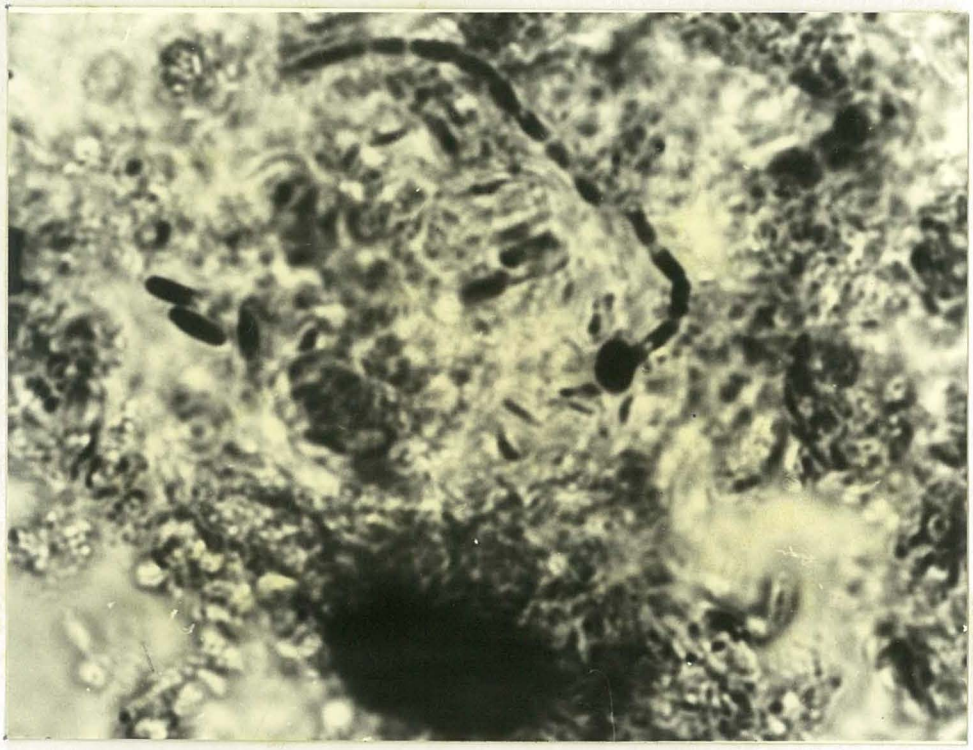


Figure 15 x 1850

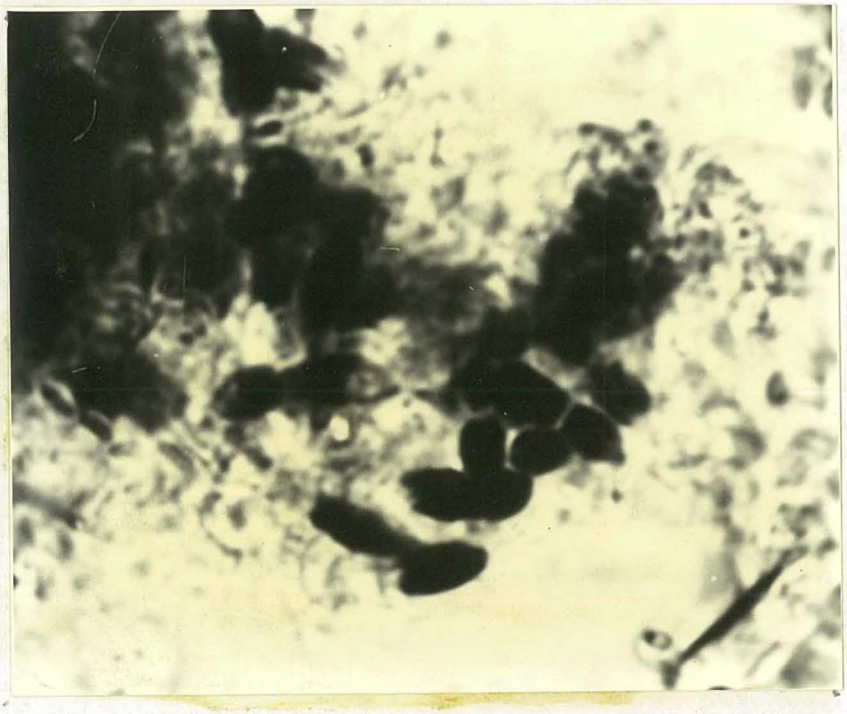


Figure 16 x 2875

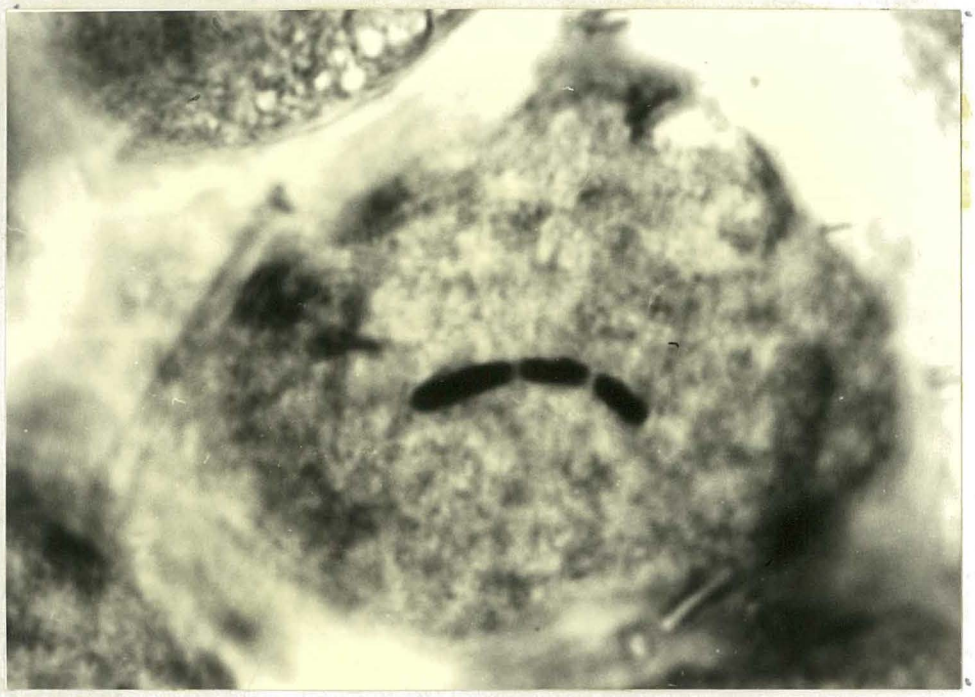


Figure 17 x 2875

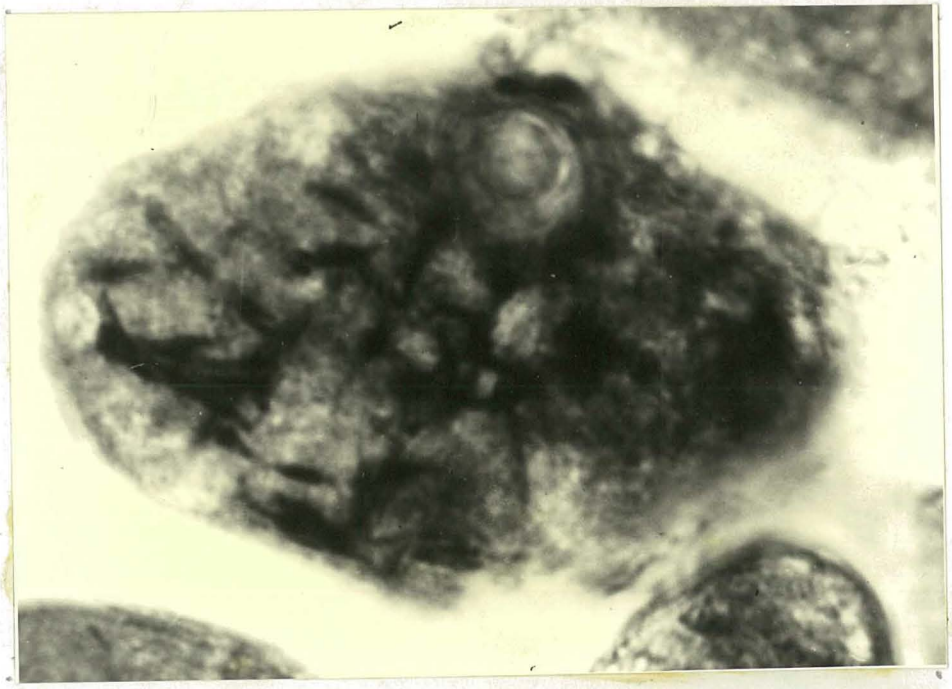


Figure 18 x 2875

Figure 19

A cyst of Balantidium coli showing the presence of small starch granules. As found in the faeces of an experimental piglet.

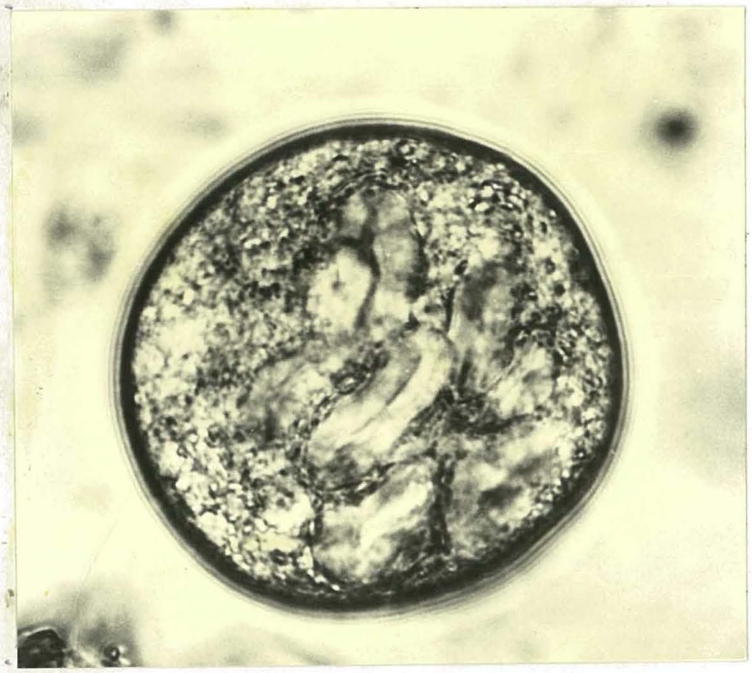


Figure 19 x 1000

Figures 20 - 22

These photographs were made from wet preparations of formalin fixed material from the caecum of a nine week old piglet.

Figure 20 An ovoid trophozoite of *Balantidium coli*

Figure 21 An elongate trophozoite of *Balantidium coli (suis)*.

Figure 22 A large ovoid trophozoite of *Balantidium coli*

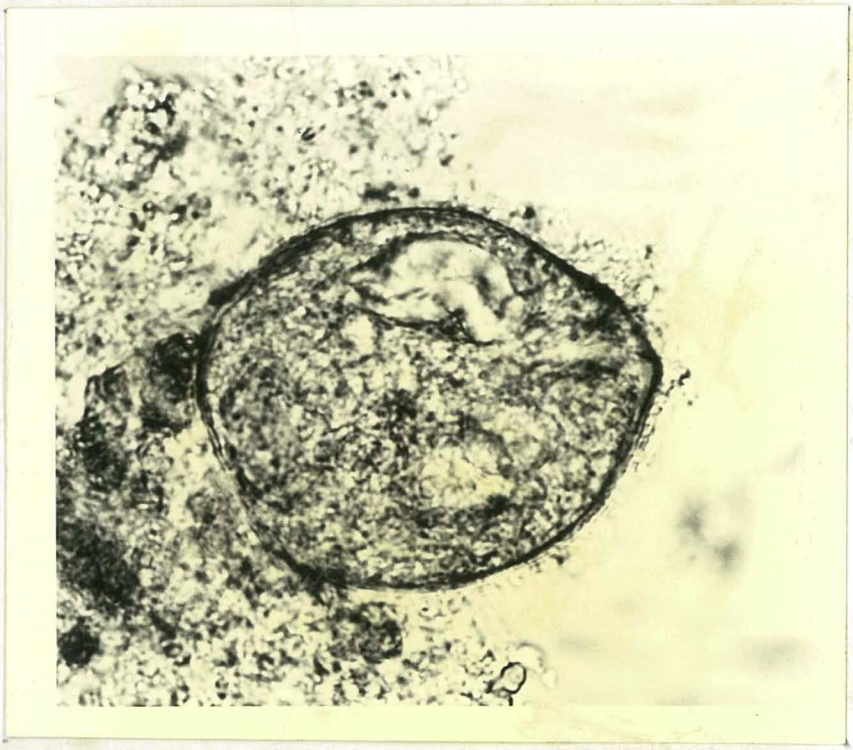


Figure 20 x 1000

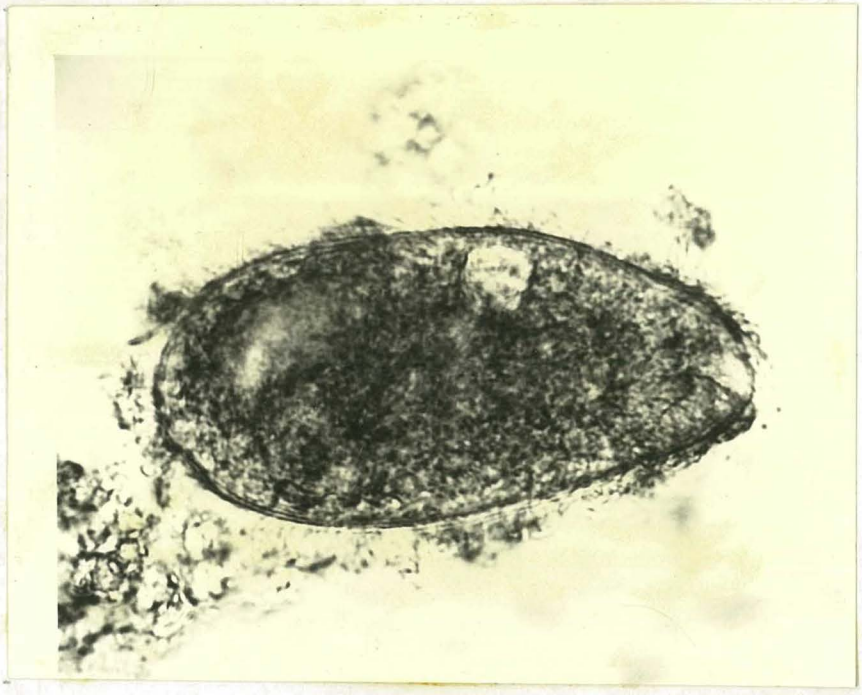


Figure 21 x 1000

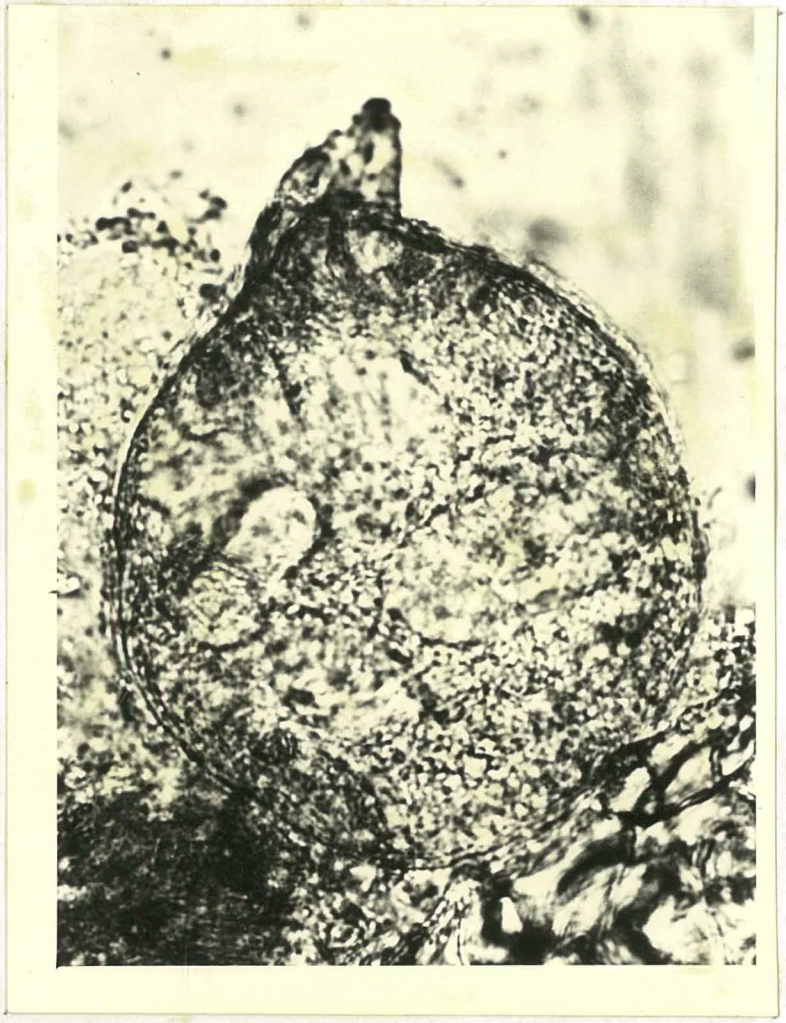


Figure 22 x 1000

CHAPTER V

DISCUSSION

1. The Use of Two Media for the Enumeration of Enterococci

During the experiments described here, eight different colony types could be distinguished on plates of Mitis-Salivarius Agar (Table VII). Organisms from only two of these colony types, namely the blue and brown circular colonies with a white periphery, were found to be gram positive diplococci. The results of the physiological tolerance tests (Table VIII) and the ability of these organisms to produce pink to dark maroon streaks on M-Enterococcus Agar indicated that they were enterococci (Breed et al 1957, Slanetz and Bartley 1957). The number of colonies of these two types only, were recorded as enterococci.

Colony types, produced by various species of streptococci from human faecal specimens, which grew on the surface of Mitis-Salivarius Agar, were described by Chapman (1944, 1946 and 1947) and are presented below:-

(i) Streptococcus salivarius produces blue smooth or rough 'gum drop' colonies from 1 to 5 m.m. in diameter which can easily be recognised by reflected light.

(ii) Streptococcus mitis produces small blue colonies 0.5mm or less in diameter which are slightly raised.

(iii) Enterococci produce dark blue (brown, Chapman 1944) or black shiny, slightly raised colonies, 1 to 2 mm. in diameter, which have a characteristic appearance and are easily differentiated from (i) and (ii) above, particularly when viewed by reflected light.

(iv) Coliforms may grow occasionally, but they produce brown or gray colonies and rarely spread.

Comparison of the colony types found on Mitis-Salivarius Agar reported here with those reported by Chapman (1944, 1946 and 1947) reveal some differences and only one similarity. These are as follows:-

(i) Colony types observed here that could be classified as streptococci or enterococci by the above descriptions, e.g. blue circular and small black pulvinate colonies, were not members of the Genus Streptococcus (Tables VII and VIII).

(ii) Organisms which were found to be enterococci came from colonies which were different from the typical enterococci colonies described by Chapman loc. cit.

(iii) In the results reported herein, the description of the appearance of colonies produced by Escherichia coli was in agreement with that reported by Chapman loc cit.

Because of the apparent discrepancy between the colony types found on Mitis-Salivarius Agar reported here and the colony types described by Chapman loc cit., a second medium, M-Enterococcus Agar, was employed for the enumeration of enterococci during the last 3 weeks of Experiment I and throughout Experiment II. The description of the colony types found on plates of M-Enterococcus Agar and the results of the physiological tolerance tests performed on organisms from these colony types (Tables VIII and IX) are in accordance with observations made by Slanetz and Bartley (1957). The last authors developed this medium and found it to be 100% selective for faecal streptococci (enterococci).

Results of the statistical analysis (Tables XVIII and XIX) show that there was close agreement between counts of typical enterococci colonies on M-Enterococcus Agar and counts of blue and brown circular colonies with a white periphery on Mitis-Salivarius Agar during Experiment II. This result and the results of the cultural and staining tests show that during the experiments reported here, the circular blue and brown colonies with a white periphery were produced by enterococci.

The discrepancy between the descriptions of the colonies produced by enterococci on Mitis-Salivarius Agar reported here with those reported by Chapman (1944, 1946 and 1947) is difficult to interpret. It is unlikely that the difference between sources of inoculum was responsible, i.e. faecal specimens from piglets or humans. A possible reason would be that some of the components of this medium, as prepared here, came from sources different from those of Chapman loc. cit., thus providing a variation in growth promoting and growth suppressing factors. Also the interaction of these components and their final reaction with the organism produced colonies of a different colour to those observed by Chapman loc. cit.

The results reported here throw some doubt on the selective ability of Mitis-Salivarius Agar for enterococci. For, while it was possible to distinguish and enumerate the enterococci colonies, the presence of other colonies on this medium did not make the task an easy one. Wilbur (1959) reported that throughout his experiments the medium provided numerous types of isolates. However, this author did not describe the colony types which were encountered.

On the other hand, M-Enterococcus Agar as used here, was found to be almost completely selective for enterococci and produced colonies of this organism which were similar in appearance to those described by Slanetz and Bartley (1957). M-Enterococcus Agar is simpler to prepare than Mitis-Salivarius Agar. The counts of enterococci from corresponding plates of Mitis-Salivarius Agar and M-Enterococcus Agar were similar. This showed that their growth promoting properties for this organism were equal. However, M-Enterococcus Agar was found to be superior to Mitis-Salivarius Agar as a medium for the enumeration of enterococci from porcine faecal samples.

2. Factors which influenced the Faecal Populations of Total Anaerobes, Lactobacilli, Enterococci and Escherichia coli.

The similarity between the weekly counts from plates of Blood Agar (total anaerobes) and Levine E.M.B. Agar (Escherichia coli) for any one piglet was due to the fact that the majority of colonies counted on the former were also Escherichia coli, a facultative anaerobe. The other organisms which grew on Blood Agar (see page 74) accounted for the slightly higher counts on this medium.

(a) Management and Interaction

Differences in the time of weaning (Management) were found to have little effect on the total faecal populations of total anaerobes, lactobacilli enterococci and Escherichia coli for the first 8 weeks of the piglet's life.

Examination of Figures 6 - 9 reveals that counts of the four organisms in the EW litters were lower the week after weaning than at weaning time. Except for enterococci, the decrease was not found to be significant and the counts were following a trend that was apparent prior to weaning. Conversely the counts of the four organisms in the LW litters were higher the week after weaning than at weaning time. A significant increase was found for counts of total anaerobes and Escherichia coli but only in litter LW1 was this increase maintained at the following week. The increase in enterococci count was significant for litter LW2 but not for LW1. Lactobacilli counts were not significantly higher and followed a similar pre-weaning pattern.

From the results reported here it can be seen that weaning may cause a significant change in the counts of some organisms at the week after weaning and the type of change may be influenced by the time of weaning. These

changes are not maintained in both litters within a Management for the following weeks and therefore tend to cancel each other out. It is also possible that litter and age effects and the interactions were great enough to mask any management effect.

Comparison of the results reported here with those of other workers (Wilbur, 1959 and Smith and Crabb 1961), for the effect of time of weaning on the faecal flora is complicated by the fact that, in all cases different management procedures and pre- and post-weaning diets were used.

Wilbur Op.cit. page 45, observed similar pre- and post-weaning faecal counts of lactobacilli, coliforms and streptococci, for piglets weaned at 2 weeks of age and fed a diet high in lactose. The suggestion by this author that the similar counts reflected the similarity between the pre- and post-weaning diets may also apply here for the piglets weaned at 3 weeks of age. The change in diet at weaning time in the experiments reported herein was from sow's milk to skim milk, the creep diet being identical for all piglets at both weaning times.

(b) Litters w Management and Interaction

Examination of Table XV shows that Litters w Management plus Age x (Litters w Management) contributed between 15 and 44% to the total variation depending on the organisms studied.

The significant difference between Litters w Management for counts of the four organisms may be related to a seasonal influence in that Experiments I and II were performed in different months of the year (Table II). Table XVI shows that the mean counts of the four organisms were higher for litter LWI than LW2 and, apart from lactobacilli, lower for litter LWI than

LW2. It is tempting to suggest that a Litter x Season interaction was responsible for these inconsistencies. However this interaction was not measured and in view of the small differences in the mean counts of any one organism between Litters w Management, the effect was probably not a large one.

Another factor which may have contributed to this source of variation was that different sow diets were used between the two experiments (Tables III - V) and piglets had access to their sow's feed. In view of the findings of other workers (Review of Literature) it is quite conceivable that dietary differences of this nature could have caused some of the observed difference in the flora between litters.

The faecal populations of the four organisms were similar for the four sows during the time they were nursing their litters. Thus, apart from providing one of the initial sources of inoculum, access to the sow's faeces probably contributed only a small amount to the observed variation.

Throughout these experiments no udder infections were observed in any of the four sows. It was assumed, therefore, that the sows milk was sterile. During the nursing period sows were allowed out to graze for one hour each day. It is possible that each sow could have brought back to its round house different concentrations and species of bacteria and transmitted them to their piglets. This could have influenced the overall difference in counts of the four organisms between Litters w Management. One mode of transmission would be the sow's teats.

The difference in counts of each of the four organisms between Litters w Management were found to change significantly from week to week (A x L w M interaction). These changes are illustrated in Figures 6 - 9.

It is probable that the cause of these significant changes is related to the factors which caused the overall Litter w Management differences. For example: After weaning the piglets do not have access to their dam and her feed. Consequently the difference in the environment between Litters w Management is reduced. It follows that the difference in counts of some organisms between Litters w Management after weaning may be significantly smaller than before weaning. This was found to be the case for lactobacilli counts in both EW and LW litters.

The significant litter influence on counts of the four organisms observed in these experiments is in agreement with Wilbur Op.cit. pp 49, 50 and 80. Furthermore Wilbur loc cit. page 79. also found in one experiment significant Age x Litter interactions for counts of lactobacilli, coliforms and streptococci. Comparison of these results is complicated by the difference in experimental design between the work reported here and that of Wilbur loc cit. page 13.

From the results reported here it would appear that the sow and the management of the sow during the nursing period have a considerable influence on the faecal counts of the four organisms present in her litter. An accurate estimate of the influence of the sow could only be obtained by carrying out further experiments in which other experimental variables are eliminated or controlled.

(c) Piglets w Litter w Management and Interaction

The similarity in the overall counts of each organism between Piglets w Litters w Management (Table XV) may be due to a number of factors. These include:-

(i) The similar genetic makeup and environment of littermates.

(ii) Access to the faeces of littermates.

(iii) The use of common feeders and drinking troughs by members of a litter.

The first factor would play a part in the development of similar physiological characteristics between littermates. Some of these characteristics would have a similar effect on the number and type of faecal organisms present.

The last two factors provided similar sources of inoculum which could have maintained an overall similarity in counts of each organism between littermates. That this is the case, is supported by the findings of Wilbur (1959) (see page 32).

The estimated contribution of the interaction Age x (Piglets w Litter w Management) to the observed variation of faecal counts of the four organisms was within the range 18 - 49% (Table XV). The interpretation of this interaction is that the differences in the counts of each of the four organisms between Piglets w Litter w Management changed significantly from week to week. This significant change is probably related to a corresponding change in the difference from week to week in the intestinal environment between littermates.

The intestinal environment of any one pig at any one time is dependant on a number of factors, the estimation of the effect of which is

beyond the scope of these studies. Some of these factors are:

(i) The selective appetite of a piglet

(ii) The health of the piglet

(iii) The stage of development of the piglet's digestive and absorptive ability.

It is obvious that the differences between littermates for each of these factors can change from week to week. However, from the results presented here it appears the effect of these factors on counts of the four organisms tend to balance out over a period of time.

(d) Age

The analyses of the pooled data (Tables XI - XIV) showed that there were significant differences between weeks in counts of each of the four organisms. From Figures 6 - 9 it is evident that these significant differences occurred within each litter. The observed differences between weekly counts cannot be attributed solely to changes in age because the presence of, and the changes in the creep diet, introduced a dietary effect. The creep diet was the same for all litters at any one time.

There was no consistent pattern during the experimental period for counts of total anaerobes, Escherichia coli and enterococci in all four litters as can be seen in Figures 6 - 8. This could be due to the factors already discussed e.g. differences in time of weaning, sows and sow management. These three factors and the type of creep diets used, may also account for the differences between the results presented here and those of other workers (Review of Literature) for piglets of the same age. It appears that only the number of lactobacilli present may have been affected by these factors as this organism followed a similar pattern in all litters. (Figure 9).

Counts of Escherichia coli and total anaerobes in both the LW litters were found to decrease with increasing age. This is in agreement with the observations made by Wilbur (1959) for counts of coliforms in piglets weaned at 2 weeks of age. For the first 6 weeks, counts of the two organisms followed a similar pattern in litter LW1 but not in litter LW2.

In litters LW1 and LW1 counts of enterococci showed a similar decrease with increasing age. The pattern for this organism in the other two litters was one of fluctuation.

In the four litters lactobacilli counts followed a cyclic pattern, being high initially followed by a fall, a rise, and finally a slight fall. The reasons for this pattern are not clear for they do not correspond with weaning times or changes in the creep diet.

It is apparent that counts of some organisms decreased with increasing age and consequent changes in diet in some litters. The other factors present could have masked this decrease in all litters for all organisms. From the experiments reported here there is no conclusive proof that age or the changes in the creep diet influenced the count of all organisms.

(c) Laboratory Error

The problems of comparing results of one group of workers with those of another group have already been discussed (see pages 19 and 101). Another problem is that in a number of cases different enumeration techniques were used.

Apart from the experiments of Smith and Crabb (1961) and those reported here most of the other groups of workers who have enumerated the

faecal and intestinal flora of pigs have used the M.P.N. and/or Agar Pour Plate techniques. As discussed in Part I these two methods can have a large sampling error (M.P.N.) and/or an overcrowding error (Agar Pour Plate). The use of these methods could significantly influence the counts obtained.

Provided that there was no bias in the data presented here because of a consistent fault/s in technique it appears that the Drop Technique is at least as accurate if not more so than the methods mentioned above. Comparison of the expected standard error for 1 sample and 2 drops for coliforms (Part I: $\pm$ 0.095) with the specific standard errors (Table XVI) for the counts of the organisms studied in Experiments I and II shows that the latter were slightly lower. Further evidence of the accuracy of the Drop Technique is found in Table XV. The contribution of laboratory error to the observed variation was approximately 1% for the four organisms counted.

3. Clostridium welchii

It was found in Experiment II that faecal counts of Clostridium welchii in both litters decreased with increasing age. After 3 weeks of age, except for three occasions, counts of this organism were less than 10^5 per g. faeces (the lower limit of the technique used). Similar results were obtained by Smith and Crabb (1961). They found that these organisms were present in the faeces after 3 weeks of age at levels within the range $0 - < 10^5$ per g. of faeces.

Fuller and Briggs (1962) have found a substance in the small intestine of pigs which is bactericidal for Clostridium welchii. They suggested that this substance may be responsible for the decline in numbers of Clostridium welchii in the intestine of pigs on weaning. The precise nature of this bactericidal substance has yet to be established. Also, the age at which this substance was found to occur in pigs was not mentioned by Fuller and Briggs loc. cit.

4. Undigested Starch, Iodophilic Bacteria and Balantidium coli

(a) Undigested Starch

In the experiments reported here it was found that undigested starch was present in the faeces of all piglets from 5 to 8 weeks of age. This undigested starch occurred as granules, or particles of grain which contained many granules. Prior to 5 weeks of age starch granules were found only occasionally in the faeces of all piglets.

From Table I it can be seen that semolina was the sole source of starch fed to the piglets from 10 to 31 days of age. The semolina, as given in the creep diet, is prepared from wheat. The commercial method of preparation consists of roller grinding the wheat to a fineness 5 grades coarser than commercial flour. Prior to grinding, the grain is moistened and left for 2 - 3 days. It is considered that moistening enables the amylase present in wheat grain (Jones and Amos, 1947) to attack the starch granules. The grain is milled before reducing substances (calculated as maltose) can be detected, and the final product still shows a blue colour reaction with iodine. The results reported herein (Appendix IX), indicate that the piglets were able to digest this form of starch almost completely.

At 32 days of age the piglets were presented with a creep diet which contained 65% grain meal (Table I). This diet was continued until the piglets were 45 days of age when it was again changed, and the grain meal component was increased to 82.5%. The constituents and the relative proportions of the grain meal were barley > maize = wheat > pollard

(>, more than). The method of preparation of these grain meals consists of hammer milling the grain so that it is broken down into particles. The resulting meals are considerably coarser in texture than semolina.

In the experiments reported here, the appetite of the piglets was not known as feed consumption measurements were not recorded. The differences between the sizes of the granules, and the numbers of granules in the particles of grain made it impossible to measure accurately the amount of undigested starch present in the faeces. It is, therefore, unwarranted to place a great deal of emphasis on the results of the statistical analysis (Table XXII) performed on the counts of starch granules. The results do indicate, however, that differences occurred between Litters x Management in the amount of undigested starch present. Also, the levels of undigested starch within any one litter remained relatively constant throughout the last four weeks of both experiments. (Appendix IX). The high numbers of starch granules recorded, within the range $10^5 - 10^7$ per g. of faeces, showed that a considerable amount of the starch was undigested.

Lucas and Lodge (1961) have reviewed recent work on the development of the digestive enzyme system in pigs. From the work reviewed it is apparent that:

> 'Pancreatic amylase activity is low at birth, but increases rapidly until the pigs are 4 to 5 weeks of age'.

Walker (1959) suggested that sufficient amylase is present, in pigs at all ages, to digest the amounts and types of starch that would be presented in a synthetic diet. Support for this suggestion is to be found

in the results obtained by Cunningham (1959), that young pigs readily digested soluble maize starch. The results reported here, for the period when semolina was the only source of starch, are in agreement with Cunningham loc. cit. and support the suggestion of Walker Op. cit.

It has been found that raw cereal starch is not digested rapidly until pigs are about 4 weeks old or even older (Review of Literature). The suggestion of Braude et al (1958) and Cunningham (1959) that the inability of the alimentary tract of young pigs to rupture the starch granule restricts the digestion of raw starch would certainly apply here. It is also apparent from the results reported here that hammer milling as a method of preparing grain meals does not cause physical damage to all the starch granules so that this form of dietary starch is not completely available to piglets 5 - 8 weeks of age. The absence of a significant rise in the number of starch granules present in the faeces from 7 to 8 weeks of age, for all litters, can be seen in Appendix IX. This indicates that the piglet's ability to digest this form of starch may have improved, because at 7 weeks of age the grain meal percentage of the creep diet rose from 65.0% to 82.5%.

The results herein are similar to those of Baker et al (1950) who found that pigs were unable to completely digest raw potato in the small intestine and large numbers of potato starch granules accumulated in the caecum. The possible reasons for the differences in digestibility of various sources of starch, suggested and discussed by Baker et al., loc. cit., have been mentioned earlier (see page 1). Baker et al. loc. cit. concluded that:

> 'In the absence of the information which systematic microscopy alone can yield, purely bacteriological and biochemical inquiries lack both a starting point and an essential control. Without

> some understanding of the significance of the many complex factors involved in the breakdown of starch within the gut of animals, workers must realise that undefined variables are introduced into all experiments whether conducted in the field, the laboratory or the hospital, in which starch-containing diets are given to animals or man'.

(b) Iodophilic Bacteria

The observations reported here are in agreement with Baker et al. (1950) who found that:

> 'In all the animal species investigated, the special bacterial facies of starch breakdown in the gut includes micro-organisms giving a colour reaction with iodine. Where the chemical composition of iodophil bacteria has been studied (Smith and Baker 1944: Hehre and Hamilton, 1946, 1948; Nasr and Baker 1949), the iodine reaction has been shown to be due to the presence in the bacteria of a hexosan polysaccharide whose chemical properties are intermediate between those of amylopectin and glycogen. Synthesis of iodophil polysaccharide attends the bacterial decomposition of sugars and of such structural polysaccharides as cellulose and starch'.

and

> 'In non-ruminants the extent to which the facies was consistently distinctive depended on the animal species investigated. In guinea-pigs and domestic pigs a high degree of uniformity was maintained; guinea-pigs showing a facies of curved iodophil rods and domestic pigs one of longer or shorter clostridial chains'.

In the experiments reported here, iodophilic bacteria were not found in the faeces of piglets during the first 4 weeks of life. These organisms were first observed in the faeces at approximately the same time as the large numbers of undigested starch granules. From the results obtained and the observations made it appeared that the presence of undigested starch in the large intestine was necessary before iodophilic bacteria became a noticeable component of the faecal flora. It is possible that

these organisms were present in the faeces prior to 5 weeks of age but their enumeration was limited by the technique employed (see page 97).

It was found that iodophilic bacteria occurred mainly in clones which were not broken down by the mixing technique used. From Figure 14 it is apparent that the iodophilic bacteria were in close association with the starch granule and were consequently resistant to release during mixing. It is possible that the apparently free clones of iodophilic bacteria were also in association with starch granules, but, because of the enzymic activity of the bacteria, these granules no longer gave a colour reaction with iodine. On the other hand the number of iodophilic bacteria present in some clones was such that it would not be possible to see the starch granule within. The above suggestions for the occurrence of clones, which were resistant to breakdown by mixing, are consistent with the observations that only iodophilic bacteria occurred in clones. Also the non-iodophilic bacteria observed, were present singly and very rarely in pairs or short chains.

During the period when the piglets were 6 - 8 weeks of age it was found that 5 of the 68 samples examined did not contain observable numbers of clones of iodophilic bacteria (Table XXIII). Also, at 5 weeks of age, none of the samples examined from piglets of Litter LW2 and only 2 from Litter EW2 contained observable numbers of clones. The reason for these observed absences of iodophilic bacteria are difficult to interpret. It is apparent that all piglets had access to sources of inoculum; the piglets of litter LW2 from the faeces of their dam (Table XXIV) and the piglets of the other litters from the faeces of some of their littermates.

It is suggested that the following factors may have been responsible

(i) The number of clones, if present in the samples collected, was below the lower limit of the enumeration technique.

and

(ii) Iodophilic bacteria become established at different rates in piglets of different litters.

The amount of undigested starch present did not appear to be a contributing factor (Appendix IX).

Althought it was impossible to count the number of iodophilic bacteria within any one clone, it was apparent from the size of the clones and the number of clones counted (Appendix X) that large numbers of these organisms were present in the majority of samples.

From an examination of Figures 13 - 18 it appears that at least two groups of iodophilic bacteria were present in the faeces; one being a much larger rod than the other. The possibility of pleomorphism, however, should not be overlooked (Baker et al 1950). As far as possible the Figures mentioned above illustrate all the forms of iodophilic bacteria and their association with starch granules that were encountered in these experiments.

The cultural investigation of the bacterial breakdown of starch in the pig's caecum performed by Baker et al (1950) has been mentioned earlier (Review of Literature, see page 34). There is a marked similarity between the iodophilic bacteria and their relationship with starch granules as depicted in the photographs presented here (Figures 13 - 18) with those presented by Baker and Harriss (1947-1948) and Baker et al (1950. This would indicate that Clostridium butyricum may have been present in the

faeces of the piglets examined in the experiments reported here. As no cultural examinations of the iodophilic bacteria were carried out, a more definite statement, based on morphological similarity alone, would be unjustified.

The investigation by Baker et al (1950) into the bacterial breakdown of starch in the pig's caecum was in the main restricted to raw potato starch. The significance of the bacterial breakdown of this form of starch has been dealt with elsewhere (see page 4[illegible]). That a somewhat similar state of affairs may occur when a diet high in grain meal is fed to young pigs is indicated from the results presented here. The full significance of the effect on, and the contribution to, the host animal of the bacterial digestion of starch within its large intestine remains to be elucidated.

(c) Balantidium coli

The observation reported herein that Balantidium coli occurred only in the encysted stage in the faecal samples of infected pigs is in agreement with McDonald (1922) cited by Rees (1927). In contrast, Rees (1927) and Shumaker (1930) rarely found encysted balantidia in the faeces of infected pigs. Also, in work on post mortem material prior to the experiments reported here, balantidia were found to occur mainly as trophozoites in the rectum of infected animals.

Descriptions of Balantidium coli cysts are presented in books by Craig (1942) and Kudo (1954). In these descriptions no mention is made of food particles being included in the cytoplasm of the encysted organism. E. A. Reynolds (pers. comm.) has observed that prior to encystment, balantidia discharge all the starch granules and other undigested food

through the cytopyge. Similar extrudation of food materials prior to encystment is known to occur in other protozoan species which inhabit the alimentary tract of the pig, e.g. Endamoeba coli (Craig 1942). The last author has found that balantidia encysts only when conditions are unfavourable for multiplication. Rees (1927) observed trophozoites in faecal samples which had been kept at room temperature (20°C) for up to 11 days.

The majority of the cysts observed here were engorged with starch granules (Figure 19). This would indicate that, although adequate substrate was present for trophozoites to survive, some factor/s caused them to encyst rapidly. It is probable that the mechanical mixing of the samples was sufficient, and that the organisms were preserved in the encysted stage, because formalin was added a short time after mixing.

An examination of Table XXV shows that balantidia were found in the faeces of only one piglet prior to 5 weeks of age. From 5 to 8 weeks of age 53 of the 92 faecal samples examined contained balantidia cysts (Appendix XI). Counts of Balantidium coli per g. of faeces from the 53 samples were within the following ranges:

No: of samples	Range
8	$10^4 - 10^5$
32	$10^3 - 10^4$
13	$10^2 - 10^3$

During the nursing period, balantidia were present in the faeces of the four sows used in these experiments (Table XXVI). It is apparent, therefore, that all piglets were exposed to infection.

From the results presented here it appears that the presence of undigested starch in the large intestine is necessary before balantidia multiply to the extent that they can be observed in the faeces. No physiological explanation can be given for the difference between litters or the difference between littermates in the first appearance and recurrence of balantidia in the faeces. It is possible that these organisms were present but in insufficient numbers to be counted. The lower limit of the counting techniques employed was 10^2 organism per g. of faeces.

The observation reported here that a diet containing grain meal, some of which was not digested by the piglets, favoured infection by balantidia is in agreement with Schumaker (1931). In contrast with the findings of Schumaker (1931), large numbers of balantidia were not always found in the samples which contained large numbers of undigested starch granules. Also, the majority of faecal counts were lower than the caecal counts obtained by Schumaker (1931) even though a similar counting technique was employed. The main reason for these differences is probably related to the difference in the source of samples, i.e. faeces and caecal material. Although it has been established that there is a close relationship between the faecal and caecal counts of some organisms (Review of Literature), no such comparisons have been reported in the literature for Balantidium coli. It is probable that Balantidium coli, an extremely motile organism, will tend to remain in the large intestine when conditions for multiplication are suitable and only relatively few will pass out with the faeces.

In the experiments reported here, scouring was observed in only two piglets, both at about 1 - 2 weeks of age. Cultural and microscopic

examination of liquid faeces from these piglets revealed that bacterial counts were similar to those of their littermates which had well formed faeces and no balantidia were found. Apart from the very hard faecal pellets from piglets 1 - 2 weeks of age, no other abnormal faecal samples were observed. This would indicate that no digestive disturbances occurred in the other 22 piglets during the two 8 week experimental periods.

At the time of the early work on the problem of scouring in young pigs at Massey University College of Manawatu Research Piggery the diet they received from 10 - 31 days of age was similar to that used in the experiments reported here except that barley meal was the source of starch. From the work reported here it is evident that this type of starch was not completely digested by piglets 31 - 56 days of age. Also, balantidia occurred in the faeces of all but 3 of the 24 piglets examined. It was observed that the balantidia were able to ingest the starch granules present (Figure 19).

Although it has not been established that Balantidium coli, by itself, is pathogenic for piglets it is evident that it is responsible for some damage to the wall of the large intestine (Review of Literature). In the earlier work by B.A. Reynolds (pers. comm.) large numbers of undigested starch granules and balantidia were commonly found in the faeces of scouring piglets. Examination of histological sections of the large intestine from such piglets showed balantidia deep in the crypts of the mucous membrane with an associated focal necrosis. On occasions they were found deep in the muscularis or within a gelatinous exudate adherent to the peritoneal surface of the serosa. These histopathological

observations are in agreement with Beck et al (1953), Dunlap (1958) and Hagan and Bruner (1961).

From the results reported here it is evident that the presence of balantidia and undigested starch granules in the large intestine do not in themselves cause scouring. Prior to the time of these experiments barley meal was fed to the piglets from 10 - 31 days of age at the Massey University College of Manawatu Research Piggery. It is possible that the surface of the large intestinal mucosa may be insufficiently resistant to damage by this sharp grain meal at this age. This could be the mechanical factor, suggested by Tempelis and Lysenko (1957), which allows balantidia to pass through the mucus and epithelial surface of the large intestine and then by hyaluronidase activity, enlarge the lesion. The host would then scour because of gut irritation and perforation of the gut wall may occur as has been observed in the earlier work mentioned above.

It may be significant that during the period covered by these experiments when semolina replaced barley meal in the diet of piglets 10 - 31 days of age, very little scouring was observed among the pigs at the Massey University College of Manawatu Research Piggery.

CHAPTER VI

SUMMARY

1. A trial was conducted to study the flora, fauna and amounts of undigested material present in the faeces during early growth of the pig and to determine how they vary with diet, age and management (weaning time).

2. The experiments involved 24 piglets, six from each of four litters, and their dams. The piglets from two litters were weaned at 3 weeks of age and the others at 6 weeks. Faecal samples were taken from each piglet weekly during the first 8 weeks of life and from the sow while it was with the litter.

3. The organisms studied and enumerated by cultural methods in these experiments were, total anaerobes, enterococci, lactobacilli, Escherichia coli and Clostridium welchii.

4. Differences in counts of each of the above organisms between litters weaned at 3 weeks of age and 6 weeks were not significant.

5. The possible causes of the observed significant differences between Litters w Management and the significant interaction with Age for counts of total anaerobes, lactobacilli, enterococci and Escherichia coli were discussed.

6. The influence of one piglet on another within the same litter was evident because of the non-significant difference in counts of the above organisms (see 5.) between Piglets w Litter w Management.

7. Changes in age and diet did not have a consistent effect on the counts of the above organisms (see 5.) between Litters w Management.

8. A comparison of two media for the detection and enumeration of enterococci from piglet faeces showed that M-Enterococcus Agar was superior to Mitis-Salivarius Agar.

9. Iodophilic bacteria, Balantidium coli and undigested starch granules were studied and enumerated by microscopy from preserved samples.

10. Differences in counts of each of the above (see 9.) between litters weaned at 3 weeks of age and 6 weeks were not significant.

11. Large numbers of undigested starch granules were first observed in the faeces of all piglets at 5 weeks of age and remained at a consistent level until 8 weeks.

12. Iodophilic bacteria were first observed in the faeces at approximately 5 - 6 weeks of age and were present in 7% of the 92 faecal samples examined between 5 - 8 weeks of age. Their relationship with starch granules is illustrated and discussed.

13. Except on one occasion Balantidium coli were not found in the faeces of piglets prior to the appearance of undigested starch.

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APPENDIX I

Log Count of total anaerobes per g. of faeces.
(Duplicates).

LITTER			RAT			
PER WEEK	1	2	3	4	5	6
1	9.09	9.19	9.23	9.24	9.32	9.76
	9.14	9.19	9.24	9.27	9.34	9.78
2	8.98	9.28	9.15	8.15	7.77	7.96
	9.00	9.32	9.28	8.20	7.79	7.98
3	8.80	8.48	8.76	8.61	8.11	9.00
	8.86	8.49	8.8[illegible]	8.64	8.18	9.11
4	8.28	8.36	6.64	8.56	8.72	8.04
	8.30	8.45	6.68	8.76	8.73	8.20
5	8.83	9.41	7.86	9.07	7.80	9.15
	8.85	9.42	7.86	9.08	7.81	9.18
6	8.00	7.85	8.08	7.81	7.42	7.42
	8.28	8. 0	8.15	7.83	7.43	7.45
7	----	----	9.15	6.83	8.40	7.66
	----	----	9.28	6.[illegible]3	8.52	7.75
8	----	----	8.30	7.45	7.18	7.20
	----	----	8.34	7.46	7.20	7.20

APPENDIX I ctd.

LITTER			SW2			
PIG	7	8.	9	10	11	12
WEEK						
1	9.32	9.15	9.00	8.30	9.54	9.18
	9.34	9.20	9.04	8.45	9.58	9.20
2	8.49	8.95	8.61	8.26	9.11	8.45
	8.59	9.20	8.65	8.45	9.15	8.46
3	7.77	9.00	8.40	7.46	8.26	8.48
	7.87	9.00	8.49	7.62	8.45	8.48
4	8.39	8.28	8.77	8.46	8.32	6.70
	8.59	8.34	8.78	8.52	8.46	6.95
5	9.12	7.91	7.18	7.00	7.48	7.11
	9.12	7.98	7.36	7.15	7.62	7.18
6	7.38	7.49	7.95	7.21	7.40	6.90
	7.65	7.52	8.00	7.53	7.49	7.26
7	7.54	8.30	6.95	8.04	8.08	8.08
	7.81	8.40	7.04	8.15	8.20	8.26
8	7.28	7.04	6.90	7.46	6.48	7.60
	7.42	7.36	6.90	7.56	6.95	7.60

APPENDIX I ctd.

LITTER			L71			
PIG	13	14	15	16	17	18
WEEK						
1	9.32	8.94	9.13	8.61	8.95	9.23
	9.49	8.96	9.25	8.89	9.00	9.28
2	8.26	8.92	9.38	8.26	8.15	7.70
	8.40	9.01	9.45	8.36	8.18	7.74
3	7.48	7.95	8.95	8.04	7.68	8.08
	8.34	8.00	9.08	8.08	7.72	8.23
4	8.01	8.10	8.26	7.59	8.62	8.00
	8.05	8.25	8.28	7.60	8.68	8.04
5	7.61	6.48	7.49	8.04	7.60	7.04
	7.68	6.58	7.58	8.08	7.60	7.20
6	6.43	6.15	7.08	6.61	7.60	6.34
	6.60	6.25	7.23	6.65	7.73	6.48
7	6.60	7.90	8.11	8.08	8.35	6.43
	6.68	7.91	8.34	8.20	8.15	6.52
8	8.29	7.81	8.04	8.48	7.95	8.34
	8.32	7.85	8.08	8.51	8.04	8.36

Appendix I cont'd.

LITTER			IN2			
PIG	19	20	21	22	23	24
WEEK						
1	9.33	8.75	8.95	9.36	9.51	8.67
	9.53	8.78	9.08	9.36	9.53	8.72
2	8.54	8.78	8.46	8.60	9.08	8.78
	8.60	8.79	8.54	8.65	9.20	8.78
3	8.26	7.90	7.70	7.70	9.26	9.25
	8.32	7.95	7.85	8.04	9.26	9.26
4	8.61	8.48	7.90	8.57	8.20	8.28
	8.63	8.48	8.28	8.57	8.62	8.28
5	9.13	8.26	8.04	8.04	9.11	8.46
	9.13	8.30	8.18	8.30	9.11	8.68
6	6.48	6.95	6.48	7.18	8.30	6.78
	6.48	7.23	6.60	7.30	8.36	7.00
7	9.28	8.73	8.28	8.82	8.46	8.11
	9.28	8.79	8.52	8.85	8.59	8.20
8	7.59	7.04	7.63	6.95	8.30	9.09
	7.62	7.36	7.64	7.08	8.40	9.00

APPENDIX II

Log Count of enterococci per g. of faeces on Mitis-Salivarius Agar. (Duplicates).

LITTER			EWI			
PIG	1	2	3	4	5	6
WEEK						
1	8.58	8.71	8.90	9.28	9.46	8.85
	8.62	8.73	8.92	9.32	9.46	8.89
2	9.04	9.26	8.89	8.28	8.04	7.48
	9.18	9.42	8.97	8.34	8.15	7.48
3	8.78	8.26	8.70	8.52	7.73	9.08
	8.85	8.48	8.72	8.54	7.75	9.15
4	7.38	8.15	6.45	8.40	8.57	7.52
	7.40	8.26	6.49	8.51	8.57	7.65
5	8.36	9.32	7.08	8.90	7.69	8.83
	8.45	9.52	7.20	8.90	7.79	8.87
6	6.79	6.70	5.48	6.34	6.48	5.85
	6.86	6.84	5.78	6.56	6.48	6.08
7	-----	-----	6.30	5.60	6.20	6.26
	-----	-----	6.15	5.48	6.08	6.20
8	-----	-----	6.30	5.00	5.78	5.00
	-----	-----	6.08	5.48	6.04	5.30

APPENDIX II ctd.

LITTER			EW2			
PIG	7	8	9	10	11	12
WEEK						
1	8.16	5.30	6.93	5.00	5.60	8.08
	8.32	5.30	6.98	5.30	5.78	8.18
2	7.11	8.66	8.58	7.62	7.90	7.89
	7.20	8.76	8.59	7.66	7.95	7.92
3	5.78	8.92	8.38	7.40	7.69	7.81
	6.00	8.94	8.38	7.43	7.71	7.81
4	5.00	5.95	5.30	6.08	5.48	5.00
	5.00	6.00	5.30	6.23	5.60	5.30
5	5.70	5.85	5.00	5.00	5.60	5.60
	5.90	5.90	5.00	5.48	6.08	5.60
6	5.70	5.00	5.00	5.00	5.00	5.70
	5.95	5.00	5.30	5.30	5.00	5.85
7	6.00	6.65	6.46	5.48	5.00	5.70
	6.20	6.67	6.56	5.78	5.30	5.70
8	6.34	5.95	5.60	6.48	5.60	6.57
	6.56	6.26	5.78	6.85	5.60	6.75

APPENDIX II cfd.

LITTER			LWI			
PIG	13	14	15	16	17	18
WEEK						
1	9.08	8.84	9.15	8.43	8.76	9.23
	9.08	8.98	9.20	8.59	8.78	9.32
2	7.95	8.91	9.42	8.43	7.52	7.59
	7.98	8.88	9.49	8.53	7.58	7.68
3	6.85	5.78	8.06	7.75	7.23	7.04
	6.94	5.78	8.08	7.86	7.26	7.20
4	7.18	5.78	6.95	6.62	6.58	7.23
	7.23	6.20	6.95	6.66	6.61	7.28
5	5.95	5.00	5.78	6.00	5.48	6.36
	6.00	5.78	6.00	6.26	5.78	6.45
6	5.00	5.00	5.00	5.00	5.78	5.00
	5.00	5.00	5.60	5.30	6.04	5.48
7	5.00	5.85	5.30	6.68	5.00	5.00
	5.00	5.90	5.30	6.71	5.00	5.00
8	5.00	5.00	5.00	6.00	5.48	6.26
	5.48	5.00	5.00	6.04	5.60	6.30

APPENDIX II ctd.

LITTER			LW2			
PIG	19	20	21	22	23	24
WEEK						
1	7.08	6.75	6.62	7.57	6.20	5.48
	7.20	6.80	6.71	7.64	6.28	5.70
2	6.60	7.15	6.40	6.52	8.42	8.53
	6.70	7.26	6.42	6.57	8.51	8.65
3	7.66	6.83	6.95	6.45	7.78	8.70
	7.68	6.84	7.04	6.58	8.18	8.70
4	6.85	8.08	7.62	6.52	7.04	6.52
	6.95	8.11	7.67	6.53	7.23	6.53
5	8.11	6.60	6.88	7.11	7.90	7.11
	8.11	6.60	6.95	7.30	7.97	7.28
6	5.30	5.95	5.48	6.00	5.30	5.00
	5.48	6.34	5.70	6.28	5.95	5.00
7	7.80	6.52	6.15	7.15	6.85	6.30
	7.97	6.42	6.15	7.26	7.04	6.38
8	5.60	5.30	5.30	5.00	5.00	5.78
	5.70	5.70	5.30	5.00	5.00	5.95

APPENDIX III

Log Count of *Escherichia coli* per g. of faeces (Duplicates).

LITTER			EWI			
PIG	1	2	3	4	5	6
WEEK						
1	9.04	9.00	8.40	9.11	9.28	8.74
	9.15	9.00	8.43	9.12	9.30	8.76
2	9.04	9.20	9.08	7.86	7.83	7.04
	9.18	9.38	9.18	7.86	7.88	7.18
3	8.78	8.46	8.49	8.51	8.05	9.04
	8.86	8.55	8.62	8.58	8.08	9.15
4	8.20	8.20	6.58	8.43	8.57	8.04
	8.26	8.36	6.68	8.54	8.61	8.04
5	8.66	9.51	7.85	8.95	7.77	8.91
	8.67	9.59	7.98	8.96	7.81	8.95
6	7.81	7.93	7.56	7.76	7.40	7.30
	7.76	8.05	7.60	7.85	7.51	7.32
7	----	----	8.97	6.51	8.40	7.48
	----	----	8.97	6.64	8.45	7.55
8	----	----	6.76	6.32	6.49	6.89
	----	----	6.76	6.83	6.54	7.00

APPENDIX ctd. III

LITTER			EM2			
PIG	7	8	9	10	11	12
WEEK						
1	8.65	8.62	8.67	7.11	9.30	8.87
	8.65	8.69	8.76	7.28	9.43	8.95
2	8.49	8.95	8.61	8.26	9.11	8.45
	8.59	9.20	8.65	8.43	9.15	8.46
3	7.77	9.00	8.40	7.46	8.26	8.48
	7.87	9.00	8.49	7.62	8.45	8.48
4	8.45	8.34	8.67	8.34	8.26	6.77
	8.70	8.43	8.77	8.51	8.43	6.73
5	9.04	7.76	6.26	6.76	7.49	7.00
	9.26	7.81	6.38	6.82	7.54	7.15
6	7.23	7.36	7.00	7.42	7.30	7.08
	6.85	7.45	7.00	7.52	7.42	7.18
7	7.58	8.26	6.43	8.05	7.81	8.04
	7.76	8.26	6.49	8.05	7.90	8.11
8	7.40	6.32	6.15	7.18	6.61	7.51
	7.46	6.40	6.15	7.45	6.62	7.56

APPENDIX III ctd.

LITTER NO.	LWI					
WEEK	13	14	15	16	17	18
1	8.87	8.91	9.11	8.72	8.89	9.38
	8.90	8.92	9.18	8.72	8.96	9.36
2	8.15	9.00	9.01	7.95	8.18	7.78
	8.18	9.00	8.90	8.08	8.23	7.81
3.	7.48	6.30	8.43	8.18	7.38	8.00
	8.04	6.72	8.45	8.30	7.38	8.11
4	7.83	8.36	8.15	7.45	8.31	7.81
	7.88	8.40	8.18	7.45	8.32	7.87
5	7.48	6.26	7.15	8.00	7.61	6.60
	7.52	6.34	7.19	8.04	7.70	6.67
6	5.95	5.78	7.11	6.65	7.31	5.95
	6.11	5.85	7.26	6.71	7.61	6.00
7	5.85	7.53	7.85	7.93	7.90	6.08
	5.95	7.58	8.07	7.98	8.02	6.08
8	8.20	7.87	7.78	8.18	7.53	8.18
	8.30	7.92	8.20	8.26	7.64	8.26

APPENDIX III ctd.

LITTER			LM2			
PIG	19	20	21	22	23	24
WEEK						
1	8.53	8.52	8.54	9.00	8.56	8.30
	8.60	8.54	8.59	9.26	8.62	8.30
2	8.52	8.57	8.53	8.36	8.77	8.89
	8.56	8.61	8.61	8.48	8.95	8.91
3	8.11	7.62	7.51	7.83	9.26	9.26
	8.15	7.68	7.64	7.85	9.30	9.28
4	8.60	8.45	7.88	8.60	8.00	8.15
	8.64	8.54	7.90	8.60	8.11	8.38
5	9.26	8.18	8.11	8.00	9.04	8.61
	9.30	8.18	8.20	8.23	9.18	8.70
6	5.78	6.66	6.53	7.23	8.36	6.52
	5.95	6.72	6.60	6.95	8.38	6.58
7	9.20	8.74	8.11	8.66	8.46	8.02
	9.32	8.80	8.36	8.88	8.53	8.05
8	7.68	7.11	7.62	6.89	8.18	8.90
	7.78	7.30	7.69	6.91	8.30	9.08

APPENDIX IV

Log Count of lactobacilli per G. of faeces (Duplicates).

LITTER			MWI			
PIG	1	2	3	4	5	6
WEEK						
1	7.11	8.54	8.54	6.90	7.32	8.20
	7.26	8.58	8.56	6.95	7.32	8.23
2	7.15	8.09	6.56	8.32	8.32	6.95
	7.20	8.15	6.60	8.32	8.61	7.11
3	8.28	7.80	7.26	6.88	6.45	7.62
	8.56	7.83	7.42	6.88	6.46	7.76
4	7.28	7.61	6.08	6.53	7.23	7.11
	7.34	7.65	6.30	6.69	7.46	7.28
5	7.26	7.51	6.70	6.57	6.57	6.78
	7.49	7.57	6.78	6.70	6.63	6.79
6	7.96	7.93	7.69	7.36	7.72	8.13
	7.98	7.94	7.70	7.36	7.72	8.30
7	-----	-----	8.34	7.00	7.53	7.08
	-----	-----	8.36	7.04	7.63	7.89
8	-----	-----	7.46	6.32	6.80	6.95
	-----	-----	7.51	6.34	6.69	7.15

APPENDIX IV ctd.

LITTER			EW2			
PIG	7	8	9	10	11	12
WEEK						
1	7.69	5.48	7.00	8.32	8.63	8.34
	7.71	5.78	7.00	8.45	8.72	8.48
2	7.30	6.11	5.30	6.20	7.04	8.18
	7.36	5.78	5.60	6.26	7.08	8.26
3	6.08	6.78	6.40	7.28	6.42	7.18
	6.08	6.79	6.56	7.32	6.48	7.18
4	6.11	6.54	5.30	6.20	5.90	5.85
	6.20	6.57	5.78	6.32	6.04	5.95
5	6.04	6.72	7.64	7.67	7.65	7.26
	6.23	6.86	7.74	7.72	7.71	7.32
6	7.71	7.82	7.61	7.65	7.85	7.08
	7.79	7.85	7.65	7.69	7.95	7.34
7	7.62	7.73	7.76	7.54	7.30	7.11
	7.69	7.81	7.89	7.58	7.36	7.26
8	6.36	7.34	7.66	6.86	7.04	6.88
	6.46	7.51	7.78	6.86	7.08	6.88

APPENDIX IV ctd.

LITTER			LWI			
PIG	13	14	15	16	17	18
WEEK						
1	7.14	8.76	6.61	7.73	8.49	7.70
	7.14	8.79	6.63	7.76	8.53	7.76
2	8.43	8.87	7.75	6.72	8.11	7.76
	8.46	8.87	7.86	6.75	8.20	7.81
3	7.40	8.04	6.93	7.74	7.07	7.88
	7.40	8.08	7.02	7.76	7.11	7.91
4	7.30	7.04	6.26	6.52	7.26	6.62
	7.30	7.13	6.28	6.54	7.28	6.64
5	6.95	7.79	6.54	7.15	7.70	6.60
	6.99	7.90	6.56	7.23	7.75	6.62
6	7.59	7.26	7.36	7.42	7.61	7.63
	7.60	7.32	7.38	7.49	7.73	7.80
7.	7.77	7.96	7.45	7.53	7.36	8.18
	7.84	8.00	7.52	7.72	7.38	8.38
8	7.36	6.95	7.45	7.30	7.48	7.43
	7.38	6.95	7.45	7.52	7.78	7.53

APPENDIX IV ctd

LITTER			IN2			
PIG	19	20	21	22	23	24
WEEK						
1	8.02	8.36	8.48	8.04	9.04	8.38
	8.84	8.38	8.62	8.30	9.08	8.38
2	6.84	7.52	5.48	7.68	7.48	6.76
	6.86	7.59	5.70	7.76	7.54	6.79
3	6.20	5.30	5.00	5.00	5.30	6.94
	6.28	5.70	5.48	5.00	5.60	7.03
4	7.01	6.30	7.34	5.78	6.18	5.70
	7.93	6.48	7.42	5.90	6.26	5.90
5	6.28	6.04	6.15	7.34	6.53	6.11
	6.32	6.18	6.30	7.42	6.56	6.26
6	8.00	7.76	6.48	8.30	7.20	7.08
	8.08	7.84	6.53	8.30	7.28	7.15
7	8.00	8.42	8.26	8.08	7.65	7.79
	8.15	8.46	8.32	8.25	7.68	7.89
8	7.97	6.85	7.18	7.18	8.08	7.34
	7.99	7.15	7.23	7.30	8.32	7.48

APPENDIX V

Log count of enterococci per g. of faeces on M-Enterococcus Agar for Experiment II (Duplicates).

LITTER			EM2			
PIG	13	14	15	16	17	18
WEEK						
1	8.11	6.00	6.96	5.00	5.00	8.08
	8.14	5.85	6.99	5.00	5.00	8.08
2	7.40	8.08	8.52	7.33	8.20	8.00
	7.26	8.11	8.45	7.61	8.11	8.00
3	5.00	8.52	6.83	7.11	7.51	5.00
	5.00	8.63	6.85	7.20	7.45	5.00
4	5.00	6.00	5.30	6.08	5.48	5.30
	5.00	5.95	5.30	6.23	5.60	5.00
5	5.78	5.78	5.30	5.30	6.20	5.95
	6.00	6.08	5.30	5.00	5.95	5.85
6	5.95	5.00	5.30	5.00	5.00	5.90
	5.90	5.00	5.78	5.00	5.00	5.95
7	6.04	6.77	6.43	5.00	5.78	5.00
	6.34	6.71	6.52	5.00	5.00	5.70
8	6.56	6.38	6.15	6.65	5.30	6.68
	6.34	6.26	6.15	6.78	5.60	6.76

APPENDIX V ctd

LITTER NO. WEEK	19	20	LW2 21	23	23	24
1	6.59	7.28	6.6	7.62	6.08	5.30
	6.57	7.20	6.63	7.58	6.26	5.78
2	6.68	7.18	6.23	6.52	8.43	8.52
	6.68	7.08	6.26	6.53	8.52	8.61
3	7.61	6.58	6.76	6.60	8.15	6.91
	7.73	6.68	6.76	6.60	8.15	6.85
4	7.11	8.00	7.67	6.57	7.28	6.77
	7.08	8.15	7.57	6.67	7.30	6.52
5	8.08	6.63	6.89	7.04	7.90	7.00
	8.23	6.65	6.80	7.15	7.91	7.26
6	5.78	6.20	5.78	6.08	5.90	5.30
	5.85	6.40	5.78	6.32	5.70	5.60
7	7.96	6.51	6.11	7.04	6.87	6.49
	7.90	6.38	6.30	7.08	6.90	6.49
8	5.85	5.60	5.30	5.30	5.60	6.00
	5.70	5.70	5.60	5.30	5.70	6.00

APPENDIX VI

Log Count of *Clostridium welchii* per c. of faeces for Experiment II (Duplicate Averages).

LITTER			LW2			
PIG	13	14	15	16	17	18
WEEK *						
1	8.74	7.38	8.70	8.33	7.45	8.30
2	8.08	7.18	6.70	6.70	8.20	8.33
3	5.48	7.57	6.90	5.30	5.30	6.95
4	0	0	5.30	0	0	0
5	0	0	0	0	0	0

LITTER			LW2			
PIG	19	20	21	22	23	24
WEEK*						
1	8.78	8.40	7.95	8.30	8.40	7.70
2	7.18	7.56	6.34	6.08	6.89	7.65
3	0	5.30	6.38	5.00	5.00	5.30
4	0	0	0	0	0	6.26
5	0	0	5.48	0	0	0

* No organisms recorded for weeks 5 to 8

APPENDIX VII

Log Count of all organisms per g. of faeces for the sows in Experiment I and II (Duplicate averages).

WEEKS (Post Farrowing)		1	2	3	4	5	6
LITTER	ORGANISM						
EXI	Total anaerobes	8.30	7.54	7.15			
	Lactobacilli	7.66	7.30	7.06			
	Enterococci (M-SA)*	6.15	6.11	7.32			
	Escherichia coli	5.85	5.48	5.70			
LXI	Total anaerobes	6.67	6.30	5.81	6.04	6.73	
	Lactobacilli	6.85	6.64	6.60	6.28	7.49	
	Enterococci (M-SA)	7.85	7.23	5.48	5.00	5.60	
	Escherichia coli	7.85	6.20	5.41	6.08	5.95	
EX2	Total anaerobes	6.26	5.95	not plated			
	Clostridium welchii	0	0	0			
	Lactobacilli	5.70	6.16	6.59			
	Enterococci (M-SA)	0	0	0			
	Enterococci (M-EA)**	0	5.00	0			
	Escherichia coli	5.30	5.95	0			
LX2	Total Anaerobes	7.82	NOT PLATED				
	Clostridium welchii	6.81	5.81	5.00	0	6.30	6.00
	Lactobacilli	7.48	6.37	5.78	7.57	7.30	6.58
	Enterococci (M-SA)	6.04	5.30	5.00	5.70	5.78	7.04
	Enterococci (M-SA)	6.11	5.70	5.00	5.70	6.04	7.08
	Escherichia coli	7.39	7.45	7.39	7.28.	6.67	7.65

* Mitis-Salivarius Agar

** M-Enterococcus Agar

APPENDIX VIII

pH levels of topsoil samples

[illegible]	[illegible]					
[illegible]	1	2	3	4	5	6
[illegible]						
2	7.0	6.9	7.3	7.6	7.4	7.5
3	7.2	6.9	6.9	6.8	7.0	7.4
4	7.1	6.8	7.2	7.0	6.9	7.3
5	7.0	7.2	7.2	6.9	6.7	6.9
6	7.0	7.3	7.3	7.1	7.1	7.5
7	-	-	7.0	7.3	7.4	7.2
8	-	-	7.3	7.3	7.1	7.3

[illegible]	[illegible]					
[illegible]	7	8	9	10	11	12
[illegible]						
2	7.3	7.2	6.5	7.1	6.9	7.5
3	6.5	7.1	7.3	7.4	6.9	6.9
4	6.8	6.1	6.3	6.5	6.1	6.2
5	7.1	7.0	6.8	7.2	7.0	7.2
6	6.9	7.0	7.2	7.3	7.1	7.4
7	6.8	7.2	7.1	7.2	6.9	6.8
8	7.3	6.9	7.3	7.5	7.2	7.1

APPENDIX VIII.cont'd.

LITTER			LW1			
PIG	13	14	15	16	17	18
WEEK						
2	7.6	7.8	7.3	7.9	7.7	7.6
3	7.1	7.2	7.1	7.3	7.2	7.4
4	7.2	7.3	7.1	7.6	7.5	7.3
5	7.3	7.2	7.4	7.5	7.2	7.0
6	7.1	7.4	7.5	7.3	7.3	7.7
7	6.7	6.6	6.6	7.1	6.7	7.1
8	6.5	6.7	7.1	6.5	6.6	7.1

LITTER			LW2			
PIG	19	20	21	22	23	24
WEEK						
2	6.9	6.6	6.6	6.5	6.0	6.1
3	7.9	6.7	7.2	8.0	7.3	7.2
4	7.6	7.4	8.1	7.4	7.7	7.6
5	7.6	7.2	7.7	7.8	7.6	8.1
6	7.3	7.2	7.1	7.6	7.0	7.0
7	7.5	7.8	7.5	6.8	6.6	7.8
8	7.5	7.4	7.3	7.4	7.2	7.3

APPENDIX IX

Log of starch granules per gram of faeces (Experiment I and II)

Age (weeks)		5	6	7	8
Litter	Pig				
EWI	1	6.24	5.95		
	2	6.15	5.52		
	3	6.34	5.86	6.02	5.03
	4	6.24	5.18	5.42	4.84
	5	5.98	5.53	5.99	5.38
	6	6.51	6.15	6.09	6.11
EW2	7	6.47	5.22	5.85	5.09
	8	6.09	5.73	4.82	5.45
	9	6.05	5.65	5.71	5.59
	10	6.32	4.65	5.41	5.78
	11	6.22	5.22	4.84	5.34
	12	5.50	5.64	5.60	5.61
EW1	13	6.04	6.17	6.12	5.61
	14	5.82	6.38	6.21	5.74
	15	5.69	6.03	5.95	5.63
	16	6.05	6.45	5.94	5.97
	17	5.99	6.41	5.91	6.07
	18	5.82	5.24	5.99	5.06
EW2	19	5.76	5.80	5.69	5.83
	20	5.32	5.47	5.21	4.38
	21	5.56	5.33	5.56	5.41
	22	5.06	5.34	5.57	4.69
	23	5.56	4.99	5.76	6.08
	24	4.86	4.71	4.68	5.39

APPENDIX IX cont.

Means and their standard errors for log counts of starch granules

Litter means

EWI	5.75
EW2	5.59
LWI	5.93
LW2	5.40

$$S.E.^{a} = \pm\sqrt{\frac{(LwM)\ M.S.}{47}}$$

$$= \pm 0.20$$

Week means

AGE (Weeks)	5	6	7	8
LITTER				
EWI	6.28	5.70	5.73	5.30
EW2	6.11	5.39	5.37	5.48
LWI	5.74	6.28	6.02	5.68
LW2	5.57	5.27	5.45	5.30

$$S.E. = \pm\sqrt{\frac{(AxPwLwM)\ M.S.}{6}}$$

$$= \pm 0.15$$

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[a] The standard error for litter EWI was calculated using the adjusted degrees of freedom.

ANNEX I

log of colonies of Eotrophilic bacteria per gram of faeces

(Experiment I and II)

Age (weeks)		5	6	7	8
Rabbit	No.				
I	1	4.49	6.19		
	2	5.00	6.10		
	3	0	5.17	5.38	5.11
	4	4.30	4.60	5.60	5.05
	5	4.76	6.10	5.28	4.20
	6	4.30	5.23	5.91	5.32
II	7	0	5.21	5.30	4.64
	8	5.00	5.30	5.54	4.25
	9	4.70	5.30	4.78	4.20
	10	0	5.41	5.22	5.40
	11	0	5.41	4.71	0
	12	0	0	4.32	5.48
III	13	0	4.23	5.17	5.62
	14	4.24	5.39	5.11	4.89
	15	0	4.26	5.30	4.39
	16	5.17	5.13	4.49	5.39
	17	5.19	4.30	4.81	4.92
	18	5.18	4.34	5.09	5.78
IV	19	0	0	5.24	0
	20	0	5.20	4.98	0
	21	0	4.19	5.18	4.25
	22	0	4.24	4.13	5.20
	23	0	5.30	5.14	4.30
	24	0	5.33	5.03	5.70

APPENDIX XI

Log of *Balantidium coli* per gram of faeces
(Experiment I and II)

Age (Weeks)		4	5	6	7	8
Litter	Pig					
EWI	1	0	0	2.00		
	2	0	0	0		
	3	0	0	0	3.64	3.11
	4	0	0	3.69	3.45	4.20
	5	0	0	3.45	3.66	3.40
	6	0	0	0	0	0
EW2	7	0	0	0	0	2.78
	8	0	3.15	2.00	2.95	4.18
	9	0	3.00	0	0	3.69
	10	0	2.00	3.20	3.74	4.07
	11	0	3.59	3.04	0	3.42
	12	2.70	2.70	2.78	2.90	3.59
LWI	13	0	3.68	0	0	0
	14	0	0	0	0	3.65
	15	0	3.20	4.25	2.60	3.88
	16	0	3.43	3.92	3.79	0
	17	0	0	2.00	0	0
	18	0	3.51	0	3.08	3.51
LW2	19	0	0	0	0	0
	20	0	0	0	3.23	3.00
	21	0	0	2.78	3.23	3.51
	22	0	0	3.54	0	3.60
	23	0	0	4.06	2.00	0
	24	0	0	4.03	4.21	4.24