AN INVESTIGATION OF FACTORS AFFECTING THE COMPOSITION OF MILK AND OF METHODS FOR THE ANALYSIS OF MILK COMPONENTS.

by

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THIS THESIS IS PRESENTED IN TWO MAIN PARTS.

(1) A study of the solids-not-fat content of milk and of some methods for the determination of Solids-not-Fat and Protein.

(2) A study of sampling frequency and the prediction of protein production in milk from dairy cows based on a restricted frequency of sampling.

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PART I.

A STUDY OF THE SOLIDS-NOT-FAT CONTENT OF MILK AND OF SOME METHODS FOR THE DETERMINATION OF SOLIDS-NOT-FAT AND PROTEIN.

PART I : INDEX

INTRODUCTION

Low solids-not-fat (S.N.F.) levels in the liquid milk industry prompted an enquiry into the problem of meeting the minimum legal requirements for S.N.F. (8.5%) and into methods for determining the S.N.F. content and the protein content of the milk. The methods, for determining the S.N.F., which were investigated were based mainly on the hydrometric or density technique which gives an indirect but rapid indication of the composition, whilst the amido black technique was examined with a view to assessing its suitability as a routine method for the protein determination.

There is a growing awareness of the nutritional advantages of milk protein and of other nutritives in the solids-not-fat content of milk.

Waite et al. have stated "In recent years the solids-not-fat content of milk has come to be regarded as of equal, or of greater importance, than the fat percentage. This change is the result of an increased awareness of the nutritive value of milk protein and other non-fatty constituents, in particular calcium and vitamins." Hansson in the same year wrote "The growing competition between fats of animal and plant origin has also led to the conclusion that the non-fat components of the milk will be emphasized more correctly in the future."

Furthermore, it is considered that there is an urgent need to maintain milk composition up to the legal requirements when it is delivered to the consumer.

Improvement in protein and S.N.F. percentage and production levels can be brought about, for example, by selective breeding. The use of S.N.F. percentage and production levels as indicative parameters for evaluating genetic improvement in protein levels is justified because of the high correlations that exist between S.N.F. and protein in milk, and because the measurement techniques for S.N.F. are simpler than those for protein.

The use of mixed breed herds will also effectively meet this need for improved S.N.F. and protein levels, but because it introduces problems in husbandry and management this is not a widely accepted answer to the problem.

A very simple and practical method for meeting minimum legal requirements, and for upgrading the protein level and nutritional value of the milk, is for low S.N.F. milks to be fortified at the milk processing plants with non-fat dried milk solids. A logical extension of this concept is the use of non-fat-dried milk solids and unsalted butter produced under maximum conditions of efficiency to provide a continuous or partial supply of reconstituted milk of standardised composition for the liquid milk market. This could readily replace the relatively inefficiently and expensively produced market milk supplies, especially winter supplies, and could result in a saving in subsidy of between £2-5 million, and at the same time allow a milk of equal or perhaps improved nutritive value to be marketed at the present prices.

This theme has been investigated and developed in a paper by the author entitled: "Meeting the minimum legal requirements for S.N.F." (Dairy Ind. 27 : 39, 114 (1962)).

The use of reconstituted dried milk solids as alternative sources of milk supply for the liquid milk industry is currently being investigated, both technologically and economically, by a committee appointed by the Minister of Agriculture. This investigation was stimulated by the publicity following the publication of the above paper which is attached to the next page.

References

HANSSON, A. (1956). Genetic variations in the content of milk constituents. VIIth Int.Zóót.Congr.,Madrid, Subj.5, 111.

WAITE, R., WHITE, J.C.D., ROBERTSON, A. (1956). Variations in the chemical composition of milk with particular reference to the solids-not-fat. I. The effect of stage of lactation, season of year and age of cow. J.Dairy Res. 23 : 65.

MEETING THE MINIMUM LEGAL REQUIREMENTS FOR S.N.F.

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Presidential Address to N.Z. Dairy Science Association, 1961

Part I

RECENTLY the Cook Committee in Great Britain recommended that a greater emphasis should be placed on the S.N.F. content of milk and that:

(ii) the efforts of the dairying industry in the years ahead should be directed towards maintaining and improving the S.N.F. content of the liquid milk supply;

(iii) the fat and S.N.F. contents of milk should continue to be the criteria used for both legal and marketing purposes;

(xii) differential payment schemes for S.N.F. should be introduced as soon as possible in all five Milk Marketing Board areas in the United Kingdom;

(xv) price penalties should be imposed in Great Britain at 8.4 per cent S.N.F. and below: gradations in the price scale should be of sufficient magnitude to provide an effective deterrent to low S.N.F. and the point at which penalties begin should be raised progressively.

The compositional quality of town milk in parts of New Zealand has caused considerable concern because of its low value, e.g. S.N.F. levels in Christchurch. The Annual Report of the Department of Health (1959), p. 29, states: "Once again the proportion of milk samples taken in Christchurch which fails to meet the standard for solids-not-fat (8.5 per cent) comprises an outstanding amount of the total non-compliance in New Zealand."

What effective measures can be taken to bring about an improvement in quality?

The problem can be handled either on the farm or in the factory.

On the Farm

(1) On the farm a testing scheme for S.N.F. and selective breeding from progeny tested stock could bring a slow but definite improvement in the quality of the milk in respect of S.N.F. Robertson, Waite and White showed there was a genetic correlation of 0.94 between percentage solids-not-fat and percentage crude protein, and also one of 0.67 between percentage solids-not-fat and percentage lactose. Thus, a breed improvement programme based on percentage solids-not-fat, instead of crude protein, could result in an increase in percentage solids-not-fat, in which part of the increase was in lactose, an expensive carbohydrate of limited nutritional significance (Ling, Kon and Porter). Our need is not to improve the lactose levels in the milk, but to increase the protein, calcium and some of the vitamin levels as milk, whether in the dried or in the liquid form, provides or could provide a very considerable proportion of the dietary needs of these constituents. Whilst it would be a foolish concept to breed for improved calcium or vitamin levels, as these can be supplemented more economically from artificial sources, there is a continuing need for proteins of high biological value and ready digestibility. Milk proteins can provide much of this need, particularly as a supplement to a predominantly cereal diet. A breed improvement programme would theoretically, therefore, be better based on protein content, not total S.N.F.

A breed improvement programme based on fat recording can bring about a limited improvement in solids-not-fat and protein levels. Thus, Robertson *et al.* demonstrated genetic correlations of approximately 0.5 between percentage fat and percentage crude protein and also between percentage fat and percentage solids-not-fat. This genetic correlation is only about half that between solids-not-fat and crude protein. Calculations using the data in their paper suggest that selection for improvement in crude protein percentage, using the indirect method of selection based on fat percentage, would give a rate of improvement of about a quarter of that possible if the protein percentage of the milk itself was used as the basis of selection.

Improved fat percentages in milk for human needs are not particularly desirable. Therefore it would be better to improve the protein percentage without increasing the fat percentage if this were possible. One of the disadantages of indirect selec-

tion on a fat basis for increased protein content is that the fat percentage will increase more rapidly than the protein percentage. Also, with indirect selection, it is possible for improvement in protein content to come to a halt if the genetic variance for percentage fat becomes small, yet there may still be unexploited genetic variance in percentage protein.

If selection for protein was based on percentage solids-not-fat, the improvement, although still indirectly controlled, would be much better, because the genetic correlation between percentage crude protein and percentage solids-not-fat is high (about 0.9); further, the increase in percentage fat would be less as the genetic correlation here is lower (about 0.5). Although this method would result in an increase in fat percentage, the rate of increase will be little different from that which would have occurred if selection had been based directly on percentage crude protein (rg crude protein % and fat % ca 0.5). Selection on solids-not-fat, or on crude protein will bring about an increase in lactose, but the increase with solids-not-fat is likely to be greater than that with protein (rg S.N.F. % and lactose % is 0.7, whereas rg protein % and lactose % is 0.4). Flux, Patchell, Campbell and McDowall have shown with Jersey cows that as solids-not-fat and fat levels increase beyond values of approximately 9.2 per cent and 5 per cent respectively, there is no comparable increase in lactose percentage levels and these, in fact, appear to drop. Thus, the increase in lactose percentage levels with breed improvement may not become a particularly serious drawback to the use of solids-not-fat as an indirect basis of selection for increased protein once a minimum level for solids-not-fat is reached.

Finally, Robertson *et al.* showed that there was virtually no correlation between solids-not-fat percentage and yield, whereas between fat percentage and yield a genetic correlation of the order of –0.2 to –0.5 is generally observed.

Stewart has stated :

(i) If a satisfactory S.N.F. recording scheme were available, the average improvement likely in closed herds, where S.N.F. was the only selection criterion, would be about 0.3 per cent at the end of the first 15 years. Where characters other than S.N.F. were considered, the improvement would be considerably less—perhaps 0.1 per cent in 15 years.

(ii) If A.I. bulls were progeny tested for S.N.F. and the best 50 per cent used extensively, without the A.I. member culling or selecting for S.N.F. and without A.I. organisation relaxing selection standards for other characters of commercial significance, there would be an improvement of about 0.22 per cent in A.I. cattle at the end of the first 15 years. (Culling 50 per cent of bulls for S.N.F. is extremely rigorous if other characters are to receive due attention.)

(iii) By basing an S.N.F. improvement plan on the existing fat-recording scheme, genetic gains could be about one-third as rapid as those possible with an S.N.F. recording scheme.

(iv) Breeding provides only a long-term answer to attempts to improve S.N.F. and if significant progress is to be made by breeding for S.N.F. other characters of commercial significance might be neglected to the ultimate detriment of the breed.

In the absence at present of a convenient field method for estimating protein, a breed improvement programme based on S.N.F. will bring about a slow but nevertheless desirable improvement in protein levels with virtually no reduction in yield. This improvement will be considerably more rapid than that which would result from an improvement programme based on fat. Furthermore, the production of protein is likely to improve at an even greater rate because of the apparently negligible correlation between yield and S.N.F., whereas there appears to be a distinct negative correlation between yield and fat.

Breed improvement programmes for percentage solids-not-fat or percentage protein without reference to production levels are completely unrealistic. A breed improvement programme must therefore either (a) increase the percentage solids-not-fat composition whilst holding solids-not-fat production constant, or preferably (b) increase the percentage solids-not-fat composition as well as increase the solids-not-fat production.

(2) A further option available to the producer is to use mixed herds to obtain a satisfactory average milk quality, but economic husbandry considerations generally indicate this to be less satisfactory than the use of herds giving a bulk of low testing milk.

In the Factory

The preparation and storage of dried non-fat milk solids results in only insignificant changes in the nutritive value of the product (Henry, Kon). In recent years the wholesale reconstitution of dry milk solids into liquid milk has been undertaken in a variety of ways in India, Bahrein, Trinidad, Hong Kong and Mexico City, and the resultant liquid milk has been well accepted. There are no valid technical or nutritional reasons why reconstitution in some form or other should not be used by milk treatment stations to bring the S.N.F. quality of the milk received up to the acceptable minimum standard and the various regulations should be modified to permit this to be done.

Reconstitution by treatment stations under strictly controlled conditions is much to be preferred to unsupervised and uncontrolled reconstitution by the farmer, a procedure which will undoubtedly be

followed by many if their S.N.F. is low.

The Cook Committee recommended that "milk should continue to be sold as it comes from the cow", and this could be discussed with considerable emotion, but only limited realism. Standardisation of the fat content (by abstraction) is already practised with homogenised milk delivered to the public and more extensively with the homogenised milk delivered to schools under the Milk in Schools scheme. This standardisation of the fat content of the milk has the blessings of the pediatricians and with the increasing interest by the medical profession and general public in low calorie diets and the possible involvement of animal fats in atherosclerosis, further standardisation of fat content is not unlikely. Surely, therefore, the addition of S.N.F. to milk to improve its nutritional quality cannot be assailed on logical grounds.

Despite their statement that milk should continue to be sold as it comes from the cow, the Cook Committee also recommended that "the fixed minimum standards should apply to milk only at the point of sale to the consumer", and if this realistic recommendation is considered in a completely practical manner the Committee must have felt that either blending with high S.N.F. content milk or in its absence addition of non-fatty dry milk solids to the low quality milk must be undertaken to protect the consumer interest.

Standards

The necessity for minimum compositional standards has been questioned. The consumer has the right of protection and no satisfactory argument has been brought forward to justify a modification of the S.N.F. standard which has been found to be of practicable attainment during the last 50 years. The producer of high quality milk, likewise, has the right of protection against the use of his milk to bolster the quality of the milk received from producers of the poorer quality milk, unless both have received payment on the basis of the quality of the milk supplied.

In this age of enlightenment the purchase of goods on the basis of quality standards is usual and there is no reason why reasonable standards of quality should not be applied to and demanded of liquid milk and, in fact, the Cook Committee has recommended the implementation gradually of increasing minimum standards for S.N.F.

(to be continued)

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Part 2

Would payment for S.N.F. supplied provide sufficient incentive to bring about the desired improvements in quality?

The estimates of the correlation coefficient between fat and S.N.F. percentages for the same sample of milk with individual cows and herds have varied greatly, e.g. Jenkins and Provan give values ranging from 0.31 to 0.54 and Wallace (unpublished data) found values of 0.71 with individual Friesian cows over a full season, 0.57 with individual Jersey cows and 0.93 when the mixed Friesian and Jersey herd was considered. There is thus the possibility of a low correlation between the weights of fat and S.N.F. supplied, and consequently in the distribution of income to each supplier, based on these respective weights. In a randomly selected group supplying the local milk treatment station, this was examined, using data originally reported in connection with an investigation into the accuracy of methods of analysis for S.N.F. (Wallace, 1961). In this study the correlation between the average fat percentage and the average S.N.F. percentage contents of the milk supplied by the ten suppliers over the whole sampling period was 0.69. When, however the correlation between the weights of fat and S.N.F. supplied over this period was determined it was found to be very high, 0.97. The distribution of income within the group was calculated on the basis of weight of fat supplied and a payout of 3s. per pound of fat. The percentage distribution of the income in the group was calculated on the basis both of fat and S.N.F. production.

Table I gives the total income for each supplier, the percentage distribution of the group income to each supplier on a fat supplied basis, and the deviation from this of the income calculated on the weight of S.N.F. supplied, both on a percentage basis and in terms of monetary value. Table I covers the whole period investigated (260 days) and shows that the maximum deviation was a decrease of 0.73 per cent, which represents a re-allocation of income amounting to £15 in a total income of £2,023 in this case. Even with the much shorter seasonal periods of 80-90 days covered also in table 1, the maximum re-allocation was only 1.40 per cent or £11 in an income of £819. The correlation coefficients

TABLE 1

Distribution of income.
TOTAL PERIOD (260 days)
Based on:
FAT SUPPLIED: S.N.F. SUPPLIED: AUTUMN PERIOD: WINTER PERIOD: SPRING PERIOD:

Suppliers:	Value of at 3/- lb. b.f. £	Percentage of group total	Deviation from percentage of group total for fat	Reallocation of income £	Value of fat supplied £	Reallocation of income on basis of SNF supplied	Value of fat supplied £	Reallocation of income on basis of SNF applied	Value of fat supplied £	Reallocation of income on basis of S.N.F. supplied
1	1,266	6·26	nil	nil	348	+0·7	411	— 1	509	+0·5
2	2,856	14·11	+0·39	+ 5	951	—6	957	+ 6	949	+7
4	2,023	10·00	−0·73	—15	525	—0·6	669	— 5	838	−9
5	2,362	11·68	+0·42	+ 8	504	—0·5	630	+ 3	1230	+1
6	2,522	12·47	+0·16	+ 4	561	+7	744	— 3	1219	−0·7
7	2,739	13·54	—0·47	—13	786	—3	819	—11	1137	+0·6
8	1,639	8·10	+0·34	+ 6	372	nil	468	+ 3	801	+4
9	1,264	6·25	+0·23	+ 3	318	nil	381	+ 2	581	+1
10	1,556	7·69	—0·50	— 7	411	—3	336	+ 0·6	807	—6
11	2,004	9·91	+0·15	+ 3	525	—3	585	+ 5	898	—1

The use of 3/- per pound of butterfat is based approximately on the current guaranteed price for butterfat. Liquid milk realisations per pound of butterfat approximate 6/- assuming an average fat content of 4 per cent and a return 1960/61 of 28.80d./gal.

The deviations of the percentage distribution of individual supplier production of S.N.F. in relation to the production of the whole group remains constant, but the actual returns and reallocations will be doubled on the basis of liquid milk prices.

TABLE 2

Supplier No:	Lb. S.N.F. supplied	Lb. S.N.F. minimum (8·5%)	Lbs. S.N.F. Under-supplied	Payout on S.N.F. supplied £	Less penalty: £	Final payout: £	Payout per lb. S.N.F. Pence
1	16,177	16,434	257	2,262	36	2,226	33·1
2	37,497	35,844		5,240		5,240	33·6
4	23,956	23,254		3,460		3,460	33·6
5	31,277	30,352		4,370		4,370	33·6
6	32,649	32,157		4,580		4,580	33·6
7	33,791	31,923		4,730		4,730	33·6
8	21,851	21,256		3,060		3,060	33·6
9	16,745	16,479		2,342		2,342	33·6
10	18,600	18,368		2,610		2,610	33·6
11	26,022	24,296		3,640		3,640	33·6

Supplier No. 1 was the only supplier sending in less than the minimum required quantity of S.N.F. He was penalised to the extent of £36 which meant that his S.N.F. was purchased at 33·1 pence per pound, instead of 33·6 pence per pound the basic price.

between the income distributed on a fat supplied basis and on an S.N.F. supplied basis were very high, being 0.990 for the total period and 0.985, 0.977 and 0.988 for the shorter autumn, winter and spring periods.

On the basis of this study, which there is no reason to consider atypical, it can be seen that the concept of distribution of income on the basis of S.N.F. supplied would provide little more incentive to improve milk quality than the incentive already present in the much more simply determined distribution of income on the fat supplied basis.

It should be borne in mind, however, that in New Zealand S.N.F. testing of raw milk supplies for town milk use is already obligatory at twice monthly intervals. Wallace, 1961, has shown that hydrometric methods of S.N.F. assessment on composite samples can give an adequate estimate of S.N.F. supplied for payment purposes. The introduction of an S.N.F. payment scheme would not therefore create very much further demand on time or expenses.

Possible method of payment for solids-not-fat incorporating penalty for low solids-not-fat percentages.

Assuming the introduction of regular S.N.F. testing for payment in the same way as the present fat testing scheme operates, the following scheme could provide monetary incentive to improve solids-not-fat percentage levels. From the weight of milk supplied in a given period the weight of S.N.F. which should have been supplied, had the milk been of the minimum acceptable composition, can be calculated. Using the present gallonage price and the present average S.N.F. content of milk, calculate the payout per pound of S.N.F. supplied. For every pound of S.N.F. below minimum required level, deduct equivalent payout. At the end of the 12 month period an adjustment should be made on any difference between the S.N.F. supplied and the minimum required supply on the one hand, and the deductions and additions paid for on the monthly basis for over or under supplying the minimum S.N.F. required on the other.

An example follows :

In ten day period 10,000 lb. milk supplied. S.N.F. test 8.0 per cent on composite.

i.e. S.N.F. supplied 800 lb.

Minimum S.N.F. required, 850 lb., i.e. 8.5 per cent on 10,000 lb.

Price per pound S.N.F. supplied is derived as follows :

Present price 2/6 gal. (should be present gallonage price) and average S.N.F. 8.6 per cent (should be present S.N.F. average).

That is, payout will be

$$= 2/6 \text{ per } \frac{8.6 \times 10.32}{100} \text{ lb. S.N.F.}$$

$$= 2/6 \text{ per } 0.887 \text{ lb. S.N.F.}$$

$$= 33.8\text{d./lb. S.N.F.}$$

Price paid for S.N.F. supplied

$$= \frac{2/6 \times 800}{.887}$$

$$= £100/.887$$

$$= £112\ 15\text{s. } 0\text{d.}$$

For every .887 lb. S.N.F. below minimum required level, deduct 2/6, e.g. in above case payout on S.N.F. supplied = £112 15s. 0d.

less 50 lb. deficient S.N.F. at 2/6 per .887 lb. = £7 1s. 3d.

i.e. net payout = £105 13s. 9d.

i.e. S.N.F. was purchased at 31.7d. lb.

At end of 12 month period an adjustment should be made on any difference between S.N.F. supplied and deductions or additions paid for on monthly basis for over or under supplying minimum S.N.F. required.

e.g. over 36 tests 400,000 lb. milk supplied.

minimum S.N.F. require at 8.5 per cent = 34,000 lb.

S.N.F. supplied in total = 32,000 lb.

i.e. S.N.F. deficiency = 2,000 lb.

S.N.F. deficiency on 10 day basis, say = 1,800 lb.

i.e. Further deduction for 200 lb. at 2/6 per .887 lb.

Using this concept, the payouts given in Table I have been recalculated, assuming 8.5 per cent as the minimum S.N.F. level and a payout of 33.6d./lb. S.N.F.

These are shown in table 2.

At current market price this deficiency in production of S.N.F. by supplier No. 1 would cost £8 11s. 4d. to replace in the form of reconstituted milk.

(continued on next page)

What is the potential of a liquid milk supply based entirely or partly on the reconstitution of non fat dry milk powder and unsalted butter or butter-oil?

The cost of reconstituted milk prepared from skim-milk powder costing £75 per ton, i.e. 8d. lb. and unsalted butter costing 3/- lb. and reconstituting to a 9 per cent S.N.F. level and a 4 per cent fat level would be :

9 lb. S.N.F. and 4 lb. fat to 10 gal.

= 6/- + 14/3 for 10 gal.

= 20/3 for 10 gal. + 2/- for processing.

= 22/3 for 10 gal. or 26.7d. gal.

A change in S.N.F. price to £95 per ton would increase the cost by 2.2d. gal. Thus the maximum cost of fully reconstituted milk of 9 per cent S.N.F. and 4 per cent fat will not be more than 2/5 gal. or to reconstitute to the minimum legal levels of 8.5 per cent S.N.F. and 3.25 per cent fat, the cost would be on present raw material values + 2/- per 10 gal. for processing, 19/- for 10 gal. or 23d. gal.

According to Section 8 of the Seventh Annual Report of the N.Z. Milk Board, the average payments to producers for the year ending 31st August, 1960, was 32.916d./gal. This return does not represent cost at the factory door since it does not include the cost of handling surplus milk nor does it include cartage from farm to the factory. These extra costs represent 4.486d./gal. Thus, the average cost of milk at the factory door was 37.402d./gal.

The average cost at the factory door does not give an entirely clear indication of cost variations with season and district. Thus, there is a premium of 5.75d./gal. paid for autumn (February-April inclusive) produced milk, and for winter (May-August inclusive) this premium rises to 12.68d./gal. Prices within districts are also subject to variation. Thus, all autumn and winter milk produced in the South Island receives an additional premium of 4.0d./gal., and in Oamaru and Southland there is, in addition, a further premium of 4.0d./gal. Several North Island districts receive additional premiums of 2-4d./gal. for limited periods. Thus, the price for winter milk at the factory door in Southland in 1960/61 will be around 48.7d./gal. Assuming an average fat content of this milk to be 3.5 per cent, this represents a cost of 146d./lb. of butterfat at the factory door.

By using reconstituted milk and reconstituting to the average butterfat 4.1 per cent and average solids-not-fat 8.8 per cent found in market milk, the cost would be 24.85d./gal. Using the 1960 average price for town milk at the factory door of 37.4 gal., this represents a minimum saving of 12.55 gal. or about 80 per cent of the subsidy, that is £3.9 million—a worthwhile amount in any government's eyes.

It may seem unrealistic in a dairying country to have to reconstitute all liquid milk, but economically it would be very sound, and technologically it is practicable. When the cost of production of winter milk is considered 43.7d./gal. (with higher costs in some districts), as compared with 24.85d./gal. for reconstituted milk, the whole of the subsidy is recoverable, together with a considerable surplus of £430,000 at least. In the winter period of 1960, approximately 26 million gal. of milk were used and the subsidy on this amounted to £1.6 million. It can be seen that the use of reconstitution as a means either of providing all milk for human consumption or more probably a substantial part of the winter consumption can bring about a major reduction in government costs through reducing the food subsidy account. It is very difficult on logical grounds to justify such excessive expenditure on the production of a product under the inefficient conditions of winter milking.

Conclusion

There is a growing awareness of the nutritional advantages of milk protein and of other nutritives in the solids-not-fat content of milk. There is an urgent need to maintain milk composition up to the legal requirements when it is delivered to the consumer.

Improvement in protein and S.N.F. percentage and production levels can be brought about by breeding. Use of S.N.F. percentage and production as an indirect means of genetic improvement in protein levels is warranted because of the high correlations that exist between S.N.F. and protein, and because of the simplicity of the tests for S.N.F.

Mixed breed herds will also effectively meet this need for improved S.N.F., but this is not generally liked from a husbandry and economic point of view.

A very simple and practical method for meeting minimum legal requirements is for milk to be purchased on the basis of its S.N.F. content and for the treatment station to fortify the liquid milk to the required level of S.N.F. with non-fat dried milk solids.

Finally, non-fat dried milk solids and unsalted butter produced under maximum conditions of efficiency can be used to provide a continuous or partial supply of reconstituted liquid milk as is done overseas. This would replace the inefficiently and expensively produced market milk supplies and could result in between £2-5 million saving in subsidy, a very worthwhile note on which to end.

REFERENCES

(1) Cook Committee: Milk Composition in the United Kingdom, Report of an Interdepartmental Committee, Chairman, J. W. Cook, Esq., Cmnd. 1147, Sept. (1960). H.M.S.O., London.

(2) Flux, D. S., Patchell, M. R., Campbell, I. L., McDowall, F. H. *Variations in the Composition of Milk*, The Dairy Research Institute (N.Z.), publication No. 277, (1955).

(3) Henry, Kathleen, M., Kon, S. K., *Proc. Nutri. Soc.* (1958), **17**, 78.

(4) Jenkins, D. I., Provan, A. L., XIVth Int. Dairy Cong. Proc. (1956). Section I, Part II, 792.

(5) Ling, E. R., Kon, S. K., Porter, J. W. G. Milk: *The Mammary Gland and its Secretion*, Vol. II, Chap. VI. Editors: Kon and Cowie; Academic Press (1961).

(6) N.Z. Milk Board: Seventh Annual report and statement of Accounts, for the Year Ending 31st August, 1960, Wellington.

(7) Robertson, Alan, Waite, R., White, J. C. D. *J. Dairy Res.* (1956), **23**, 82.

(8) Stewart, Alan (Massey College) private communication.

(9) Wallace, G. M., *J. Dairy Res.* In the press (1961).

ANALYTICAL INVESTIGATIONS

In an endeavour to facilitate and extend the testing of milk supplies for recording and for commercial purposes, aspects of the following methods of milk analysis were investigated:

(A) Fat testing using techniques based on Sulphuric Acid.

(B) S.N.F. testing using the density hydrometer method.

(C) Protein determination using amido black.

A. The quantitative relationship in the dilution of Sulphuric Acid for fat testing.

A small but nevertheless important problem in the testing of milk for S.N.F. has been the associated test for fat and the need in this test for a simple means to calculate the amount of dilution water necessary to add to the concentrated sulphuric acid used in the testing procedure to adjust the density of the acid to within the required limits. In view of the hazards involved in adding water to concentrated sulphuric acid, the accepted techniques for adjusting the composition of solutions by taking a known weight of the material and diluting to a predetermined volume cannot be followed.

Because of the large amount of heat of dilution and the major deviation of the final volume from that of a purely additive relationship, the normal dilution formulae cannot be applied in these circumstances.

This investigation showed that a relationship existed between the amount of contraction that occurred and the

difference between the energy stored as molal free energy of the dilution water and the energy liberated as heat of dilution. Using this relationship a volume contraction factor was derived for each 0.001 gms/ml. change in density between 1.836 gms/ml. and 1.794 gms/ml., at which latter point and less the dilution relationship became simply additive. Using these volume contraction factors, dilution curves were established permitting accurate dilution of concentrated sulphuric acid to another predetermined density.

This investigation has been reported in the following paper: "The Dilution of Sulphuric Acid in the Region of Density 1.84 - 1.80." (Dairy Ind. 25 : 753 (1960)).

A copy is attached.

THE DILUTION OF SULPHURIC ACID IN THE REGION OF DENSITY 1.84—1.80

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IN the testing of milk to determine its fat content by the Gerber (1) method, sulphuric acid of density 1.815±0.002 g/ml. at 20° C. (S.G. 60/60° F., 1.820-1.825) is required. In the Babcock (2) method sulphuric acid of density 1.822±0.005 g/ml. at 20° C. (S.G. 60/60° F. 1.823-1.833, S.G. 20/20° C., 1.820-1.830) is required.

Concentrated sulphuric acid normally has a density of approximately 1.84 and it is necessary therefore to dilute this with water to obtain the specified densities. In view of the hazards involved in adding water to concentrated sulphuric acid, the accepted techniques for adjusting the composition of solutions by taking a known weight of the material and diluting to a predetermined volume cannot be followed.

The method generally followed is a somewhat haphazard one of adding the sulphuric acid to small quantities of water, with quite extensive delays whilst waiting for the mixed acid to cool, until the density or specific gravity has been adjusted to within the required limits.

Because of the large amount of heat of dilution and the major deviation of the final volume from that of a purely additive relationship, the normal dilution formulae cannot be applied under these circumstances. Scott (3) and Domke (4), Rogers (5) have published dilution tables, but in no case are these applicable in the dilution range discussed.

An investigation of this problem showed that a relationship existed between the amount of contraction that occurred and the difference between the energy stored as molal free energy (E) of the dilution water and the energy liberated as heat of dilution (H) (6).

Table 1 gives this difference in energy (E-H) and the corresponding volume contraction factor for various densities in the range of interest. This data can be used in conjunction with Fig. 1 to calculate the volume of water required to produce a dilute acid of known density.

Using the data available in BS 753/1959 (7), Fig. 1 has been constructed so that it is possible to read directly the volume of strong acid required to produce a dilute acid of known composition and density at 20° C. when diluted to 1 litre.

Method

Measure the density of the strong sulphuric acid and note the temperature.

Calculate the density of this acid at 20° C. by adjusting the observed density by 0.001 gms/ml. for each one degree centigrade difference from 20° C., increasing the observed density if the observed temperature was greater than 20° C. or decreasing the observed density if the observed temperature was less than 20° C.

Using Fig. 1, determine the *volume*, in millilitres, of this strong acid to be taken to dilute to 1,000 ml. to obtain acid of the desired density, by following or interpolating the curve corresponding to the density of the strong acid to its intercept with the vertical line corresponding to the density desired. Perpendicular to the vertical line at this intercept read off the volume of strong acid to be taken.

The volume, in millilitres, of water to be added to this predetermined volume of strong acid to give acid of the desired density on mixing is calculated by multiplying the difference between the volume of acid taken and 1,000 ml. by the factor

TABLE 1

Difference between relative partial molal free energy of water and heat of dilution at 0·001 gms/ml. incremental changes in density

D20°C. gms/ml.	E-H cals. deg.-¹mol.-¹	Volume Contraction factor	D20°C. gms/ml.	E-H cals. deg.-¹mol.-¹	Volume Contraction factor
1·836	3925	1·92	1·814	2640	1·29
1·835	3850	1·88	1·813	2600	1·27
1·834	3775	1·85	1·812	2570	1·26
1·833	3700	1·81	1·811	2540	1·24
1·832	3625	1·77	1·810	2510	1·23
1·831	3550	1·74	1·809	2480	1·21
1·830	3475	1·70	1·808	2450	1·20
1·829	3400	1·66	1·807	2420	1·18
1·828	3325	1·63	1·806	2390	1·17
1·827	3270	1·60	1·805	2360	1·15
1·826	3215	1·57	1·804	2330	1·14
1·825	3160	1·55	1·803	2300	1·12
1·824	3105	1·52	1·802	2270	1·11
1·823	3050	1·49	1·801	2240	1·10
1·822	3000	1·47	1·800	2210	1·08
1·821	2950	1·44	1·799	2180	1·07
1·820	2900	1·42	1·798	2150	1·05
1·819	2850	1·39	1·797	2120	1·04
1·818	2800	1·37	1·796	2090	1·02
1·817	2760	1·35	1·795	2060	1·01
1·816	2720	1·33	1·794	2030	1·00
1·815	2680	1·31			

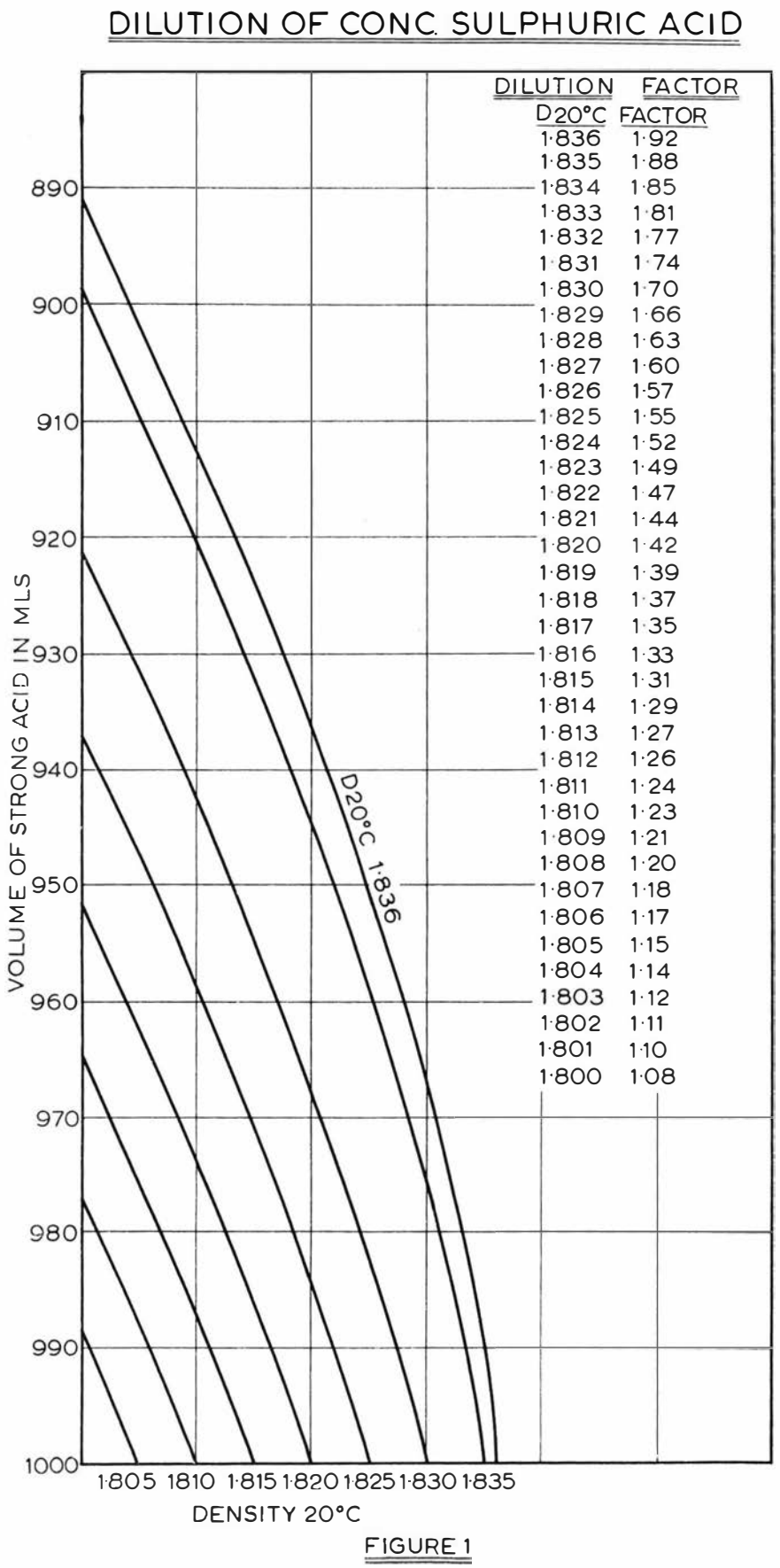

FIGURE 1

for the density representing the mean of the starting and finishing acid densities.

The correct volume of acid is then poured carefully with stirring into this calculated volume of water.

Example.

Density of acid 1·834 gms/ml at 22°C.	
Density of acid at 20°C.	$=1{\cdot}834+2\times{\cdot}001$
	$=1{\cdot}836$
Required density at 20°C.	$=1{\cdot}815$
Mean density	$=1{\cdot}825$
Factor for D_{20} 1·825	$=1{\cdot}55$ from table
Volume of strong acid needed	$=925$ ml from Fig. 1
Volume of water needed	$=(1000-925)\times 1{\cdot}55$
	$=75\times 1{\cdot}55$
	$=116$ ml.

This investigation has been supported in part by a grant from the Department of Scientific and Industrial Research and this is gratefully acknowledged, as is the technical assistance of Miss S. Quin.

REFERENCES

(1) British Standard 696-1955.
(2) Australian Standard N26-1958.
(3) Scott's Standard Methods of Chemical Analysis 5th Edn. Editor N. H. Furman vol. 2. p. 2237.
(4) Domke, J. Dilution Table for Sulphuric Acid—based on data of J. Domke Wis. Abh. der K. Normal-Eichungs-Kommission 1904 vol. V. as published in Handbook of Chemical Data 1957 F. W. Atack, p. 78.
(5) Rogers, J. P. Improved Dilution Table for Sulphuric Acid—*Industrial Chemist* 1929, June 223.
(6) Wallace, G. M. Relationship Between Contraction in Volume, Change in Density and Evolution of Heat on the Dilution of Concentrated Sulphuric Acid.
(7) B.S. 753/1959. Density Composition Tables for Aqueous Solutions of Sulphuric Acid.

B. The development of the hydrometric method for determining the S.N.F. content as a more precise analytical method.

(a) Design of a Nomograph relating S.N.F., Fat and Density.

A recurring source of error in determining the S.N.F. content of milk was in the calculations involved in converting observed hydrometer readings into S.N.F. percentages. The methods available were slow and complicated even when the Richmond slide rule was used and these frequently led to errors of computation. In an endeavour to eliminate any complex manipulative technique or computation, the relationship between the fat percentage, the hydrometer reading at 20°C and the solids-not-fat content was plotted in the form of a nomograph. The cursor was engraved not only with the basic ruling but also with a set of other rulings, which enabled the S.N.F. content to be read directly and without further manipulations when the hydrometer readings were made at temperatures other than 20°C (Standard). With this device it is possible to read the solids-not-fat content directly from the nomograph as soon as the cursor has been properly located over the fat percentage and hydrometer reading corresponding with those of the sample being tested.

This nomograph and cursor were described in a paper entitled: "A nomograph for calculating S.N.F. content of milk from density hydrometer readings and fat percentage." (Dairy Ind. 22 : 1030 (1957)).

A copy of this paper is attached. A slightly modified version of the nomograph and cursor (described in a note attached to the paper) was subsequently developed by the author and this form is universally used by the Town Milk Industry in New Zealand.

A Nomograph for Calculating S.N.F. Content of Milk from Density Hydrometer Readings and Fat Percentage

by G. M. Wallace, Biochemistry Department, Massey Agricultural College (University of New Zealand), Palmerston North, N.Z.

Summary.

A NOMOGRAPH and cursor which simplifies the calculation of the solids-not-fat content of milk from the density hydrometer reading and fat percentage is described. The cursor is so calibrated that independent correction of the density hydrometer reading for temperatures other than 20° C. is obviated.

Introduction.

The present methods of calculating the solids-not-fat content of milk from hydrometer reading and fat content are slow and complicated.

Although the British Standard (1) (appendix D, table 4) gives a series of tables for the derivation of S.N.F. and a separate table (table 3) for correcting hydrometer readings at temperatures other than 20° C. to density at 20° C., time and care are required to obtain the result. The density hydrometer slide rule developed from Richmond's slide rule necessitates various manipulations before giving the result in total solids content. Incorrect use of the slide rule in the adjustment of hydrometer readings to density at 20° C. is not unusual with technicians. Further, the S.N.F. percentage must be derived from the total solids percentage, thus presenting more opportunities for error.

In the older lactometer technique it was unusual for standardisation of the previous temperature history of the milk to be undertaken to control the effect of the Recknagel contraction. This is an integral part of the British Standard procedure. Because of the extra handling necessary it is desirable to compensate for the time involved by simplifying the procedure wherever possible.

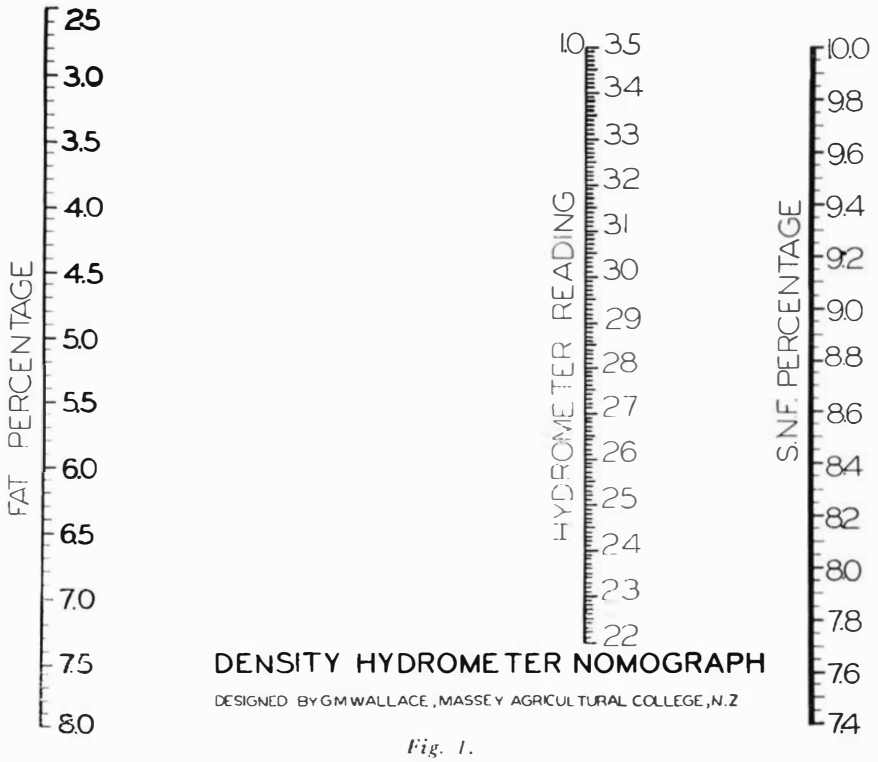

Fig. 1.

This nomograph and cursor save time and because of their simplicity, increase accuracy.

Description.

Using the data given in appendix D, tables 3 and 4, of the British Standard, a nomograph relating the fat content of milks between 3.0 per cent and 7.55 per cent and hydrometer readings between 25 and 35 degrees was constructed so that the S.N.F. percentage could be read off directly to the nearest 0.05. This is shown in fig. 1.

The perspex cursor (fig. 2) has been so engraved that the S.N.F. content may be read without the need for a preliminary adjustment of hydrometer reading taken at tem-

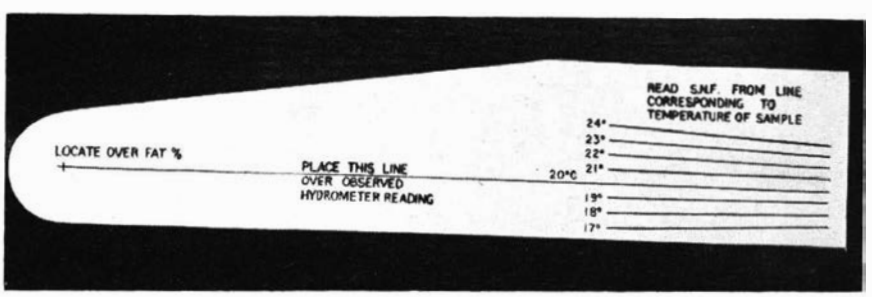

Fig. 2. Perspex cursor.

peratures other than 20° C. to density at 20° C. The range of temperature compensation is 17-24° C. in 1° increments.

Along the left border of the nomograph a strip of perspex can be fitted so that when the cursor, which has been radiused in relation to the fat-percentage-locating-mark, is in contact with this strip it will always bring the locating mark directly over the fat percentage line of the nomograph, thereby assisting in its rapid manipulation.

Method of Use.

(1) The cross at the left of the cursor is placed over the appropriate fat percentage.

(2) The main line on the cursor, marked 20° C., is located over the observed hydrometer reading.

(3) The S.N.F. percentage is read off under the appropriate temperature line on the right of the cursor.

Discussion.

Some simple method of compensation for hydrometer readings at temperatures other than 20° C. is essential, since it is not always convenient to adjust the temperature of the milk exactly to 20° C. after heating to 40° C. for 5 minutes as prescribed in the British Standard. The correction converting hydrometer readings to density at 20° C. varies with the fat content (British Standard table 3). The correction for 4 per cent fat has been used in preparing the cursor, but the error introduced for fat percentages outside this range is very small and can justifiably be ignored.

The accuracy of the nomograph and cursor is within 0.03 units expressed as percentage S.N.F., which means that readings to the nearest 0.05 will be sufficiently accurate for all practical purposes.

Cursors and nomographs are available from the author.

I wish to acknowledge my indebtedness to Dr. W. A. McGillivray, of this Department, who suggested the possibility of using a nomograph in this connection.

REFERENCE

(1) British Standard 734:1955, Density hydrometers for use in milk.

Since this paper was published, B.S.734 has been amended and appropriately amended versions of the nomograph for use when the fat is in the solid state and for use when the fat is in the liquid state were prepared and were used by Nameplate Engravers Limited as a template for commercial production of the nomographs. The cursor has been modified by making the mark for locating the fat percentage on the cursor a sharpened point. The nomograph has been provided with a rule notched at every 0.1% fat increment to locate the pointed cursor. This nomograph and cursor is universally used by the Town Milk Industry in New Zealand and has proved very satisfactory.

B. (b) Examination of the hydrometric technique

Because of the indirect basis of measurement of S.N.F. when using density and fat content as the measured parameters, the method is subject to errors other than those inherent in the method of analysis. These other errors which are primarily due to variation in the lactose/protein ratio of milk may be appreciable (S.D. ± 0.2 S.N.F.%) with samples from individual cows and will vary with the stage of lactation. The bulking of milk and the use of 10-day composite samples was shown to reduce this variation to a much lower value (S.D. ± 0.05 S.N.F.%). Bias in the estimate occurred, the extent and direction of the bias changing with season. The importance of this bias was not easily assessed due to the limited information available but it was possible to ignore it, for the estimate over an extended period of sampling (9 months) showed no bias.

Using the hydrometric method, when the S.N.F. content of 10-day composite samples of milk taken from individual herds were compared, after making due allowance for the effect of the preservative present, with the weighted mean S.N.F. content calculated on a daily basis, it was shown that the 10-day composite sample could be used successfully for estimating the S.N.F. percentage composition of the milk samples and S.N.F. production of the herd.

A comparison of the S.N.F. production estimated by gravimetric and hydrometric methods using 10 commercial herds gave a mean difference in the estimate of 0.14% (S.D. ± 0.4). The standard deviation expressed as a percentage error of the estimate of S.N.F. production based on 10-day composite samples using the hydrometric method of estimation was ± 0.56 which was not appreciably more than that for the Gerber estimation for

fat (S.D. ± 0.5) and was less than that for the Babcock estimation for fat (S.D. ± 0.85), both of which have been accepted without question as satisfactory methods of analysis for a long time. This hydrometric technique using 10-day composite samples was of a satisfactory accuracy and precision for routine analysis. These results are presented and discussed in two papers.

In the first paper entitled: "Application of the hydrometric method of analysis to the estimation of solids-not-fat production." (J.Dairy Research 29 : 11 (1962)), the experimental results and their evaluation are presented.

In the second paper entitled: "Use of hydrometric technique in estimating S.N.F. content of milk." (Dairy Ind. 28 : 600 (1963)), the accuracy of this technique is further considered in relation to the accuracy of other methods of sampling and analysis for S.N.F. and some of the practical applications of the hydrometric technique are examined.

Copies of these papers are attached. The work reported in these papers forms the basis of S.N.F. testing studies at present under investigation by several sections of the Town Milk Industry in New Zealand.

Application of the hydrometric method of analysis to the estimation of solids-not-fat production

By G. M. WALLACE

Massey Agricultural College, Palmerston North, University of New Zealand

(*Received* 28 *July* 1961)

SUMMARY. The effect of preservative tablets added to milk on its composition and density is discussed. A comparison between the S.N.F. content of 10-day composite samples of milk from individual herds after making due allowance for added preservative, and the weighted mean S.N.F. calculated on a daily basis, showed that the 10-day composite sample could be used successfully for estimating S.N.F. hydrometrically. A comparison of the weights of S.N.F. supplied when estimated by gravimetric and hydrometric methods was made for ten herds supplying a commercial plant over a period of 260 days. The mean difference was $-0{\cdot}14\%$ of the gravimetrically estimated weight, S.D. 0·4, and the range was $\pm 0{\cdot}6\%$. The result is discussed and is considered of sufficient accuracy to justify, on a technical basis, using the hydrometric method of estimating S.N.F. for determining and, if necessary, paying for the S.N.F. supplied.

With the realization of the increasing nutritional and economic importance of the S.N.F. components of milk there has come an increasing interest in methods of estimation of the S.N.F., or preferably protein, fractions of the milk. This study has been undertaken to establish whether the relatively simple hydrometric method for estimating S.N.F. in milk will give a sufficiently accurate estimate for commercial purposes. Claesson (1958) described a method for the rapid gravimetric estimation of total solids using infra-red drying. This procedure and equipment was intended for use in centralized laboratories. Preliminary studies on the effect of added preservative on the composition and density of milk and on the analytical differences to be expected between daily sampling and analysis, and daily sampling with analysis of the 10-day composite samples were also undertaken since an understanding of the effects of these modifications, normally used in commercial fat estimation procedures, was essential in the final assessment of the major study.

EXPERIMENTAL

All samples were taken by the Palmerston North Milk Treatment Station staff on the milk receiving stage.

(1) *Correction for preservative*

Duplicate half-pint samples of the milk in the weigh vat were taken from fourteen individual supplies. The density of one sample of each pair was determined in accordance with BS 734 (1955), Amendment no. 1 (1957) as soon as the sample had

undergone the necessary pretreatment to ensure that the fat was in the solid state. The other sample was preserved with one 2-grain tablet of mercuric chloride (B.D.H. corrosive sublimate tablets, 2-grain, coloured blue) and was then examined at the same time as the unpreserved sample after undergoing identical pretreatment. The preserved sample was then held for 4 days in the refrigerator to determine the effect, if any, of storage on the density of preserved samples. The density of these samples was again determined as before. No deterioration of these preserved samples was evident.

(2) *Composite versus daily sampling*

Half-pint samples of milk from the herds selected for the main study were taken daily for testing.

A sample dipper full (about 25 ml) of the same milk was also taken daily and placed in a composite sample jar containing one 2-grain mercuric chloride preservative tablet per half pint of sample. Where more than one weigh vat full of milk was received from a supplier proportionate sampling was used for both daily and composite samples. The composite samples were kept in a cool room when sampling was completed. Density was determined with the fat in the solid state and fat content percentage was estimated using the Babcock procedure. The analyses were carried out entirely at the Palmerston North Milk Treatment Station during May 1959.

(3) *Accuracy of hydrometric method of estimating* S.N.F. *production*

A further series of 10-day composite samples of the milk from the herds of selected suppliers were taken into sampling bottles containing a 2-grain tablet of mercuric chloride as preservative. These composite samples were stored in the cool room when not required on the stage. At the end of the 10-day period the samples were analysed at Massey Agricultural College. Density was determined with the fat in the solid state. Fat was estimated in duplicate by the Gerber procedure (BS 696). Total solids were determined on duplicate 2 g samples using disposable aluminium dishes and drying in an electric oven with fan-assisted circulation at 101 °C for 3 h.

The selection of suppliers was based entirely on the estimated likelihood of their maintaining a continuous supply of milk to the factory throughout the sampling period. They were otherwise a completely randomized sample of town milk producers.

RESULTS AND DISCUSSION

Correction for preservative

With an aged preserved sample there was an average increase in density due to the addition of preservative of 0·00094 g/ml at 20 °C with a standard deviation of 0·00012.

Using the formula given in BS 734, Amendment no. 1, for determining the solids-not-fat percentage from the hydrometer reading when the fat is in the solid state and from the fat percentage:

$$\%\ \text{S.N.F.} = 0{\cdot}25D + 0{\cdot}22\ \text{fat}\ \% + 0{\cdot}55,$$

where $D = 1000(\text{density} - 1)$, the effect of an increase in density of 0·00094 g/ml at 20 °C amounts to 0·235 % S.N.F.

The average weight of a preservative tablet was 0·265 g (average of sixteen, range 0·258–0·282 g). If all this tablet were soluble it would increase the density of a half pint of milk (284 ml) by 0·00093 g/ml at 20°C. There is thus excellent agreement between the average observed contribution of the added preservative to the density of the milk and the theoretically deduced contribution.

The extent of the contribution to the hydrometric S.N.F. percentage of a sample of milk when one 2-grain preservative tablet is added to each half pint of milk amounts to 0·235 %. The contribution of the preservative to the gravimetric estimate of the S.N.F. amounts to 0·095 % since the additional material added amounts to 0·27 g in 284 ml which is equivalent to 0·095 %.

In the main study preserved samples were used throughout and the density was determined with the fat in the solid state. The effect of the added preservative on the hydrometric estimate of the S.N.F. percentage would, therefore, amount to an increase of 0·235, but there would also be an increase of 0·095 on the gravimetric estimation, due to the effect of the added preservative. The hydrometric S.N.F. percentage should, therefore, have exceeded the gravimetric S.N.F. percentage by an average of 0·14.

In the first examination of the results it was calculated that 284 217 lb of S.N.F. were supplied using the density method of estimation of S.N.F. %, whereas on the basis of the gravimetric estimation 276 751 lb of S.N.F. were supplied in 3 145 370 lb of milk. Thus, the average S.N.F. content by density equals 9·036 %, whereas the average S.N.F. obtained gravimetrically equals 8·799 %. Correcting these values for the effect of the added preservative gives a density S.N.F. of 8·801 % and a gravimetric S.N.F. of 8·704 %, a difference of 0·097 % S.N.F.

Our original results were calculated using the formula given in BS 734:1955 for use with milk in which the fat was in the liquid state. In our samples, the fat was in all cases in the solid state when the density determinations were made. The observed over-estimation of 0·097 in the S.N.F. percentage as a result of this difference in procedure is considerably greater than the over-estimation of 0·07 % suggested in Amendment no. 1 to BS 734, but agrees well with an observed over-estimation of 0·1 % determined on a herd of mixed Friesian and Jersey cows (Wallace, 1958).

However, in view of its recognition in Amendment no. 1 to BS 734, the standard correction of −0·07 was applied to all the original results to compensate for the over-estimation introduced by using a procedure different from that for which the formula in BS 734:1955 was derived.

In summary, the results used in the study of the accuracy of the hydrometric method of estimating S.N.F. production have been adjusted by subtracting 0·21 from the S.N.F. percentage to allow for the effect of added preservative and 0·07 to allow for the differences in procedure.

Composite versus daily sampling

This particular aspect of the investigation was carried out entirely at the Milk Treatment Station using their equipment. An examination of Table 1 indicates that the adjustment of −0·235 to the hydrometric S.N.F. percentage for the added preservative in the composite sample was slightly over-estimating the correction to be applied since all but one of the composite estimates are biased in the direction of under-estimation. The gravimetric estimation of S.N.F. in the composite sample was

also biased, in this case completely, in the direction of an apparent under-estimation, but this can be explained since the hydrometer used in this aspect of the studies was found, on calibration, to be giving a consistently high reading.

Table 1. *Comparison of* S.N.F. *estimates based on weighted mean estimate of daily samples and on estimate of corresponding* 10*-day composite sample (nine herds)*

(The composite sample analyses given below have been corrected for added preservative, the hydrometric estimates by reducing apparent S.N.F. percentages by 0·235 and the gravimetric estimates by reducing the apparent S.N.F. percentages by 0·095 (see Results and Discussion).)

Mean S.N.F. %, daily basis (hydrometric) (1)	S.N.F. %, composite (hydrometric) (2)	Difference (1)−(2), % S.N.F.	S.N.F. %, composite (gravimetric) (3)	Difference (1)−(3), % S.N.F.
8·988	8·845	0·143	8·81	0·18
8·894	8·885	0·009	8·73	0·16
8·945	8·965	−0·020	8·87	0·08
8·747	8·685	0·062	8·55	0·20
9·075	9·015	0·060	8·95	0·13
8·642	8·595	0·047	8·48	0·16
9·256	9·145	0·111	9·06	0·20
8·877	8·785	0·092	8·66	0·22
9·162	9·065	0·097	9·05	0·11
		Mean +0·068	—	+0·16
		S.D. 0·051	—	0·046
Mean of the differences ignoring signs		0·071	—	—

Table 2. *Comparison of estimates of weights of* S.N.F. *produced based on daily testing and on test of corresponding* 10*-day composite sample (nine herds)*

S.N.F. produced daily testing, lb (1)	S.N.F. produced 10-day composite sample, lb (2)	Difference, lb (1)−(2)	Difference, % $\frac{(1)-(2)\times 100}{(1)}$
451·8	444·5	7·3	1·62
770·1	769·1	1·0	0·13
932·6	934·4	−1·8	−0·19
945·1	938·4	6·7	0·71
1231·1	1223·0	8·1	0·66
498·0	495·3	2·7	0·54
732·0	723·0	9·0	1·23
498·7	493·0	5·7	1·14
555·0	549·1	5·9	1·06
Total 6614·4	6570·4	44·0	0·67
			Mean +0·77
			S.D. 0·57
		Mean of the differences ignoring signs	0·81

The interest in this study is, however, primarily in the precision to be expected when the results of a composite sample analysis are compared with the weighted mean results of the daily estimates. Comparing the results of the hydrometric method for daily and composite samples, the mean difference between the two estimates of S.N.F. percentage was 0·068, S.D. 0·051, and the range was from −0·02 to +0·14. Comparing the hydrometric estimates on daily samples with the gravimetric esti-

mates on the composite samples, the mean difference of the two estimates of S.N.F. percentage was 0·16, S.D. 0·046, and the range was from +0·08 to +0·22. The precision of the hydrometric method on a composite sample is thus as good as that of the reference method.

Table 2 shows the difference in estimates of pounds of S.N.F. supplied on a daily testing basis and on the basis of using a 10-day composite sample, all samples being analysed hydrometrically. The difference in the estimates of total S.N.F. supplied amounted to only 0·67 %, despite the bias discussed above. The mean percentage difference between the daily and composite methods of estimating the weight of S.N.F. supplied was +0·77, S.D. 0·57.

In a study of the relationship between the mean of a daily series of fat tests and the fat test of a composite sample, McDowall (1936) found that there may be a slightly lower estimation of the fat percentage of the composite sample amounting to 0·02 (i.e. about 0·5 % of an estimated fat content of 4 %).

Despite the obvious bias, the under-estimation of 0·068 in the S.N.F. percentage in this investigation (Table 1) amounts to only 0·76 % for a S.N.F. level of 9 %, not greatly different from the data of McDowall for fat. The accuracy of the hydrometric method of S.N.F. estimation on a composite sample is thus of the same order as that accepted as satisfactory in New Zealand for the now well-established techniques of composite sample testing for fat content of milk and for estimating the quantity of fat supplied for payment purposes.

Table 3. *Comparison of estimates of weights of* S.N.F. *produced based on gravimetric and hydrometric methods of analysis during period* 20. iii. 59 *to* 10. xii. 59

Supplier	No. of tests	Total milk supplied, lb	Total S.N.F. supplied (gravimetric), lb (1)	Difference in S.N.F. supplied (hydrometric), lb (2)	Difference in S.N.F. supplied (hydrometric), $\frac{(2) \times 100}{(1)}$ %	Average S.N.F. % (gravimetric)
1	26	193360	16177·24	− 1·45	−0·009	8·366
2	26	421689	37496·83	−103·68	−0·277	8·892
3	14	118990	10689·52	− 99·43	−0·930	8·984
4	26	273571	23956·20	− 98·26	−0·410	8·757
5	26	357085	31278·34	+ 9·48	+0·030	8·759
6	24	378314	32649·36	+ 83·13	+0·255	8·630
7	25	375564	33796·69	−121·56	−0·360	8·999
8	26	250069	21820·47	−138·10	−0·633	8·726
9	12	80940	7519·73	−114·59	−1·524	9·290
10	26	193864	16745·03	+108·54	+0·648	8·638
11	25	216091	18600·16	− 1·24	−0·007	8·608
12	26	285833	26021·78	−164·70	−0·633	9·104

Accuracy of hydrometric method of estimating S.N.F. *production*

In Table 3 the total estimates of S.N.F. supplied by each of the twelve suppliers during the period under test are given and the difference in pounds S.N.F. over- or under-estimated by the hydrometric method are shown (column 2). These differences are also expressed as percentages of the gravimetric estimate in the next column. The results indicate that there was no regular pattern when the difference in estimates and

the average S.N.F. percentage composition calculated from the gravimetric data are compared. There was a significant negative correlation coefficient of 0·57 between the two sets of results ($P < 0{\cdot}05$). The correlation coefficient between the individual gravimetric and hydrometric estimates of S.N.F. supplied were calculated for suppliers nos. 1 and 2, and were found to be +0·995 and +0·996 respectively.

In Table 4 the effect of shorter and longer sampling periods and of season are summarized both in respect of the total S.N.F. produced by the group and in respect of the individual suppliers. The most important result is that there was a difference of only 428 lb of S.N.F. between the amount estimated by the hydrometric method and the 260000 lb estimated on a gravimetric basis. This is equivalent to 0·17 % of the total gravimetric estimate. For the ten suppliers whose milks were tested throughout the period under investigation the mean percentage difference of the weight of S.N.F. supplied (gravimetric minus hydrometric) was −0·14, S.D. 0·404, the range being from +0·648 to −0·653.

Are these differences between gravimetric and hydrometric estimates significantly different when the errors of sampling and testing are considered, and at what level of difference do they become significant?

Let us assume we are estimating the fat content of a milk containing 4 % fat. Using equipment complying with BS 696:1955 for the estimation of fat in milk by the Gerber method when the permissible tolerances are cumulative it is possible for the maximum permissible error to amount to ± 1·52 % of the estimate, and similarly using the Australian standard S.A.A. N 26:1958 Babcock equipment the maximum permissible equipment error amounts to ± 2·79 % of the estimate.

In discussing factors affecting the precision of the Babcock test, Heinemann (1953) showed that an error amounting to 2·5 % of the estimate was to be expected and he also showed that problems involved in obtaining representative sampling introduced an error amounting to 0·94 % of the estimate.

Radema & Mulder (1948) showed that with a single Gerber test the standard deviation of the estimate was 0·44 %. Roeder (1940), discussing the reproducibility of the Gerber test, stated that 88 % of the tests would agree within ± 1·25 % of the estimate.

In a discussion on the difficulties inherent in the adequate mixing of milk in weigh tanks, Henningson & Bird (1959) gave the following data as representing the differences between the highest and lowest butterfat test of samples taken from the four corners of a weigh tank under commercial conditions of usage, with and without agitation:

Average differences expressed as a percentage of the mean fat content, between samples taken from the corners

	Maximum	Minimum
Agitated	2·9 %	0·3 %
Not agitated	2·3 %	0·8 %

In an examination of milk sampling from weigh tanks, Edwards & Simpson (1958) demonstrated the extent of the poor mixing frequently encountered and showed that the fat estimation was more variable than the S.N.F. estimation with a coefficient of variation for the fat, when compared with an adequately mixed sample from the same supply, ranging between 0·53 and 4·64 with different herd supplies as against a

Table 4. *Comparison of estimates of weights of* S.N.F. *produced based on gravimetric and hydrometric methods of analysis during periods as indicated*

Period	No. of tests in period	No. of suppliers tested	Total S.N.F. supplied (gravimetric), lb (1)	Total S.N.F. supplied (hydrometric), lb (2)	Percentage difference in S.N.F. supplied $\frac{(1)-(2)\times 100}{(1)}$	Distribution of difference in weights of S.N.F. supplied, gravimetric minus hydrometric as a percentage of gravimetric, for individual suppliers over the period		
						Range	Mean and (S.D.)	Mean of the differences ignoring signs
Autumn, 20. iii to 31. v.	8	11	76040	74661	−1·81	−0·676 to −2·923	−1·85 (0·625)	1·85
Winter, 10. vi. to 31. viii.	9	11	79392	79270	−0·15	+2·124 to −1·136	+0·06 (0·892)	0·67
Spring, 10. ix. to 10. xii.	9	10	113071	114010	+0·83	+2·063 to −0·015	−0·76 (0·536)	0·76
Autumn } Winter }	17	10	145494	114109	−0·95	+0·197 to −1·929	−0·90 (0·625)	0·93
Winter } Spring }	18	10	187987	188879	+0·47	+1·569 to −0·087	+0·49 (0·521)	0·51
Spring } Autumn }	17	10	183648	183287	−0·20	+0·519 to −0·737	−0·22 (0·359)	0·33
Autumn } Winter } Spring }	26	10	258542	258114	−0·17	+0·648 to −0·633	−0·14 (0·404)	0·33

range between 0·56 and 1·78 for S.N.F. estimations in the same supplies. They estimated that the probable deviations from the true means for fat amounted to 3–5 % of the fat estimate and 1·7–2·3 % of the S.N.F. estimate.

Thus, the methods which have been extensively used in estimating the quantity of butterfat supplied are not more accurate in their estimation than within ± 1·25 % of the true value and frequently could be considerably in excess of this. Furthermore, sampling errors alone may amount to 2–3 %. Bearing in mind the much smaller variation sampling has on S.N.F. composition as compared with that on fat composition and the observed deviation of only ± 0·6 % with a standard deviation of 0·4 in the estimates of S.N.F. supplied on a hydrometric basis as compared with that estimated gravimetrically, the hydrometric method for estimating the quantity of S.N.F. supplied compares very favourably with the methods used for estimating the quantity of fat supplied.

The question that now arises is, will the supplier who sends in milk for only a limited period be unduly penalized using the hydrometric method of estimating the quantity of S.N.F. supplied? Both Tables 3 and 4 indicate that the greater the number of tests available, the closer the estimate is to the true parameter, which is what would be expected on a statistical basis. However, Table 4 suggests that, although there may be seasonal effects which affect the accuracy of the estimate, eight or nine test samples give as good an estimate as seventeen or eighteen, except when these samples are confined entirely to the winter period, and even under these most adverse conditions the range of estimates was between 2 % over-estimated and 1 % under-estimated with a standard deviation of 0·9, a relatively small variation.

From an entirely different approach, what effect would these observed differences have on the percentage distribution of income assessed on the quantity of S.N.F. supplied as estimated by the two methods?

When the values of the calculated quantities of S.N.F. supplied over the total period of observation were compared on a gravimetric and hydrometric basis, the maximum deviation observed between the percentage distribution of incomes was 0·05 %, or 25s. in £2500 of income in this case. With the shorter sampling periods involved in the autumn, winter and spring comparisons, the maximum deviations were 0·13, 0·15 and 0·15 % respectively, or 25s. in £950, 12s. in £380, and 38s. in £1200. These represent maximum deviations, so the majority of suppliers would be affected considerably less than even these trivial amounts indicate.

CONCLUSIONS

1. The hydrometric method for estimating the S.N.F. content of milk to obtain the weight of S.N.F. supplied will give an entirely adequate estimate of the weight for commercial purposes, the accuracy of estimation increasing with the number of sampling periods.

2. The use of composite samples for the hydrometric estimation of S.N.F. is satisfactory provided due allowance is made for the effect of the added preservative on milk density.

3. There is no technical reason why a system of payment based on the quantity of S.N.F. supplied which has been determined by the hydrometric method on 10-day

composite samples could not be instituted in place of or in conjunction with present systems of payment based on the quantity of fat supplied.

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REFERENCES

BRITISH STANDARDS INSTITUTION (1955). B.S. 696. Gerber method for the determination of fat in milk and milk products.

BRITISH STANDARDS INSTITUTION (1955). B.S. 734. Amendment no. 1, 1957. Density hydrometers for use in milk.

CLAESSON, O. (1958). *K. Landbrukschögskolans Annaler*, 24, 417.

EDWARDS, R. A. & SIMPSON, M. E. (1958). *J. Soc. Dairy Tech.* **11**, 30.

HEINEMANN, B. (1953). *J. Dairy Sci.* **36**, 450.

HENNINGSON, R. W. & BIRD, W. P. (1959). *J. Milk Fd Tech.* **22**, 10.

MCDOWALL, F. H. (1936). *N.Z. J. Sci. Tech.* **18**, 137.

RADEMA, L. & MULDER, H. (1948). *Neth. Milk Dairy J.* **2**, 210.

ROEDER, G. (1940). *Milchw. Forsch.* **20**, 200.

S.A.A. No. N. 26:1958. The Determination of Percentage of Fat in Milk by the Babcock Method.

WALLACE, G. M. (1958). *Proc.* 1958 *Market Milk 'Week' Conference*, Wellington: N.Z. Milk Board.

Printed in Great Britain

Use of Hydrometric Technique in estimating SNF content of Milk

SUMMARIES

Etant donné la base indirecte de la mesure des solides non gras quand on utilise la densité et la teneur en graisse, cette méthode est susceptible d'erreurs autres que celles qui sont inhérentes aux méthodes d'analyse, Ces erreurs, qui sont dûes avant tout aux variations de la proportion lactose-protéine du lait, peuvent être appréciables (SD ±0,05% de solides non gras). On a constaté une déviation dans les estimations dont la mesure et le sens changent suivant la saison; il est possible que ce soit plus étroitement dépendant du stade de lactation de la majorité des troupeaux que de la saison.

Il n'est pas facile d'évaluer l'importance de cette déviation, car l'on ne dispose que de renseignements limités. On estime que cette déviation peut varier suivant les troupeaux et il semblerait que ce soit le cas, d'après la publication de plusieurs équations de régression différentes (2), (3), (7), montrant un rapport entre densité, graisse et solides non gras. Il serait difficile de tenir compte de cette variation et il serait peut-être très équitable de n'en faire aucun cas, puisque l'estimation couvrant une longue période d'échantillonnage (9 mois) n'a pas révélé de déviation.

Enfin le Tableau III indique qu'en dépit de diverses sources d'inexactitude, le calcul de la teneur en solides non gras du lait, en se servant d'échantillons composés sur 10 jours de lait de troupeau ne s'écarte pas bien plus (SD ±0,56) comme pourcentage d'erreur de l'estimation totale que l'estimation Gerber pour la graisse (SD ± 0,5), acceptée sans réserves depuis longtemps.

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Wegen der indirekten Messungsbasis der ffrTM bei Verwendung von Dichte und Fettgehalt, unterliegt die Methode Irrtümern, die unterschiedlich sind von denen, die den Analyseverfahren anhaften. Diese Fehlerquellen, die in erster Linie auf die Schwankung im Laktose:Protein Verhältnis der Milch zurückzuführen sind, mögen abschätzbar sein (SD ±0,05 ffrTM%). Unzuverlässigkeiten bei den Schätzungen wurden festgestellt, deren Ausmass und Richtung jahreszeitlich schwanken. Dieses hängt möglicherweise mehr vom Zustand der Milchabsonderung der Mehrzahl der Herden als von der Jahreszeit ab.

Das Ausmass dieser Unzuverlässigkeiten lässt sich nicht leicht ermitteln, da die verfügbaren Hinweise begrenzt sind. Man nimmt an, dass die Unzuverlässigkeiten wahrscheinlich von Herde zu Herde schwanken, und dass dieses von der Veröffentlichung verschiedener Regressionsgleichungen (2), (3), (7), bezügl. Dichte, Fett und ffrTM, her geschieht. Es dürfte schwierig sein, diese Schwankungen in Anrechnung zu bringen, und wahrscheinlich wäre es nur zu gerecht, sie allesamt zu ignorieren, da die Bewertung iiber einen längeren Zeitraum der Probensammlung (9 Monate) keine Unzuverlässigkeiten aufwies.

Schliesslich zeigt Tabelle III, dass trotz einer Vielzahl von Quellen für Ungenauigkeit, die Ermittlung des ffrTM-Gehalts von Milch bei Verwendung gemischter Proben von Herdenmilch über 10 Tage als prozentualer Fehler der Gesamtschätzung nicht wesentlich mehr (SD ±0,56) abweicht als die Gerber-Schätzung für Fett (SD ±0,5), die seit langer Zeit unwidersprochen akzeptiert wird.

Summary in English on page 601

G. M. WALLACE,

Senior Lecturer in Food Chemistry at New Zealand's Massey University College of Manawatu, reviews its accuracy and limitations

IN VIEW of the considerably increased interest being shown by several countries in the solids-not-fat component of liquid milk supplies, a review of the variability to be expected in the use of hydrometric procedures for the indirect determination of solids-not-fat is desirable, so that the administrators of the various schemes for quality payment or improvements are in a position to assess the method and its usefulness for the work proposed.

Accuracy of hydrometric or lactometric method

The density method of estimating solids-not-fat is subject apart from errors of determination (1), (9), to two main types of error:

1. An error in accuracy due to some fallacy in the equation used to predict composition from the observed data, i.e. fat percentage and density at 20°C. This bias varies with different investigators and with the methods used in determining fat and density. With the introduction of B.S. 734 amendment No. 1 (1957) a closely detailed standard method for the determination of density of milk is available and the equations used in the calculation of S.N.F. are based on a broad spectrum of samples. Similarly B.S. 696 gives a standardized procedure for the determination of fat by the Gerber method.

   It is thus possible to reduce this error to a minimum by the use of appropriately standardized procedures.

2. An error inherent in the assumptions that the composition of the solids-not-fat component of milk is invariant and that the density of the individual components is likewise invariant. Of these two assumptions the one that the

composition of the solids-not-fat component of milk is invariant is very unsoundly based. This can be seen readily in Table 1.

TABLE I

Breed	Average % Solids-not-fat	Average % Protein	Ratio SNF/Protein
Jersey	9.3	3.4	3.66
Friesian	8.6	2.9	3.37

Recalculation of the data of Heinemann, et al. (5) to establish the correlation between SNF/Protein ratio and the difference between the calculated and weighed total solids gave a correlation of —0.62 showing that protein to solids-not-fat ratio does affect the calculated SNF value.

Similarly within a particular herd over a twelve month period the ratio of solids-not-fat to protein ranged from 3.8 to 4.6 (12A).

As the density of lactose is approximately 1.6 and of milk protein 1.3, changes in the ratios of these two components will cause changes in the mean density of the SNF component, and thus the observed differences in SNF/Protein or more specifically lactose/protein ratios occurring between breeds, between individual cows of the same breed, and between milks from the same cow at different times in her lactational period can all affect the mean density.

As a consequence of this compositional variation in the solids-not-fat component of milk the formulae used are not always capable of predicting with precision the solids-not-fat content of a milk sample.

The differences in the composition of the solids-not-fat component of samples of milk from individual cows become less when milks from a herd of cows is bulked and become less again when these herd samples are composited for a 10 day period.

Available data, Table II, gives some indication of the range of variation observed by different investigators. All data has been converted to a standard deviation and are based on comparison with gravimetrically determined total solids.

TABLE II

Comparison between calculated and gravimetrically determined SNF

	Individual cows[3,6,7,8,9]	Herd[3,10,11]	10 day composite [12] on herd milks
Standard deviation, i.e. 66% of tests lie within these limits.	±0.13 to ±0.18	±0.09 to ±0.16	±0.05

It is thus possible to estimate the composition of a bulked milk supply using a ten day composite sample with between a half and one-third of the error inherent in the testing of individual bulk samples.

It has been pointed out (12) that error introduced in the taking of samples and in the testing of milk for its fat contents can be considerable. Table III summarizes data on the extent of the errors introduced by sampling, fat testing and

SUMMARY

Because of the indirect basis of measurement of SNF when using density and fat content, the method is subject to errors other than those inherent in the methods of analysis. These errors which are primarily due to variation in the lactose/protein ratio of the milk may be appreciable (SD ± 0.2 SNF%) with samples of milk from individual cows and will vary with stage of lactation. Bulked milks and 10 day composited samples can reduce this variation to a reasonable value (SD ± 0.05 SNF %). Bias in the estimates has been observed, the extent and direction changing with season; this is possibly more closely related to stage of lactation of the majority of the herds rather than with season.

The importance of this bias is not easily assessed as the information available is limited. It is considered that the bias is likely to vary from herd to herd and there is some indication that this is the case from the publication of several different regression equations (2), (3), (7), relating density, fat, and SNF. It would be difficult to allow for this variation and it would possibly be most fair to ignore it altogether since the estimate over an extended period of sampling (9 months) showed no bias.

Finally Table III indicates that despite a variety of sources of inaccuracy the calculation of the SNF content of milk using 10 day composite samples of herd milk does not deviate appreciably more (SD ± 0.56) as a percentage error of the total estimate than does the Gerber estimation for fat (SD ± 0.5) which has been accepted without question for a long time.

SNF testing, the latter being based on hydrometry (B.S. 734 Amendment No. 1). The extent of error is given as a standard deviation and is expressed as a percentage of the average value for the component estimated using 4% for fat and 9% for solids-not-fat.

TABLE III

Standard deviation of estimate due to method
(expressed as a percentage of the estimate)

Fat		Hydrometric Solids-not-fat			Sampling	
Babcock	Gerber	Individual	Herd	10 day composite on herd milk	Fat	SNF
±0.85	±0.5	±1.8	±1.3	±0.56	±2	±1

The combined errors due to variation in composition of solids-not-fat and inherent in the methods of analysis are not appreciably greater with the hydrometric estimation of the solids-not-fat of a 10 day composite sample of bulked milk than with the Gerber test for butterfat.

Estimating compliance with S.N.F. standards

It has already been shown that errors due to compositional variation in the solids-not-fat component are two to three times greater with individual herd samples than with composite samples.

The range of fluctuation in fat and solids-not-fat in day to

TABLE IV

Supplier No.	Av. daily quantity of milk supplied. lbs.	Min. SNF %	Max. SNF %	Weighted Mean SNF %	SD of daily variation about mean	10 day composite SNF %
1	630	8.90	9.41	8.99	0.176	8.91
2	1,850	8.85	9.06	8.95	0.083	
3	870	8.74	9.10	8.91	0.147	
4	870	8.54	9.35	8.89	0.233	8.95
5	1,040	8.87	9.11	8.95	0.082	9.03
6	1,200	8.67	8.80	8.75	0.045	8.75
7	1,360	8.95	9.18	9.08	0.084	9.08
8	580	8.55	8.78	8.64	0.071	8.66
9	790	9.18	9.35	9.26	0.066	9.21
10	560	8.57	9.20	8.88	0.217	8.85
11	600	9.06	9.30	9.16	0.065	9.13
12	930	9.23	9.53	9.38	0.084	

day samples can be quite wide. Thus with 12 supplies coming into a local treatment station and examined daily over a ten day period using a hydrometric method for estimating SNF the above fluctuation was observed (12a).

Av. SD for individual daily variation about the mean 0.113

SD 0.05 about weighted mean of daily SNF testing

Mickle (11), et al. discussing the results of a twelve month survey of the composition of mixed herd milk states: "These data show the relatively large week to week variations which occurred in the composition of this milk, particularly in the fat and lactose content. The daily variations were even greater than the variations in the weekly averages, and it appears that these daily variations are often greater than the seasonal changes".

It could thus be grossly unfair to assume from individual day samples of bulked milk analysed hydrometrically, that a supplier has failed to comply over a period with minimum S.N.F. composition regulations and it is recommended that 10 days composite samples should be used.

If this is done the standard deviation of the estimate by comparison with gravimetrically determined solids-not-fat should be of the order of 0.05% S.N.F. after due standardization of the hydrometer and adjustment for the effect of added preservatives.

Determining compliance with minimum SNF requirements

In view of the standard deviation of 0.11% SNF (Table IV) that must be allowed for individual day samples of milk used for calculating SNF content, it is possible for one sample in 100 to be 0.33% SNF over or under calculated and for one sample in 20 to be 0.22% SNF over or under calculated. This makes individual day sampling for compliance subject to considerable unfairness.

With 10-day composite samples used for the calculation, the errors are reduced to one sample in 100 being 0.15% SNF over or under calculated, and to one sample in 20 being 0.10% SNF over or under calculated.

A variable bias is introduced to the calculated SNF value at different times of the year, presumably as a result of consistent changes in the composition of the solids-not-fat and possibly of the fat in the milk due to seasonal and lactational effects. Using data collected and reported (12) it is possible to indicate the extent of this bias (Table V).

Thus over a nine month period of testing of composite samples variations in the extent of the bias have been eliminated for practical considerations, and 66% of the supplies will have been estimated to within ± 0.015 SNF % of their gravimetric estimate, 95% to within ± 0.030 SNF %, and 99% to within ± 0.045 SNF %. It is thus possible to estimate with fairness and accuracy the SNF content of a milk supply using a hydrometric method and calculating the SNF from the results, provided a reasonable number of sampling periods is utilized.

This approach may not be entirely acceptable as most

TABLE V

	Autumn Winter Spring	Autumn Winter	Winter Spring	Spring Autumn	Autumn	Winter	Spring
No. of suppliers tested	10	10	10	10	11	11	10
No. of 10 day periods involved	26	17	18	17	8	9	9
Bias of calculated SNF content	—0.020	—0.085	+0.047	—0.030	—0.160	—0.011	+0.073
Standard deviation of calculated individual SNF contents about gravimetric estimates	±0.015	±0.042	±0.027	±0.019	±0.080	±0.109	+0.046

regulatory authorities require compliance at any individual test period rather than over the extended period of supply.

For illustration using 8.5% SNF as minimum acceptable composition, and using the limited data available, Table V indicates that variable allowances must be made for bias and standard deviation if the supplier is to be treated fairly at all times of the year. Basing the estimate of calculated SNF on 8 or 9 ten day composite samples tested by the standardized hydrometric technique, and using this admittedly limited data, it would be necessary to accept an SNF content of 8.1% SNF during the autumn period, September to November inclusive, if the probability of one unfair decision in 200 is acceptable. This limit could be raised to 8.2% SNF if the probability of one unfair decision in 40 is acceptable.

In the winter period, December to February inclusive, the corresponding limits would be 8.15% SNF and 8.2% SNF respectively, whilst in the spring the limits would be 8.40% SNF and 8.5% SNF. No data is available for the summer period.

Problem of standardization of bulk supplies

Since individual day samples would be used and the quantities of milk to be standardized would vary it is difficult to assess the error involved in using a calculated value for the SNF content of the milk to be standardized.

It would be logical to allow for the bias in the estimate as given in Table V. The likely standard deviation of this adjusted estimate will reduce with increase in the quantity of milk being standardized but no data is at present available for determining the extent of this standard deviation. To standardize to a minimum SNF content of 8.5% when determined gravimetrically and using the bias data from Table V and the averaged standard deviation of 0.13% SNF for herd milk from Table II, it would be necessary to adjust the SNF content to a calculated value of 8.7% SNF in autumn, 8.9% in winter, and 8.95% SNF in spring, to ensure that no more than one sample in 200 failed to meet these requirements. If a probability of one in forty is acceptable, then the acceptable level of adjustment could be reduced to 8.6% in autumn and 8.8% in winter and spring.

It must be realized that these apparently high figures merely represent tolerances that have to be allowed to ensure a reasonable probability of standardization being made to the desired figure. This anomaly would be removed if the formula used for calculating SNF was modified to allow for changing values of the bias through the year, but allowance of 0.13 SNF % would still have to be made for the natural standard deviation of the individual samples.

References

(1) DAVIS, J. G. *The Chemical Composition of Milk between* 1900 *and* 1950: Analyst, (1952), **77**: 499.

(2) WATSON, P. *Determination of the solids in milk by lactometric method at* 102°*F:* J. Dairy Sci., (1957), **40**: 394.

(3) PASCOE, J. V. *The Determination of total solids in milk by Richmond's formula:* Aust. J. Dairy Tech., (1956), **11**: 145.

(4) BODEN, S. M. and CAMPBELL, C. H. *The Estimation of solids in milk, Part I. Determination of solids-not-fat by various methods of hydrometry:* J. Dairy Res. .(1942), **13**: 45.

(5) HEINEMANN, B., COSIMIN, J., JACK, E. L., WILLINGHAM, J. J. and ZAKARIASEN, B. M. J. Dairy Sci., (1954), **37**: 869.

(6) ERB, R. E., ASHWORTH, U. S., and MANUS, L. J. *Field testing for solids-not-fat and protein:* J. Dairy Sci., (1960), **43**: 1534.

(7) ROEDER, G., *Die fehlerquellen bei der berichnung des trockenmasse gehaltes der milch:* Milchwissenschaft, (1953), **8**: 125.

(8) EDWARDS, R. A. *Comparison of the hydrometric and gravimetric methods for the determination of solids-not-fat when applied to the milk of individual cows in a single herd:* J. Dairy Res., (1961), **28**: 109.

(9) Annual Report of National Institute for Research in Dairying (Gt. Britain): (1947), p.36: (1948), p.42.

(10) LAWRENCE, A. J. *Lactometric determination of solids-not-fat in milk of individual cows:* Aust. J. Dairy Tech., (1958), **13**: 144.

(11) MICKLE, J. B., EGERMEIER, E. R., MUSGRAVE, S. D., MORRISON, R. D., GRAYBILL, F. A., and MACVICAR, R. *Evaluation of formulas for predicting various components of mixed herd milk:* Oklahoma State University Tech. Bul. No. T-74, (1958).

(12) WALLACE, G. M. *Application of the hydrometric method of analysis to the estimation of solids-not-fat production:* J. Dairy Res., (1962), **29**: 11.

(12*A*) WALLACE, G. M. Unpublished data.

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C. <u>An investigation of the amido black technique for protein analysis and of its potential as a routine method</u>.

This technique has received considerable publicity and is being used in one of its modifications for a very extensive protein testing scheme in Friesland. The various factors contributing to the precision of the original Steinsholt form of the technique were examined, particularly as they affected the blank readings. A precision based on comparison with Kjeldahl values for protein at least as good as that claimed by other investigators was obtained but there was considerable variability in the blank determinations. Miniaturisation of the method was considered desirable as the quantities of reagents used and the size and quantity of the equipment needed made the method unsuitable for use as a routine method. The macro technique was miniaturised simply by reducing the quantities taken at the various stages of the procedure but maintaining the same ratios in these quantities. An endeavour was made to determine any major controllable sources of error in this miniaturised technique but no consistent source of variation was observed over 40 tests and the standard deviation was only $\pm$ 0.003, about a mean value of 0.527 optical density units. Application of this miniaturised technique to 70 preserved composite samples of milk from 12 herds and comparison with Kjeldahl protein results gave a standard deviation of $\pm$ 0.19% protein (5.2% of the protein content). This standard deviation was considered far too high for routine testing and so further modifications were made to the technique, but they brought about only a small reduction in the standard deviation (to $\pm$ 0.17%). Since a similar technique was being used in Friesland and a standard deviation of only 0.06% claimed, it was decided that the

technique should be again tried on commercial samples using Kjeldahl determined protein contents for comparison. Ten bulk supplies were examined using ten day composite samples over a three month period but the results were no more encouraging, the standard deviation being ± 0.21% protein and the correlation coefficient between the two protein values for any sampling period ranging from 0.30 to 0.96. It was considered that in the miniaturised form, as developed, the amido black technique for protein was unsuitable as a routine method using 10-day composite samples of bulked milk.

This work is further discussed in an unpublished paper entitled: "The use of the amido black technique for protein analysis."

This is attached. (p.23).

CONCLUSION

It has been shown that it is economically practical and, in fact, advantageous to control the compositional S.N.F. quality of milk by supplementation with reconstitutable dried milk solids. In a study of some of the analytical procedures routinely used in the dairy industry, a relationship between the amount of water added to concentrated sulphuric acid and the resulting density has been derived and its practical use demonstrated. The hydrometric (density) method for determining solids-not-fat on 10-day composite samples has been shown to give results in good agreement with results based on daily sampling and testing; furthermore, it was shown that if the S.N.F. content of 10-day composite samples of bulk (herd) milk supplies are determined by the hydrometric method, the resultant estimate of the quantity of S.N.F. supplied is measured with about the same accuracy and precision as the quantity of fat supplied based on the Gerber method of fat estimation, providing the testing has extended over at least 6 sampling periods. The amido black method for protein analysis has been examined and a modified miniaturised technique has been developed as being more suitable for routine use. However, the S.D. of $\pm$ 0.19% protein about the Kjeldahl derived protein values was considered too high for routine use, particularly when the correlation coefficient between the protein values determined by the Kjeldahl and amido black techniques fell as low as 0.3 on two out of ten sampling occasions.

Economic and practical methods of S.N.F. supplementation and of measurement have thus been demonstrated.

In view of the lack of success with the amido black technique for measuring protein, it appeared that a more useful line of investigation for estimating protein production was to use Kjeldahl determined values based on samples taken on a specified few occasions and to predict total production by means of a regression equation from this limited data. This then is developed and examined in Part II.

THE USE OF THE AMIDO BLACK TECHNIQUE FOR PROTEIN ANALYSIS

In acid solutions of protein a stoichiometric reaction occurs between certain anion active dyes and the cation active sites on the protein giving a dye-protein precipitate and thus the change in the optical density of the dye solution gives an inverse measure of the protein content of the system. It is necessary to separate fat and precipitated protein by centrifugation prior to determining the optical density of the residual dye solution. The technique using amido black as the dye was developed in a usable form by Steinsholt (1957), although Udy had published a similar technique using the dye Orange G., whilst the potential for the technique was first indicated by Fraenkel-Conrat and Cooper.

Dolby (1958) reported on the amido black method then being investigated in Europe and this, with an earlier report by Stewart on the adaptation of the Steinsholt technique proposed for the extensive protein testing schemes to be used in Friesland, were used as a basis for investigating the application of the technique to New Zealand conditions.

The Steinsholt technique (Appendix (a)) requires relatively large quantities of samples and reagents, and apparatus which would be inconvenient in commercial practice. It was decided, therefore, that the Friesland modifications (Appendix (b)) of the technique should be investigated, particularly in relation to miniaturising the technique.

RESULTS

I. STEINSHOLT TECHNIQUE

A. Suitability of Dye

A scan of the absorption spectrum of the sample of the Amido Black 10B Merck (available) gave a maximum at 620 mμ which was sufficiently close to the 612.5 mμ recommended, as well as comparable spectra, to indicate the dye solution was comparable to that used in other investigations.

B. Determining Blank

Using the dye concentrations recommended and a Beckman DU Spectrophotometer, the intensity of the dye solution in the blank sample which had been treated in a manner that was identical with that for milk, was such that less than 11% of the incident light was transmitted. At this level of transmission, sensitivity is low and increased sensitivity had to be provided. The blank was, therefore, prepared as a weaker solution by taking 5 ml. of the bulk dye solution instead of the indicated 10 ml. The rest of the procedure was not changed. The resultant reading for the optical density of the blank was increased 1.33 times to allow for the change in concentration of the dye (doubling the value would give an incorrect adjustment due to subsequent operations altering the original proportions). Using this technique the precision of the blank determination was examined.

Number of tests (N) = 12

Mean value = 1.895 OD units (O.D.= optical density).

Standard Deviation (S.D.) = 0.020

Standard Error (S.E.) = 1.07% $\left(\text{S.E.} = \frac{\text{S.D.} \times 100}{\text{mean value}}\right)$

C. Relationship between Kjeldahl determined protein and amido black values and effect of blank variations on amido black values.

Using the Steinsholt technique but using only 5 mls. of dye for the blank, the change in dye concentration was determined for twelve 10-day composite herd milk samples preserved with mercuric chloride. Kjeldahl protein values were also determined on the same samples.

It was observed that the values for the blanks using amido black were not consistent and the method for deciding which blank value to use was varied to observe its effect on the estimating (regression) equation relating the changes in the amido black readings (Y) to the Kjeldahl protein values (X).

	Method of assessing blank value	Standard Deviation of Estimate (% Protein)
(1)	Common blank solution - readings taken between every two samples - each pair of sample readings subtracted from their own blank.	0.03
(2)	As for (1) (repeated).	0.06
(3)	Common blank solution-readings taken between every two samples - all blank readings pooled and mean value determined. All sample readings subtracted from mean value.	0.03
(4)	As for (2) (repeated).	0.03
(5)	Using the mean of the results for (3) and (4).	0.06

The estimating (regression) equation for (5) was:

X (% protein) = 0.073 + 2.632 Y (OD units)

Y (OD units) = 0.117 + 0.343 X (% protein)

Despite a disturbing variability in the blank values, the standard deviation of these regression equations were not high. To examine whether there was a significant difference in the estimates given by these different regression equations, the differences in O.D. units for dye precipitated in various hypothetical protein solutions was calculated for each equation. (Protein concentrations of 2,3,4 and 5% were used). The values for equation (5) which was based on mean differences in readings were used as the reference.

SIGNIFICANCE OF DIFFERENCES BETWEEN VARIOUSLY DETERMINED MEAN VALUES FOR BLANKS

Mean Values Compared	Mean difference OD units	"t" value
(5) - (1)	0.025	1.42
(5) - (2)	0.014	1.19
(5) - (3)	0.008	0.68
(5) - (4)	0.015	1.28

There were no significant differences resulting from the different methods for determining the blank values and their effect on the change in optical density of the dye solution.

The estimating (regression) equation indicates that a difference of 1% protein caused a change in optical density of the dye solution of about 0.34 units. The standard deviation

of the estimate was of the order of 0.02 O.D. units or about 0.06% protein, i.e. about 1.5% of the mean protein content of 3.9%. The standard deviation of the blank was also of the order of 0.02 O.D. units, but using the mean of a series of blank determinations reduced this latter source of deviation.

D. The effect of delays in reading.

Readings for blanks and samples were made immediately and after 18 hours' storage in the refrigerator.

Results for both the blanks and the samples showed highly significant differences between the readings at 0 and 18 hours. Despite these changes, the differences between blank and sample did not differ significantly at 0 and 18 hours, thus indicating that provided both blank and sample are treated in an identical manner and are read at the same time, appreciable delays in reading will be without significant effect on the dye protein relationship.

	Blank	Sample	Blank-sample
Difference between mean values (0 and 18 hours).	0.109	0.083	0.026
S.D. difference (0 and 18 hours).	0.05	0.038	0.036
Probability of significance "P".	0.005	0.001	<0.025

E. Investigation of factors affecting constancy of blank reading.

(1) In view of the difference in results which is directly dependent on variations in the blank values, a fresh bulk sample of concentrated dye solution was

prepared and this was diluted by both the 5 ml. technique and a new 2 ml. technique, and the results were compared with samples similarly prepared from the first concentrated dye solution. The 2 ml. technique which involved 2 ml. of dye instead of 5 or 10 ml., but with all other procedures common except for multiplying the reading by 3.33 to obtain the equivalent blank value was introduced so that the readings for both blank and sample were taken in the same area of the scale.

	No.of readings	Mean reading	SD	SD as % of reading
e.g. 2 ml.new dye solution	13	0.610	0.008	1.31%
2 ml.old dye solution	11	0.604	0.006	0.99%
5 ml.new dye solution	5	1.496	nil	nil
5 ml.old dye solution	2	1.459	0.017	1.16%

There was no consistent improvement in precision using the fresh dye solution or the smaller quantity of dye.

It appears that the standard deviation about the mean blank reading amounts to about 1% of the reading, although at times the precision is unexplainedly very much better than this.

(2) Instead of reducing the amount of concentrated dye taken as had been done above (b) and (E) (1), the same quantity (10 ml.) of concentrated dye was used as for the sample but smaller volumes of the centrifugate were taken for dilution prior to making the readings.

Only half the quantity of blank centrifugate when compared with the sample centrifugate was used. It was hoped that under these conditions the known non-linearity with Beer's Law would be reduced to a minimum as both sample and blank would then give readings of the same order. The blank reading was then multiplied by two.

The methods thus modified are given in Appendix (c). For the blanks the following mean values were obtained.

	No.of readings	Mean reading	SD
Old dye solution			
Series (1)	5	0.818	Nil
Series (2)	5	0.816	Nil
New dye solution			
Series (1)	1	0.759	-
Series (2)	1	0.713	-

The precision appears to be better using this method, but there is still appreciable variation between the blank preparations.

ii. MINIATURISED TECHNIQUE

The technique detailed in Appendix (c) was further modified by reducing the quantities taken at the different stages, but the ratios were not altered. The possible sources of variation were examined and these are given in Appendix (e). It was considered that the main variation was probably in the various

volume measurements made including the use of a Cornwall syringe, or in the cells used in the spectrophotometer. Forty tests were made on the sample with the following results.

O.D.Difference mean value : 0.527 S.D. 0.003

No consistent pattern of variation was observable in these variations of technique. The use of the Cornwall syringe may have been a source of some slight variation but its use was justified in terms of speed if the method was to be commercially useful.

III. APPLICATION OF MINIATURISED TECHNIQUE TO COMMERCIAL SAMPLES.

The method described in Appendix (d) was applied to 70 preserved composite samples of milk collected from the same 12 herds during six ten-day sampling periods, and the regression equation relating the difference in Optical Density (Y) and the Kjeldahl protein values (X) was derived and was as follows:-

X (% protein) = 1.652 + 4.974 Y (O.D.units) S.D.0.19% protein

(mean blank value was 0.793 S.D. 0.015)

The higher values in the equation were consequent upon the smaller differences in optical density resulting from the changed technique.

The standard deviation of 0.19% protein was equivalent to 5.2% of the mean protein content.

This large standard deviation of the regression equation made the technique unacceptable as an analytical method. However, a similar technique was being used as an analytical method in Friesland and so further modifications were introduced to try to improve the precision of the technique. These modifications were the use of larger quantities of a weaker dye solution and the use of larger quantities of centrifugate, whereby errors in volume measurement would be reduced in extent. These modifications are detailed in an addendum to Appendix IV.

Using 32 further samples, the regression equation for this modified miniaturised technique was:

X (% protein) = 1.221 + 6.303 Y (O.D. units) S.D. 0.17 and the mean blank value was 0.818 S.D. 0.006.

These modifications brought about only a small reduction in the standard deviation.

IV. ESTIMATION OF PROTEIN CONTENT OF COMMERCIAL SAMPLES BY THE MODIFIED MINIATURISED TECHNIQUE.

A limited comparison of estimates of protein production by the modified miniaturised amido black technique and by the standard Kjeldahl method was undertaken using mixed herd milks collected as 10-day composite samples over approximately 3 months from a common group of suppliers. The correlation coefficients between the two methods for the data for each 10-day sampling period were as follows:-

Period	No.of suppliers test	Correlation Coefficient
1	10	0.2971
2	11	0.6852
3	9	0.7568
4	11	0.6528
5	11	0.9630
6	10	0.9370
7	9	0.2960
8	8	0.8983
all	bulked	0.4723

In periods 1 and 7, two results were discarded since with them present negative correlations were obtained. Even with these two samples discarded the correlations in these two periods were extremely low.

Using this data a new regression equation was calculated (the two results discarded for the correlation studies were again discarded).

X (% protein) = 3.051 + 1.794 Y (O.D. units) S.D. 0.21

The standard deviation was greater than that observed in Section III when the miniaturised technique was first applied to commercial preservatised samples and, in consequence, the method as modified was still too imprecise for use in estimating the protein content of preservatised mixed herd milks.

If all data in periods 1 and 7 were discarded, the precision of the regression equation improved. The regression equation then became:

X (% protein) = 1.580 + 6.467 Y (O.D.units) S.D. 0.18 and the correlation coefficient was 0.7382.

Thus, even under these highly selective conditions the protein was estimated with a standard deviation of 0.18% which was too high to be acceptable since it represented 5% of the average protein content.

DISCUSSION

Steinsholt (1960) showed there was a linear relationship between the protein content and the amount of amido black precipitated only when the amido black was present in excess. He showed that a ratio of 0.486 gm.dye per gm.protein was necessary to ensure linearity at all protein levels. In the technique used in these studies a dye to protein ratio of 1:1 was used; consequently the variations observed were not due to a deficiency of dye but may have been related to the excess of dye present.

The accuracy of the Steinsholt technique, when compared with the Kjeldahl method, appears to be fairly well documented.

Steinsholt	S.D.	0.12%	protein
Vogt	S.D.	0.07%	"
Dolby	S.D.	0.07%	"
Macro method (IC)	S.D.	0.06%	"
Miniature method (III)	S.D.	0.17%	"

The macro method used in this study was thus of the same precision as that of other investigators, but the miniaturised method had a consistent error of about 3 times the extent of the macro method and it was not possible to reduce it appreciably.

Raadsveld modified and simplified the Steinsholt technique in order to make it more suitable for mass analytical purposes. He standardised the dye strength to a predetermined optical density and then read the optical

density of the clear centrifugate. He claimed that the standard error of the estimate using this technique was 0.06% protein when compared with the Kjeldahl determination and the standard deviation between duplicates for the amido black technique was 0.05% protein. Posthumus reported the automation of the Raadsveld technique to handle 1000 tests per hour and claimed a standard error of estimate of the same order, but he considers it necessary to run regular checks against the Kjeldahl method.

Ricordeau et al., Dolby and Vanschoubrek also reported standard deviations of the same order, although disagreeing on the presence or absence of lactational effects on the relationship.

Hashimoto et al. reported a survey of the protein determination in 997 milk samples by the amido black and Kjeldahl techniques and their correlation coefficients between the two methods ranged between 0.706 - 0.990 for their 10 groups of samples; whereas in the study reported here the range was from 0.296 - 0.963. They also observed that the following factors did not affect the determination, source of dye, pH range 2.2 - 3.2, filtration or centrifugation, mercuric chloride preservative, thus indicating that the method is not particularly sensitive to conditions. This is disputed by Ashworth and by Vogt.

Kiermeier and Reimer established a regression equation different from the original equation determined by Steinsholt as have all the other investigators.

In Denmark the method has been commercialised under the name Pro-Milk. Reviewing the Pro-Milk technique, McNeil

et al. noted that this technique gave higher values for protein %: the differences ranged from -0.26% to +0.34% total protein.

It appears that the various modifications of the Steinsholt technique should give protein estimates that have a standard deviation from those determined by the Kjeldahl technique of around 0.1% or less, but this has not proved consistently so, nor is there consistent agreement on the effect of varying conditions of analysis. Investigations on miniaturised techniques reported in this paper showed that these techniques were not capable of producing results maintaining a consistently high correlation with the Kjeldahl results, and although the inconsistency of the blank determination is responsible for some of the variation, this is by no means the only source as is obvious from a perusal of the correlation coefficients in Section IV. The cause of this excessive variation has not been determined. The modified amido black methods for the determination of protein in milk discussed herein are not suitable for use on commercial 10-day composite samples in their present form, although their use consistently over an extended period of supply should give as accurate an estimate of protein supplied as does the hydrometric technique for solids-not-fat.

AMIDO BLACK REFERENCES

ASHWORTH, V.S. (1964). Buffer and pH effects on the dye binding capacities of milk protein. J.Dairy Sci. 47 : 672.

DOLBY, R.M. (1958). Personal communication.

DOLBY, R.M. (1961). Dye binding methods for estimation of protein in milk. J.Dairy Res. 28 : 43.

FRAENKEL-CONRAT, H. and COOPER, M. (1944). The use of dyes for the determination of acid and basic groups in proteins. J.Biol. Chem. 154 : 239.

HASHIMOTO, Y., ARIMA, S. and SAITO, Z. (1965). The determination of the protein content of milk by the colorimetric amido black method. Dairy Science Abstracts 27 : Nos. 1302, 1303.

KIERMEIER, F. and RENNER, E. (1960). The rapid estimation of the protein content of fresh milk. Z.Lebensmitt-Untersuch, 113 : 1.

McNEIL, J.E., STINE, C.M. and HEDRICK, T.I. (1963). Pro-milk dye binding and Kjeldahl methods for determining protein content of cow's milk. Quart.Bull.Mich. Agric.Exp.Sta. 46 : (2) 162-4.

RAADSVELD, C.W. (1958). The rapid estimation of the protein content of milk. Dairy Science Abstracts 20 : No. 1458.

RICORDEAU, G., AURIOL, P., MOCQUOT, G., BLANC-PATIN, E. and JEUNET, R. (1962). Ecarts observès entre les taux de protèines du lait obtenus par la mèthode Kjeldahl et al mèthode au noir amido, en fonction de la saison et du stade de lactation. J.Dairy Res. 29 : 191.

STEINSHOLT, K. (1957). A colorimetric method for the quantitative determination of protein in milk. Meieriposten 46 : (14) 259, (15) 279 (46) 901.

STEINSHOLT, K., DØVLE,H. and SOLDBERG, P. (1960). Studies on the quantitative colorimetric determination of milk proteins by use of the dye amido black 10B. Meld.Norg.Landbr. Høgsk. 39 : (18) 1.

STEWART, A. (1958). Personal communication.

UDY, D.C. (1956). A rapid method for estimating total protein in milk. Nature, Lond., 178 : 314.

VANSCHOUBROEK, F.X.E. (1963). Determination and variation of protein, fat and lactose in cow's milk, and selection on these factors. Neth.Milk and Dairy J. 17 : 12.

VOGT, K.F. (1962). Amido black determination of protein in milk. S.Afr. J. Agr. Sci. 5 : 433.

APPENDIX (a)

PROTEIN ANALYSIS BY AMIDO BLACK (STEINSHOLT)

Steinsholt, K. Meieriposten 46:(14) 259-64 (15) 279-84 (46) 901-05 (1957).
"A colorimetric method for the quantitative determination of protein".

Reagent : 0.6165 g amido black 10B (Merck for electrophoresis) is dissolved in 1 litre of buffer solution pH 2.35 consisting of:

825 ml. 0.1 M citric acid solution
175 ml. 0.2 M disodium hydrogen phosphate

In later experiments, 0.3M citric acid at pH 2 replaced the mixed buffer.

Method : 15 ml. milk diluted with distilled water to 200 ml.

To 10 ml. of the dilution is added 20 ml.amido black solution.

After 10 min. the mixture is centrifuged for 5 min. at 2,500 RPM in a Wifug (Model H) centrifuge.

5 ml. of centrifugate is diluted with distilled water to 200 ml. and light transmission measured at 612.6 m/u.

Distilled water used as blank.

Kjeldahl method using 6.37 x N% was used as reference method.

Results : Correlations between amido black method and Kjeldahl.

(a) 64 preserved herd milk samples 0.982

(b) 62 fresh (unpreserved) herd milk samples 0.975

Regression equation - linear

% protein = -3.132D + 4.738 where D = optical density.

APPENDIX (b)

OFFICIAL FRIESLAND METHOD (POSTHUMUS)

(based on report by Stewart)

Reagent : 0.888 g amido black 10B

2.080 g $Na_2 HPO_4 2H_2O$

15.78 g citric acid

are dissolved in 1 litre distilled water.

pH is adjusted to 2.35

Dilute 38 ml. of this solution to 100 ml. with distilled water.

Determine optical density of this and adjust with citric acid - phosphate buffer pH 2.35 to predetermined standard O.D.

Method : Warm sample to 40°C mix and cool back to 20°C using Cornwall pipette transfer 0.95 ml. to test tube.

Add 19 ml. amido black solution and mix 10 mins.

Centrifuge 1500 RPM/5 mins.

Determine the colour in the intermediate layer (i.e. minus fat and sediment).

Results : Using 40 samples and comparing with Kjeldahl.

Regression equation - curvilinear

% protein = $1.091D^2 \times 10^6 - 3.6787D \times 10^{-3} + 4.27$

Day to day fluctation of $\pm$ 0.05%

Check samples using Kjeldahl are necessary.

S.D. claimed to be $\pm$ 0.05%

Cost : About 9d per test.

APPENDIX (c)

MODIFIED STEINSHOLT TECHNIQUE

Sample : Dilute 3 ml. milk to 100 ml. with water and to 5 ml. of this add 10 ml. dye solution, shake, stand 10 min. Centrifuge at 2500 RPM for 5 min., then dilute 4 ml. of supernatant to 100 ml. with water. Read at 620 m/u in optical density units and subtract from blank.

Blank : To 5 ml. of water add 10 ml. dye solution, shake, and dilute 2 ml. of this to 100 ml. with water. Read at 620 m/u and multiply optical density reading by two.

APPENDIX (d)

MINIATURISED MODIFIED STEINSHOLT TECHNIQUE

Sample : Dilute 3 ml. of milk to 100 ml. with water, to 1 ml. of this add 2 ml. of dye solution, shake, stand 10 min. Centrifuge at 2500 RPM for 5 min. and to 0.83 ml. of the supernatant add 20 ml. of water using Cornwall syringe. Read at 620 mμ in optical density units and subtract from blank.

Blank : To 1 ml. of water add 2 ml. of dye solution, shake, and to 0.415 ml. of this add 20 ml. of water using Cornwall syringe. Read at 620 mμ multiplying optical density reading by two.

Further modifications introduced:

(a) the concentrated dye solution was diluted 1:5 and 10 ml. of this was used in place of the 2 ml. of concentrated dye.

(b) 3 ml. of centrifugate was used in place of 0.83 ml. and to this, using a Cornwall syringe, 80 ml. of water was added in place of 20 ml.

(c) the blank was prepared as follows: to 1 ml. of water add 10 ml. of dye solution diluted 1:5, shake. To 1.5 ml. of this add 19.5 ml. of water. Read at 620 mμ, multiply optical density reading by two.

APPENDIX (g)

EFFECT OF VARIATION OF TECHNIQUE ON PRECISION OF MEASUREMENT

Test No.	O.D. Difference	Technique variation			
1	.533	1 ml. pipette A 2 ml.pipette C	0 - .415	pipette D	20 ml. pipette F
2	.528		.415 - .830	"	"
3	.528	1 ml. pipette A 2 ml.pipette C	0 - .415	"	"
4	.529		.415 - .830	"	"
5	.530	1 ml. pipette B 2 ml.pipette E	0 - .415	"	"
6	.530		.415 - .830	"	"
7	.532	1 ml. pipette B 2 ml.pipette E	0 - .415	"	"
8	.530		.415 - .830	"	"
9	.540	as for 1			
10	.519	as for 1 but 20 ml. Cornwall syringe replaced 20 ml. pipette F			
11	.529				
12	.522				
13	.531				
14	.519				
15	.530				
16	.510				
17	.538	as for 5 and 6 but taking particular care with .415 ml.			
18	.531				
19	.535	quantity using full length of pipette D and 20 ml. Cornwall syringe			
20	.525				
21	.533	as for 7 and 8 but using only 0 - .415 length of pipette D and 20 ml. Cornwall syringe			
22	.512				
23	.525	as for 21 and 22 but cells reversed			
24	.533				
25	.532	repeat on 21 using third cell			
26	.530				
27	.531	repeat on 21 using fourth cell			
28	.530				
29	.528				
30	.521	as for 7 and 8 but using matched cells			
31	.527				
32	.520				
33	.520				
34	.519				
35	.531	fresh sample using only 0 - .415 ml. portions pipette D and 20 ml. Cornwall syringe			
36	.527				
37	.527				
38	.528				
39	.529				
40	.525				

MEAN 0.527 S.D. 0.003 S.D. as % of reading 0.6%

PART II

A STUDY OF SAMPLING FREQUENCY AND THE PREDICTION OF PROTEIN PRODUCTION IN MILK FROM DAIRY COWS BASED ON A RESTRICTED FREQUENCY OF SAMPLING.

PART II : INDEX

INTRODUCTION

For effective use of prediction of protein production in herd improvement and in milk production, it was considered that the following requirements must be met.

(1) For herd improvement on an individual cow basis

(a) One should be able to select the best and worst 10% of the cows with confidence - the main requirement is one of ranking rather than an accurate knowledge of individual production.

(b) If several groups of sampling times gave approximately equivalent estimates for the purposes of (a), this would be an advantage, as it would spread the sampling and analysis requirements. This would be an advantage both within and between breeds.

(2) For estimating production on a whole herd basis

One should be able to give an estimate of total production as this would be of value as an experimental aid in assessing the effects of changes in husbandry on the production of protein. A knowledge of the error of the estimate would assist in determining the significance of observed changes.

(3) For determining monetary return to the farmer

One should be able to make a reliable estimate of the protein supplied during a dairying season on a whole herd basis so that a reasonably accurate payment can be made for it.

For all of these it was considered that the desirable frequency of sampling should be some combination of 2, 3 or 4 sampling times which gives the best estimates of the total production for the individual animals and for the herd. It was considered desirable that the partial regression coefficients for the selected sampling times should not differ greatly from year to year and it would be useful as well if these coefficients did not differ greatly between breeds.

The investigation has been centred on protein production since it is considered that the protein content of milk will be of increasing nutritional significance in a situation where the available sources of palatable and acceptable protein are not increasing at the same rate as the world population. There are also analytical problems, as the quicker methods of protein analysis are still of only marginally adequate accuracy and reliability, and the Kjeldahl technique with its requirements for specialised laboratory facilities continues to be used both as the reference and as the routine method.

If the number of tests needed to predict protein production can be reduced, then it becomes a practical possibility to test for protein production since regional central laboratories could be established to do these analyses. In this way the cost of the testing programme could be kept to a minimum.

The results and discussion that follow were the consequence of examining the feasibility of these concepts. Following the introduction is a literature review of the

effects of variations in sampling frequency on the accuracy of prediction. First the results from various short interval sampling frequencies were compared with the results from daily sampling and it was established that a frequency of fortnightly samplings could be used to provide an adequate basis for this study. Secondly, the results from long interval or restricted frequency samplings have been recorded for comparison with the results reported herein and the advantages and disadvantages of regression equations and simple proportion ratios for estimating total production from a limited amount of data have also been noted. The methods section then follows and in it the source of the data, the methods of analysis and of computation are described, and a list of the statistics used is given.

In the results and discussion, it has been necessary for purposes of argument to examine and discuss the results stepwise. Thus the first sections (A), (B) and (C) deal with the selection and evaluation of the sampling times for use in predicting protein production. They include a consideration of the correlation of protein production at particular sampling times with the total production of protein, together with a consideration of the intercorrelation of protein production between individual sampling times, since it was considered that these two correlations would be of assistance in selecting the sampling times to be examined for their ability to provide data suitable for accurate prediction (or estimation) of production. A comparison with correlation data reported in the literature is also included.

In Section D the results of the manual determination of the partial regression coefficients for data from

particular sampling times and their efficiency for predicting production, as measured in terms of the standard error of the estimate (for definition, see p.70), are presented and considered.

In Section E an examination similar to that in Section D is made, but in this section the partial regression coefficients and standard errors of estimate used were computer derived and further the merits and demerits of the computer programme are examined. Finally, in this section, there is a comparison of results obtained by the manual and computer methods of regression analysis.

In Section F the problem of selecting from the large number of partial regression coefficients available for any particular sampling time or for any group of sampling times, those partial regression coefficients to use in a general purpose predicting or estimating equation is presented. The effect of various methods of combining data, or of combining partial regression coefficients, on the accuracy of the prediction and on practical application is considered. A review of the accepted accuracy of the prediction of production is also included.

In Section G an examination is made of, procedures for ranking cows in terms of production, and, the effect on the ranking of the cows of using the selected partial regression coefficients for predicting production. The use of partial regression coefficients which are applicable to data derived from cows within a breed over an extended range of sampling times is then considered in terms of its effect on the prediction of total production for a herd, the prediction of total production for individuals within a herd, the ranking

of the individuals and the effect on culling practices.

In conclusion, it is shown that a system of predicting protein production from only three samples taken during specific but broadly defined periods within a lactation seems practicable.

An interim report on this investigation was presented to the XVII International Dairy Congress and has been published as the following paper:-

> "Prediction of Protein Production based on a restricted frequency of sampling." Proc. XVII Int.Dairy Cong.Sect. A1, Paper No.378, p.37. (1966).

A copy is attached as Appendix, Introduction (1).

REVIEW OF EFFECTS OF VARIATIONS IN SAMPLING FREQUENCY ON ACCURACY OF PREDICTION

(A) Short interval sampling

In a recent review, Voigtlander (Appendix Introduction (2)) discussed the number of tests necessary to assess the milk yield and its composition. Most investigations which have been reported have been based on milk yield and butterfat yield and so far little work appears to have been reported on the number of tests necessary to determine protein yield. However, a consideration of these reports on milk and fat yield can be of value in (a) establishing whether a fortnightly sampling routine was a suitable system on which to base the investigations into the estimation of protein yield and (b) establishing the level of errors in prediction which are acceptable.

With regard to milk yield, examination of the data listed in Appendix Introduction (3) showed that the mean maximum percentage difference between actual and estimated production at different sampling intervals was as follows:-

for milk yield -

	Sampling frequency			
	7 days	15 days	21 days	30 days
Mean maximum difference %	6.3	8.0	10.5	12.2
Standard deviation (S.D.)	±4.3	±4.3	± 3.6	± 6.2
No. of reports used (N)	12	12	9	15

and for butterfat yield -

	Sampling frequency			
	7 days	15 days	21 days	30 days
Mean maximum difference %	5.4	8.3	8.9	12.0
Standard deviation (S.D.)	±2.0	±2.9	±4.0	± 4.3
No. of reports used (N)	4	4	2	9

With regard to fortnightly sampling, Campbell, basing his report on the results of daily testing, showed that the percentage error to be expected and its standard deviation, was as follows:-

Frequency of test	Average % error and S.D.		
	Milk Yield	Fat %	Fat Yield
Twice weekly	-0.79 ± 1.11	-0.53 ± 1.64	-1.29 ± 2.42
Weekly	+0.52 ± 1.10	-0.38 ± 2.41	+0.14 ± 2.65
Fortnightly	+1.52 ± 1.71	-0.33 ± 3.52	+1.18 ± 3.90
Monthly	+1.35 ± 3.72	-0.65 ± 4.12	+0.56 ± 6.45

Dick, after a more detailed examination of some of the data on milk yield used by Campbell, states that "bias in the sampling results may be safely neglected for all periods (i.e. from 2 up to 28 days), as the highest average percentage error does not exceed 1%. The relationship between the interval of the sampling period and the standard deviation of the percentage error follows a straight line law very closely."

Dick's figures in summary were:-

Frequency of test	Average percentage error and S.D. Milk Yield
Every 3 days	+ 0.17 ± 0.49
" 7 "	- 0.07 ± 0.98
" 14 "	- 0.11 ± 1.20
" 28 "	- 0.55 ± 2.39

Dick also examined whether the accuracy of sampling depends not only on the interval in the period used but also on the point, in time, within the period at which the sample was taken. His results given above were based on samples taken on the last day of the period studied. Dick stated "for each period there were no significant differences between the standard deviations of the percentage errors obtained by using different starting points." His results also showed no bias in the average percentage error due to lactational effects with the use of different sampling days within the sampling period. Johansson, on the other hand, in a review of earlier work reported results which suggest increases in both the systematic error and the standard deviation when the sampling day was moved from mid-period to end of period.

It seems reasonable to summarise these findings as follows:- there is no bias introduced into results using fortnightly samplings instead of daily, the average percentage error in most investigations has been around 0.1% with a standard deviation around 2% and there appears to be little significance in the day, within the fortnightly period, on which the sample is taken.

Dick examined the daily variability in milk yield, breaking the change in yield down into two components (a) that which occurs as a result of a normal lactational change (his percentage variability); (b) that which occurs due to random factors (his standard deviation).

For the 52 cows examined the percentage variability was 8.13%, the standard deviation ± 2.65, and the correlation between the percentage variability and the average daily production was zero.

There is thus a considerable uncontrolled variability from day to day which only daily recording could obviate and since such a procedure is for the majority of purposes impracticable, an extended period between samplings becomes logical, provided the extent of the errors so introduced are known.

With respect to protein sampling, Senft, using daily protein determinations as a basis, found that the protein yield could equally well be determined by sampling at 4-weekly intervals as by sampling every 10 days.

Politiek, in a discussion on the influence of heredity and environment on the composition of the milk of Friesian cows, stated that the protein percentage appeared to be very constant during a milking and there appeared to be little difference between the percentage of protein in the morning milk and the evening milk. Other factors such as in-season, incomplete milking-out, etc., had practically no effect on the percentage of protein.

Vanschoubrek reported the daily variation for protein

percentage during 10 successive days to be 2.6%, considerably less than the 4.2% for milk yield and 6.2% for fat.

A study in the Netherlands reported by the International Dairy Federation indicates that sampling once a fortnight, when compared with daily sampling taken over a period of a year, will yield an estimate of protein production having a standard deviation of 0.013% about the actual.

The error introduced by fortnightly sampling is therefore small.

(B) Long interval or restricted frequency sampling

Czakó and Csukas considered it unnecessary to perform monthly protein analyses during lactation as the milk protein production during lactation could be adequately assessed by sampling and analysis on only three occasions, namely in the 2nd, 6th and 10th month of lactation. Using this combination of sampling times the correlation coefficient (r) with the monthly data was 0.86. The daily examination, for one month, of the protein content in the milk of 14 cows showed that reliability of the analysis was not influenced by a deviation of a few days or even 1-2 weeks from the centred sampling dates, the range in observed protein values being less than 3% during this period.

Lemvigh, in an early paper, suggested sampling in the 5th and 6th months, with the 2nd or the 4th in addition. (Cited by Voigtlander, Vanschoubrek).

Senft, using daily protein determinations as a basis, found that the protein yield could equally well be determined by sampling once every 4 weeks as by sampling at 10-day intervals. Sampling at 4-weekly intervals gave a better estimate of protein production than did Lemvigh's sampling pattern. Senft recommended that samples taken on the 50th, 150th and 250th day of lactation gave a fairly satisfactory prediction of protein production. No correlation coefficient was given.

Voigtlander compared prediction of milk yield and protein percentage based on weekly sampling with that based on 14, 21, 28, 42 and 56-day sampling frequencies, and also with limited frequency sampling systems, as follows:-

(a) Czakó and Csukas' method - 2, 6 and 10 months

(b) Horn's method - 3, 6 and 9 months

(c) Lemvigh's method - 4, 5 and 6 months

(d) Pjanovskaya's method - 2, 5 and 8 months

(e) Senft's method - 50,150,250 days.

Samples were taken mid-month in systems (a) to (d). The average yield over the three test days was determined and this was multiplied by the length of the lactation period to determine total protein yield. This simple averaging and multiplication approach is common to almost all systems of predicting production from partial lactation records (Carrè et al., Lamb and McGilliard, Alexander and Yapp).

Voigtlander's results for the average of 29 lactations of German Friesian cows are given in Table 1 and 2 of his translated paper, Appendix Introduction (2).

In summary, he found that when the production data for the whole herd, using samples taken at 7-day intervals as his reference, were compared, there was an almost linear increase in the mean value of the estimated milk yield as the interval between the regular sampling dates increased reaching 0.36% in excess of the 7-day total at 56-day intervals. With mean protein percentage there was likewise an increase in the mean value, but whereas this value was only 0.57% in excess of the 7-day value at both 28 and 42-day intervals, it had increased to 1.71% in excess at 56-day intervals. With the sampling frequencies previously mentioned (a) to (e) above, the following results for variation of the mean protein percentage from the 7-day value were obtained:-

2 + 6 + 10 mth.	-0.28% of the mean value
3 + 6 + 9 mth.	-1.14% " " " "
4 + 5 + 6 mth.	-4.84% " " " "
2 + 5 + 8 mth.	-2.85% " " " "
50 + 150 + 250 days	-1.14% " " " "

The above results represented the deviations of the mean values from the 7-day values when all the lactation data were combined. When individual lactations were examined for milk yield, the mean error tended to increase with increasing sampling interval and the standard deviation of the mean error increased almost linearly from ± 1.36% of the mean value at 14 days to ± 3.54% of the mean value at 56 days. With protein percentage, although the error of the mean value increased with longer sampling intervals, the standard deviation of the error did not vary appreciably for 28, 42 or 56-day intervals being of the order of ± 1.4% of the mean value. With the limited frequency-of-sampling studies, the following variations and their standard deviation from the 7-day values for individual lactation data in terms of protein percent were obtained:-

2 + 6 + 10 mth.	-1.50 ± 3.58%	of the mean value
3 + 6 + 9 mth.	-1.17 ± 3.49%	" " " "
4 + 5 + 6 mth.	-5.48 ± 4.86%	" " " "
2 + 5 + 8 mth.	-3.03 ± 3.84%	" " " "
50 + 150 + 250 days	-0.97 ± 3.57%	" " " "

The results given in Table 2 of the original data* indicate that sampling regularly at 42-day intervals gives an estimation of protein production for an individual cow with a maximum error of + 0.79 ± 14.76% of the mean value. Unfortunately, the effect of the limited frequency sampling times on milk yield was not given so an estimate of the maximum error in protein yield is not possible, but if the 50, 150, 250-day data for protein % is used with the 56-day sampling interval data for milk yield, an estimate of the maximum error is - 0.45 ± 21.33% of the mean value.

Voigtlander suggests that a maximum error range of ± 10% would be acceptable for most uses of the predicted production.

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* ABSTRACT FROM TABLE 2. Relative sampling errors using individual lactation data. Data for 42-day interval relative to 7-day interval.

	MEAN	S.D.
Milk Yield Kg/Lactation	+ 0.05	3.48
Protein %	+ 0.74	1.44
Protein Yield	+ 0.79	4.92

Examining the effect of different sampling times on the ranking of individual cows, Voigtlander found the following rank correlations.

Rank correlation of cows using protein % values based on different sampling intervals

7 day	14 days	+ 0.9844
	21 "	+ 0.9916
	28 "	+ 0.9788
	42 "	+ 0.9547
	56 "	+ 0.9675
	2 + 6 + 10 mth.	+ 0.8049
	3 + 6 + 9 mth.	+ 0.8670
	4 + 5 + 8 mth.	+ 0.8706
	2 + 5 + 8 mth.	+ 0.8783
	50 + 150 + 250 days	+ 0.8659

The above work on the prediction of protein production, most of which has been reported since the investigation reported herein was commenced, has been approached differently from that used here in that no use has been made of a regression equation to weight the data gathered at the specified sampling times.

Discussing the value of a regression equation for prediction of production, Lamb and McGilliard observed that there were two basic methods for estimating total lactation production, either (1) from a single test or (2) from cumulative production. The simplest method was to extend the data using a simple proportion ratio based on days-on-

test projected to 305 days.

The other method was to obtain the regression of total production on partial production. Both linear and quadratic regression equations have been used but linear regression is considered to provide a satisfactory means for extending part production.

Choice between the two methods depended on the:

(1) purpose of the prediction.
(2) ease and simplicity of use.
(3) comparative accuracy.

The advantages and disadvantages of the two methods were considered.

The ratio method is far simpler and easier to derive, to use and to understand. It under estimates the total production of low producers and it over estimates the total production of high producers since time is the only variable. However, the variation in the estimated production is closer to that observed in actual production records, whereas the regression method of prediction tends to narrow the extent of this variation. The regression method does, however, correct more adequately for an incomplete lactation.

Either method should rank cows in the same order but since records extended by regression differ less than actual, this tendency to group the records more closely about the mean may make selection decisions more difficult.

Cianci examined the average difference between the actual 270-day production and the production predicted, using in one case a simple ratio factor and in the other case regression factors, with the following results:-

	Average difference from actual production			
	Simple ratio factor		Regression factor	
Breed of cow	Brown Alpine	Simmental	Brown Alpine	Simmental
Based on 30-day yield	8.2%	10.5%	7.3%	11.7%
Based on 150-day yield	3.3%	4.1%	3.0%	4.4%

In this study the average difference was not reduced by the use of regression factors in place of the simple ratio factors.

In 1955, Patchell reported briefly that samples taken at the end of the 10th, 18th and 26th week after calving could be used, with the appropriate regression equations to predict milk yield and fat yield with an accuracy differing little from that based on monthly testing.

Comparing his results against daily testing, he found the extent of the errors to be as follows:-

	Prediction by regression	Prediction based on monthly testing
Milk yield	± 8% of true value	± 7% of true value
Butterfat yield	± 9% of true value	± 7% of true value

This report of Patchell's prompted the present investigation.

METHODS

(A) Sampling and Analysis

1. Source of data

The milk production and composition of individual cows in the Massey University Jersey and Friesian herds was measured during two consecutive full lactational periods for each cow. All data were kept separate by year and by breed.

The number of cows finally used in the computation were:

Year	1957/58	1958/59
Jersey	29 (set 101)	23 (set 102)
Friesian	35 (set 103)	42 (set 104)

The reasons for changes in the cows used in the different sets within a breed are detailed in Appendix (Methods (a)).

2. Milk collection and sampling

The milk from individual cows was collected, weighed, and sampled at fortnightly intervals using the p.m. milking and the following a.m. milking. Composite samples were prepared by mixing together the well-mixed p.m. and a.m. samples in volumetric proportion to the weights of milk produced. The composite samples were preserved with 0.06 g $HgCl_2$ per 250 ml. of milk (0.2 ml. of a saturated alcoholic solution of mercuric chloride per 250 ml. of milk). The samples were held in a refrigerator until required for analysis.

Samples were then warmed to room temperature (approx. 15°C) before proceeding with the analysis.

3. Protein determination

This was based on the Kjeldahl method using mercuric sulphate solution as catalyst and reducing with powdered zinc in the distillation process. The distillate was collected in saturated boric acid and the titration was made with standardised approximately 0.1 N H_2SO_4 using methyl red as the indicator. Five ml. of the well-mixed milk sample was digested and the protein content was calculated on a weight/volume basis.

Protein production was calculated from the weight of milk produced and percentage protein found. This introduces a slight error in that the protein content was calculated on w/v basis, whereas the production calculation assumed a w/w basis. The extent of the error introduced by this approach is relatively small since the maximum and minimum densities observed at 20°C were 1.032 and 1.026 and these give a spread of $\pm$0.015 g in the weight of milk assumed to be represented by the 5 mls. taken (an error of $\pm$ 0.3% in the weight assumed).

No attempt was made to "age correct" the production data.

(B) Calculation and evaluation of prediction equations

1. Calculating the sampling period

All cows in milk were sampled at each sampling date. The first sample for each cow (representing a stage of lactation between 1 and 13 days) was not used in the statistical analysis as it was not possible to entirely eliminate the variability due to "colostrum effects" in this sample. The 16 sampling times used in this study were consecutive and followed immediately after the rejected 1st sample. These sampling times, therefore, refer to the following stages of lactation.

Sampling time	Stage of Lactation
1	14 - 27 days
2	28 - 41 "
3	42 - 55 "
4	56 - 69 "
5	70 - 83 "
6	84 - 97 "
7	98 -111 "
8	112- 125 "
9	126 -139 "
10	140 -153 "
11	154 -167 "
12	168 -181 "
13	182 -195 "
14	196 -209 "
15	210 -223 "
16	224 -238 "

Only cows giving 16 consecutive tests were used in the computation. A lactation length of at least 224 days was thus the minimum used in this investigation. The New Zealand Dairy Production and Marketing Board (1965) considers any lactation over 200 days to be satisfactory when evaluating sire survey data. For the years involved in this study, the average length of lactations based on Herd Improvement data, as reported in the Annual Reports of the Board, were:-

1957/58	258 days
1958/59	260 days

The lactations used in this investigation were, therefore, normal in character.

2. Calculating correlation coefficients

The correlations between the individual sets of fortnightly data and total production, and the inter-correlations between the individual sets of fortnightly data, were calculated using an I.B.M.650 Computer. These correlations were determined on each of the four sets of data, namely Jersey 1957/58, Jersey 1958/59, Friesian 1957/58, Friesian 1958/59.

3. Selecting combinations of periods for regression analysis - manual calculation

Using the determined correlation information, the ten out of sixteen individual sampling times most highly correlated with the total production were ranked in descending order of their correlation coefficient.

Intercorrelations between these ranked sampling times were then examined, and various combinations of the sampling times most highly correlated with the total production and with minimal inter-correlation were selected for calculating the appropriate parameters of the multiple regression equations for predicting total production.

4. Regression analysis by computer

Subsequently a modified Tape Regression Analysis Programme (TRAP) was used on the I.B.M. 650 for the regression analysis, and as only a limited capacity was available, sampling times were selected and examined and their contribution to the regression equation was tested by determining the Student "t" value. The TRAP programme was modified to reject the lowest value in terms of "t" until all values of "t" were in excess of 2 which was just over the 5% significance level.

All odd sampling times were first examined. All even sampling times were then examined and finally the odd and even sampling times retained on the "t" basis were examined as a group. The procedure was repeated for each of the four sets of data.

Finally the odd and even sampling times retained on the "t" basis for Jersey year 1 data were used to examine Jersey year 2 data and vice versa, and likewise this was done within the Friesian groups. Because this procedure did not give a clear indication that any specific sampling times were consistently capable of giving a satisfactory basis on which

prediction could be based, the TRAP programme was modified to take every combination of 4 sampling times out of 9 sampling times selected on the basis of their relatively high correlation with total production and their spread over the lactation period, namely sampling times 4,6,7,10,11,12,14,15, 16. This programme was run using the Jersey data for both years together and the Friesian data for both years together.

5. Basis of evaluation of groups of sampling times used in regression analysis.

Each multiple regression equation was evaluated in terms of the standard error of the estimated value. Each group of sampling times was ranked in terms of this standard error for each set or combination of sets. The ranked sequences were then examined for those groups having the lowest standard errors over all sets or combinations of sets. A set was one year's data for one breed, thus there were four sets of data available.

6. Statistics and methods used in manual calculations

(a) Multiple Regression Analysis and standard error of estimate (Croxton and Cowden, p.546 et sq).

(b) Standard error of estimate expressed as percentage of the mean production of protein per cow for the set or sets of data being used. Whenever standard error of estimate is mentioned in the text, it is this statistic that is referred to. This statistic is also known as the Coefficient of Variation.

(c) Spearman's Rank Correlation Coefficient (Croxton and Cowden, p.478).

(d) Limits for sums of squares of rank differences (Bennett, p.284).

(e) Average standard error of estimate:

Average standard error of estimate =

$$\sqrt{\frac{SE^2(a) \times df(a) + SE^2(b) \times df(b) \text{ etc.}}{df(a) + df(b) \text{ etc.}}}$$

where $SE^2(a)$ = square of the standard error of the estimate for set (a).

df(a) = degrees of freedom in set (a).

7. Sampling time V Sampling period

In this investigation the effect of sampling time in relation to sampling period was of no significance since no account was taken of the total fortnightly production, only the production at the specified sampling times being used in the correlation studies and regression analyses. In the discussion on ranking and predicted production, the total production values used for the regression analyses or predicted by regression have been multiplied by a factor of 14 so that they approximate actual production values.

RESULTS AND DISCUSSION

(A) (a) Basis of selection of sampling times for use in predicting protein production.

The criteria used to select sampling time groups for examining their suitability for predicting protein production were:-

(1) High correlation with total production.

(2) Low intercorrelation among the individuals of the group of sampling times.

(3) Reasonable spread in time between sampling times to reduce temporary interactions of weather, feed, husbandry conditions, etc.,and to enable a reasonable sampling and testing organisation to be planned, which is easier with a spread of sampling times.

(b) Basis of evaluation of sampling times selected for use in predicting protein production.

It was considered that a group of three sampling times would be the most satisfactory grouping. In this way the number of samplings needed would be kept to a minimum and yet three sampling times would reduce the effects of inaccurate sampling and analysis, and uncontrollable local variations of weather, feed or husbandry on the data used for prediction.

It was considered that a prediction equation based on a regression analysis using data from the sampling times selected would be the most suitable form of prediction to use.

The practical limitation in the use of prediction equations is the accuracy of their prediction and this is indicated by the standard error of estimate of the equation.

This statistic is thus in the form of a standard deviation but, since it is expressed in every case in the form of a percentage, it can be used directly for the comparative assessment of the efficiency of a regression equation for prediction, irrespective of the magnitude of the data on which the regression equation was based.

Data from various groups of sampling times, selected on the basis of the correlation study, were examined to establish the standard error of the estimate. The smallest standard error of the estimate was used as the best measure of the efficiency of a group in predicting protein production.

It was also considered that where two groups of sampling times gave comparable standard errors of the estimate, then the group in which the individual coefficients of regression were as uniform as possible should be used so that undue weighting of results from any single sampling time would not occur.

(B) (a) Correlation of protein production at particular sampling times with the total production of protein for each set of data.

The correlation coefficients derived from the computer matrix are presented as a histogram in Fig.1. The correlation coefficients for each set at each sampling time are shown in sequence as continuous lines. The interrupted line in each group represents the mean correlation coefficient using all data for each sampling time. The mean value was derived as the arithmetic mean.

The mean values and their standard deviation for all sampling times having a mean correlation coefficient greater than 0.75 were as follows:-

Sampling time	Correlation with total production	
	Mean value of Coefficient	Standard Deviation of Coefficient
12	0.86	± 0.06
11	0.85	± 0.13
10	0.81	± 0.10
15	0.79	± 0.08
7	0.78	± 0.04
16	0.77	± 0.06
6	0.77	± 0.05

The above table and Fig.1 clearly indicate that protein production in only a limited number of sampling times is consistently highly correlated with the total protein production. Thus, sampling times 6, 7, 10, 11, 12, 15 and 16 appear to be those that

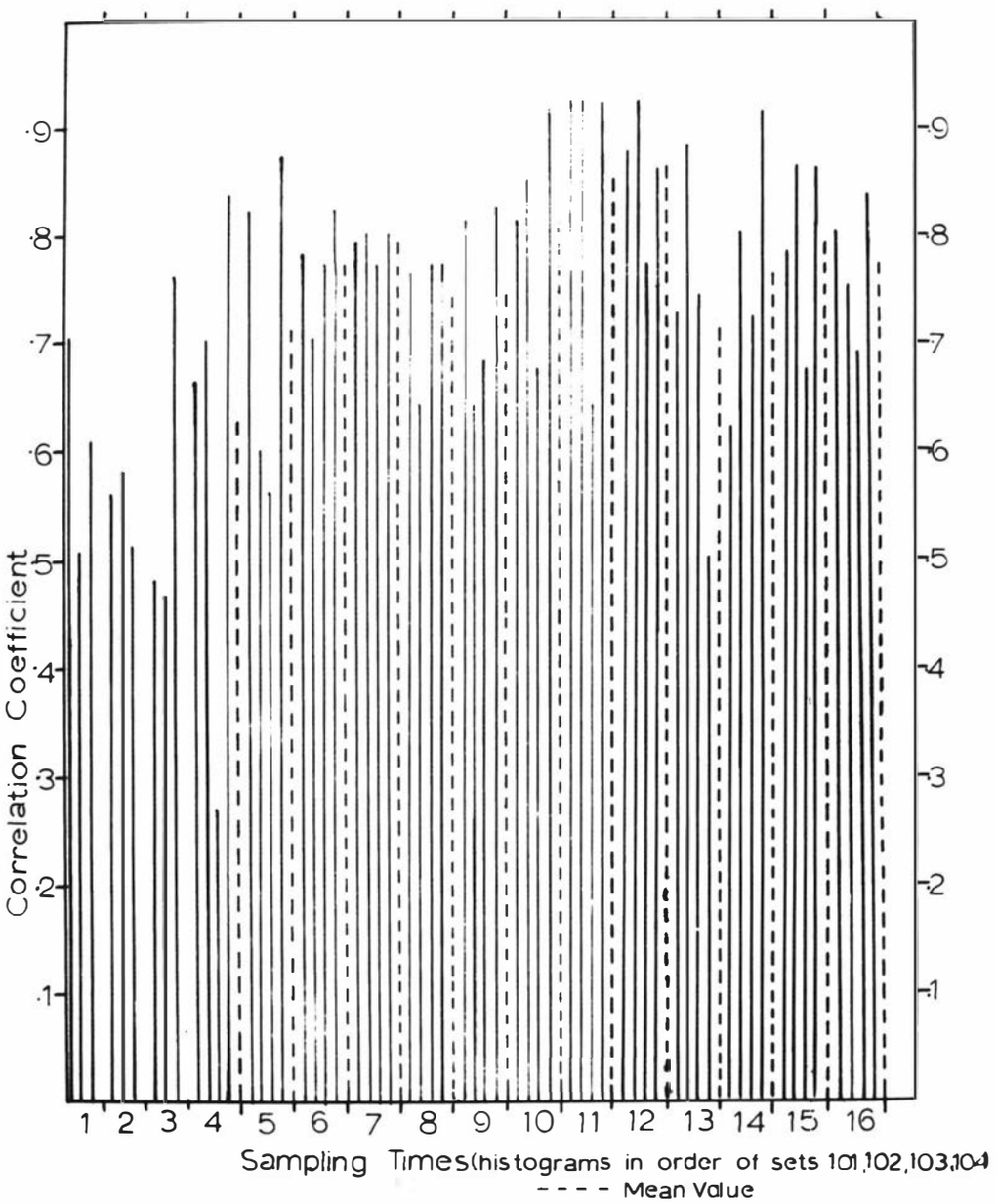

Fig 1 Correlation with total production

warrant a more critical examination to determine their value as sampling times for prediction of protein production.

(b) Intercorrelation of Protein production values between particular sampling times.

These are shown in Fig.2 (a) where the intercorrelation of protein production in sampling time 6, with that in sampling times 7, 10, 11, 12, 15 and 16 and similarly for sampling time 7 are plotted. In Fig.2 (b), the plots for intercorrelation coefficients for sampling times 10,11,12,15 and 16 are presented. In each group the intercorrelation for each set in the individual sampling time is shown as a continuous line, whereas the interrupted line represents the arithmetic mean value of intercorrelations for all data for the particular sampling time.

The table below is the intercorrelation matrix using mean values for each of the selected sampling times. Values in brackets are the standard deviations about the mean values.

	7	10	11	12	15	16
6	0.68 (±0.14)	0.71 (±0.09)	0.65 (±0.14)	0.65 (±0.07)	0.57 (±0.13)	0.51 (±0.15)
7	--	0.73 (±0.07)	0.70 (±0.08)	0.68 (±0.09)	0.59 (±0.13)	0.57 (±0.04)
10	--	--	0.79 (±0.18)	0.72 (±0.12)	0.61 (±0.15)	0.61 (±0.13)
11	--	--	--	0.82 (±0.06)	0.78 (±0.05)	0.72 (±0.12)
12	--	--	--	--	0.77 (±0.09)	0.70 (±0.10)
15	--	--	--	--	--	0.78 (±0.09)

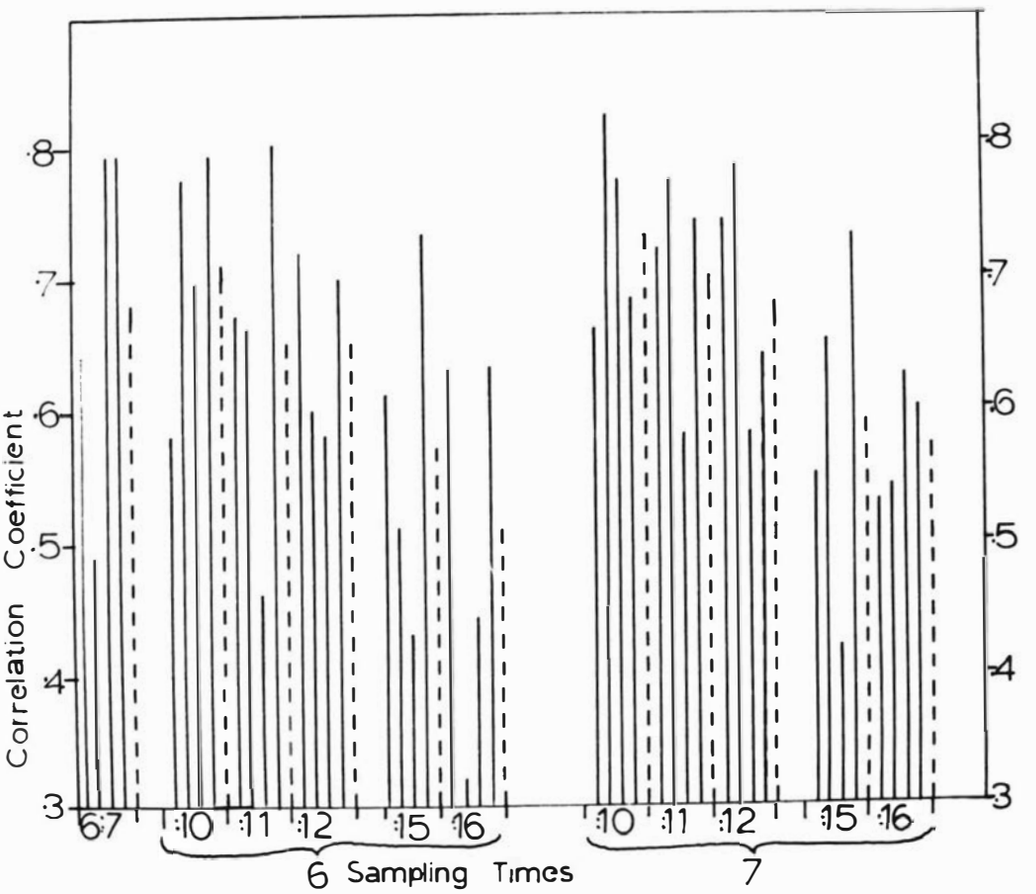

Fig.2a Intercorrelation of sampling times

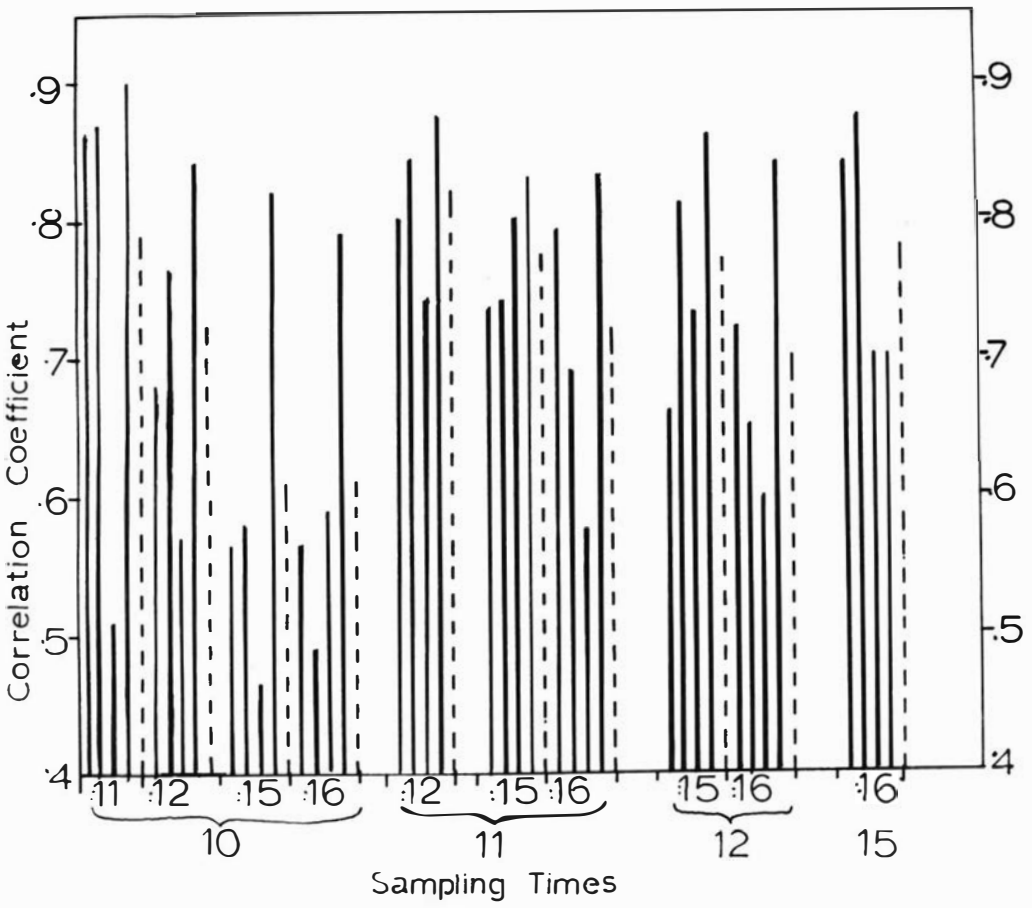

Fig 2b Intercorrelation of sampling times

The intercorrelations between the sampling times selected because of the high correlation of their protein production with total production vary and are, at times, fairly high. Despite this, sampling times 6 and 7, although not themselves highly intercorrelated, have intercorrelation coefficients with the other sampling times that do not differ greatly and that follow the same general trend in value. Sampling times 6 and 7 may, therefore, be found to be interchangeable as sampling times.

Sampling times 10, 11 and 12 are all fairly highly intercorrelated and sampling times 11 and 12 follow one another very closely in their intercorrelation with sampling times 15 and 16, and so sampling times 11 and 12 should likewise be interchangeable. On the basis of the intercorrelation coefficients, sampling time 10 does not fit as neatly into the scheme with sampling times 11 and 12.

Sampling time 10 produced intercorrelation coefficients of considerably lower value for the 1957/58 Friesian data (set 103) than with the other data, as can be seen in Fig. 3, and this may explain its non-conformity with the pattern for sampling times 11 and 12.

Sampling times 15 and 16 are reasonably highly intercorrelated. The intercorrelation between these and the other sampling times selected, with

the exception of sampling time 10, show a characteristic common trend.

From the correlation data, it has been possible to select several sampling times, namely 6,7,10,11,12, 15 and 16 which:

(1) are fairly highly correlated with the total when the values for protein production at the individual sampling times and in total are examined.

(2) can be grouped into three groups of contiguous times, namely 6 and 7; 10,11 and 12; 15 and 16 having fairly common trends for intercorrelation values (with the exception of 10) and with intercorrelations with members of the other groups not unreasonably high.

(3) when grouped are reasonably spread throughout the lactation.

It has thus been possible to meet the original criterion defining the suitability of selected sampling times from this preliminary survey of the correlation data.

(C) Reported values for correlations between production at individual sampling times and total production.

Madden et al have published a correlation matrix for milk production of Holstein-Friesian cows

giving the correlations with total production and intercorrelations between the production estimated using monthly test days. Their month of test, say M, can be related to the sampling times (S) reported in this study by the equation S = 2M - 1. They further calculated their correlation data for two groups within the herd (a) under 3 years of age, and (b) 3 years of age and over. Some of their values were:-

Ranking in terms of highest correlation between milk production at individual times with total milk production.

	Under 3 year old		3 year old and over	
Rank	Sampling time	$\bar{r}$(TOT)	Sampling time	$\bar{r}$(TOT)
1	9	0.93	9	0.91
2	11	.93	11	.90
3	7	.92	7	.89
4	13	.91	13	.88
5	15	.90	5	.86
6	5	.89	15	.84
7	3	.86	3	.80
8	17	.85	17	.78
9	1	.75	19	.68
10	19	.74	1	.67

Intercorrelations of some of the more highly ranked sampling times.

Sampling time	Sampling time	$\bar{r}$ (INT) Under 3 yr.	$\bar{r}$ (INT) 3 yrs.& over	Rank for lowest inter-correlation.	
7	9	.89	.87	7	7
	11	.85	.79	4	4
	13	.80	.73	2	2
	15	.75	.65	1	1
9	11	.89	.87	7	7
	13	.85	.80	4	5
	15	.81	.73	3	2
11	13	.89	.87	7	7
	15	.85	.80	4	5
13	15	.89	.88	7	8

These correlations indicate the same trend observed with the data used in this study, namely that sampling times 7,11 and 15 give estimates that correlate highly with total production; 9 and 13 also had high correlation coefficients. The intercorrelations between sampling times were considerably higher than those reported here for protein. Although cow age affected the magnitude of the correlation coefficient, it had no significant effect on the rank order of production at individual sampling times, either with the total production or between sampling times. The age of the cows used in this study was not considered as a variable factor but it seems unlikely, in view of Madden's data, that this would have affected the findings significantly.

(D) Regression analyses and determination of the standard error of the estimate based on manual computation.

(a) Evaluation of results using combined data for all the Jersey cows and combined data for all the Friesian cows.

The group of three sampling times having the highest arithmetic mean correlation coefficient with total production ($\bar{r}$ TOT) was selected for the first trial. The mean intercorrelation coefficients ($\bar{r}$ TOT) were also determined and the various groupings of sampling times were then ranked in descending order of mean total correlation and mean intercorrelation. The regression equations for this series of groups were not all calculated since it was argued that when the mean total correlation of a group was the same or only 0.01 less than the next group above it, but the mean intercorrelation was higher than the group above, then the standard error of the estimate would be greater than the previous group.

When the groups of sampling times were assembled in descending order, of mean correlation with total and mean intercorrelations, no obvious order or pattern was observable in the standard error of the estimate (see Appendices D (a) and (b). The data were, therefore, rearranged as in Tables D (a) and (b) (pgs. 88 and 89). where the groups are assembled in increasing order of their standard errors of the estimate. These tables indicate that high mean intercorrelations frequently counteract any expected advantage associated with groups of sampling times having high mean correlations with total production. This was particularly apparent

when sampling times 10:11:12 and 7:11:12 in Table D (a) were compared with 7:11:15. There were anomolies in the effect of the intercorrelation on the standard error of the estimate. Thus in Table D (a) sampling times 10:12:16 had a greater standard error of the estimate than did groups 10:11:16, 11:15:16 and 7:12:15, despite the appreciably lower mean intercorrelation for 10:12:16. This lack of consistency is also apparent in Table D (a) in a slightly different form when the standard errors of estimate for 7:11:12:15 and 10:11:15 are compared. In this case, both groups have the same mean correlation with total and the same mean intercorrelation. The inclusion of an extra sampling time has no doubt influenced the result so that the group of four sampling times has an appreciably lower standard error of the estimate than the group of three. The inclusion of a fourth sampling time does not, however, always result in this appreciable improvement in the standard error of the estimate. This is apparent in Table D (b) with sampling times 6:10:12:14 and 10:12:14 and with 6:7:10:12 and 6:7:12, and in Table D (a) with 7:11:12:16 and 11:12:16.

The use of the mean correlation coefficients and mean intercorrelation coefficients to select the most likely groupings of sampling times to give a low standard error of the estimate was justified, but the method cannot be depended on to critically delineate the best possible grouping of sampling times.

In an endeavour to improve the ability to select those groupings of sampling times likely to have the

smallest standard error of the estimate, the regression equation relating changes in the average intercorrelation coefficient with changes in the average correlation coefficient and with total production was determined and is given in Appendix D (e). It was found that an increase in the value of $\bar{r}$ (TOT) by 0.01 will generally result in a lower standard error of the estimate provided $\bar{r}$ (INT) has not also increased by about 0.04.

It is of interest to note that an individual sampling time, e.g. 11 in Table D (a), that is highly correlated with the total production can give a fairly low standard error of estimate, but even here the mean correlation coefficient was not an infallible guide to the suitability of the sampling time for prediction as was shown by sampling times 15 and 7 in Table D (b). The inclusion of a fourth sampling time in a group did not consistently improve the group's ability to predict with precision, and so it was decided that groups of three sampling times would probably meet the needs of the study best.

When all the Jersey data was combined and examined Table D (a), the group of sampling times 7:11:15 gave the best prediction. When all the Friesian data was combined and examined Table D (b), sampling time 14 occurred frequently in the groups of sampling times having the least standard errors of estimate. Unfortunately, whenever sampling time 14 occurred in a group its regression coefficient was strongly negative; it was, therefore, rejected from further consideration. The group of sampling times

6:12:15 gave the best prediction for the Friesian cows but the standard error of the estimate was considerably greater, 5.5% as against 3.5% for 7:11:15, the best grouping for the Jersey cows, but it was not greatly different from the 5.1% for 6:12:15 for the Jersey cows. On the other hand, the group 7:11:15 for the Friesian cows had a standard error of the estimate of 8.4%. In the group 6:12:15, using all the Friesian data, sampling time 6 had a negative regression coefficient and so it too was discarded.

This comparison is extended in the following table which also includes the standard errors of the estimate when all data was used in determining the regression equation for several groups of sampling times.

Sampling times	Standard error of estimate			
	Sets 101 & 102 %	Sets 103 & 104 %	Av. %	All-data %
7:11:12:15	3.6	7.4	6.1	--
6:11:16	4.4	7.4	6.4	--
7:12:15	4.9	7.4	6.5	7.0
6:12:16	5.1	7.2	6.5	--
7:12:16	5.3	8.0	6.5	--
6:11:15	4.5	7.3	6.8	--
7:11:15	3.4	8.4	6.9	--
11:12:15	4.2	8.8	7.4	8.0
12	6.6	10.1	8.9	9.6
11	5.5	11.1	9.3	10.0
15	8.0	10.8	9.8	10.2
10	7.9	11.2	10.0	10.7
7	8.4	11.3	10.4	10.7

This table has been assembled in increasing

order of the average of the standard errors of estimate for sets 101 and 102 combined and sets 103 and 104 combined. The group of sampling times 7:11:12:15 has the lowest average standard error and then groups 6:11:16, 7:12:15, 6:12:16 and 7:12:16 have very similar average values.

In Appendix D (c) the regression coefficients, standard errors of estimate and related data are listed for all combinations of three sampling times from the groupings 6 or 7; 10,11 or 12; 15 or 16 for each set of data.

The following table lists these various groups of sampling times in the order of their increasing average standard error of the estimate. No combination of sampling times gave an obvious and consistent pattern of high prediction.

Individual S.E. of Estimate

Sampling time	Set 101 %	Set 102 %	Set 103 %	Set 104 %	Av.S.E. of Estimate %
6:12:16	5.3	4.2	4.0	7.7	5.779
6:10:16	4.8	5.3	7.0	6.2	6.012
7:10:15	4.9	4.2	7.2	6.7	6.123
6:11:16	4.3	5.0	6.6	7.2	6.130
6:11:15	4.2	4.3	7.1	7.3	6.216
6:10:15	5.0	4.2	7.1	7.2	6.297
7:12:16	4.8	4.9	6.6	7.5	6.316
6:12:15	5.2	4.6	6.5	7.5	6.319
7:12:15	4.9	4.8	6.5	7.6	6.331
7:10:16	4.4	5.5	8.1	6.2	6.342
7:11:15	3.5	4.3	7.4	8.1	6.537
7:11:16	4.1	5.0	9.1	6.7	6.735
Mean & S.D.	6.62 ± 0.52	4.67±0.67	6.58±1.24	6.73 ± 0.81	

The above table suggests that various combinations of sampling times 6 or 7; 10,11 or 12; 15 or 16 will give a very uniform estimate of production irrespective of breed or year of sampling. Only the combinations 6:12:16 and 7:11:16 lie outside the limits of the mean of the Average Standard Error of the Estimate ± 5% of the S.E. which contains all other values. Calculated data thus substantiates the observations made on the basis of the correlation data that combinations of sampling times 6 and 7; 10,11 and 12; 15 and 16 should give reasonable estimates of production and this section shows that the estimates are reasonably uniform.

In Appendix D (d), the results are given of a limited examination of regression data and standard error of estimate for selected groups of sampling times using data aggregated by years rather than by breeds. Once again the introduction of the Friesian data increases the standard error of the best estimates derived to about 7%. It was considered that this method of grouping data did not warrant further investigation.

Finally in this series, test regression analyses were made using as data the differences between the mean and actual production values at each selected sampling time in any one set of data. Using this approach it was hoped that there would be an improvement in the predicted relationship between the production at an individual sampling time and total production, for by taking only the differences from the mean value, common factors, such as weather and

feed conditions should be eliminated from consideration and only individual cow variability should be affecting the results. The improved relationship was not apparent, e.g. with the group 7:11:15 and set 101 data, the coefficient of determination was 0.93 using actual values, but only 0.71 when the differences from the mean value were used. With set 102 data, the coefficients of determination were 0.92 and 0.79 respectively. This approach was not proceeded with.

TABLE 8 (a)

Standard error of the estimate for groups of sampling times selected because of their high mean correlation coefficients. Data used were the combined data for the Jersey Cows. (Sets 101 and 102 combined).

Sampling times	Mean correlation with total production	Mean intercorrelation between sampling times	S.E. of Estimate
	$\bar{r}$ (TOT)	$\bar{r}$ (INT)	%
7:11:15	0.85	0.69	3.4
7:11:12:15	.86	.73	3.6
11:12:16	.87	.75	4.1
7:11:12:16	.85	.71	4.1
11:12:15	.88	.77	4.2
4:7 :11:16	.80	.59	4.2
7:11:16	.83	.67	4.3
10:12:15	.85	.68	4.4
6:11:16	.81	.63	4.4
6:11:15	.83	.66	4.5
10:11:12	.88	.80	4.6
7:11:12	.87	.77	4.6
10:11:15	.86	.73	4.7
10:11:16	.84	.71	4.7
11:15:16	.84	.71	4.7
7:12:15	.84	.70	4.9
7:10:15	.82	.64	4.9
10:12:16	.84	.65	5.1
12:15:16	.83	.69	5.1
6:12:15	.81	.65	5.1
6:12:16	.81	.61	5.1
7:12:16	.83	.66	5.3
11	.92	-	5.5
7:10:16	.80	.60	5.6
7:15:16	.80	.59	5.9
12	.90	-	6.6
10	.83	-	7.9
15	.82	-	8.0
7	.80	-	8.4
16	.79	-	8.9

TABLE D (b)

Standard error of the estimate for groups of sampling times selected because of their high mean correlation coefficients. Data used were the combined data for the Friesian Cows. (Sets 103 and 104 combined).

Sampling times	Mean correlation with total production	Mean intercorrelation between sampling times	S.E. of Estimate
	$\bar{r}$ (TOT)	$\bar{r}$ (INT)	%
7:10:12:14	0.80	0.66	3.9
6:10:12:14	.81	.65	4.3
10:12:14	.81	.72	4.4
6:12:15	.80	.67	5.5
7:12:14	.81	.61	6.3
6:12:14	.81	.59	7.1
6:12:16	.80	.63	7.2
6:7:10:12	.80	.70	7.3
7:12:16	.79	.65	7.3
6: 7:12	.80	.68	7.4
7:11:12:15	.79	.71	7.4
7:12:15	.79	.66	7.4
6:11:16	.78	.62	7.4
7:11:16	.78	.66	7.9
6:11:15	.78	.67	8.0
11:12:14	.81	.80	8.3
7:11:15	.78	.69	8.4
11:12:15	.79	.81	8.8
12	.82	-	10.1
15	.77	-	10.8
6	.80	-	11.1
11	.79	-	11.1
16	.77	-	11.1
10	.79	-	11.2
7	.79	-	11.3
14	.81	-	16.5

(D) (b) Comparison of partial regression coefficients for a common group of sampling times.

If the groupings 7, 11 or 12 and 15 were selected as those which could logically be used to give the best prediction of total production, then all that is necessary is to decide on the appropriate partial regression coefficients and a value for the constant (a). Examination of the Table D (b) (1) indicates the problems involved in this decision. Thus the regression coefficients derived for sampling time 7 range from 2 to 9, for sampling time 11 from -2 to plus 11, and so on. It appears that the only possible answer to this is to use the regression coefficients derived by using all the data or at least combined data for a single breed, and to accept the increase in the standard error of the estimate inherent in this procedure.

TABLE 9 (b)

(1) Comparison of partial regression coefficients for a common group of sampling times

Sampling times and data used in regression analysis	a	Partial regression coefficients byl.m	byn.ln	kyn.ln	S.E. of Estimate
	-				%
7:11:15 Set 101	3.45	3.940	3.890	10.580	3.5
102	3.25	2.203	8.640	6.610	4.3
103	5.54	9.390	-1.650	9.240	7.4
104	0.42	3.550	1.400	12.570	8.1
101/102 combined	3.70	5.995	2.300	8.987	3.4
101/103 combined	nd	8.164	1.440	6.923	7.0
103/104 combined	4.65	5.979	5.656	6.039	8.4
102/104 combined	nd	3.232	11.751	4.904	6.8
All-data	4.33	5.526	5.929	5.902	6.9
7:11:12 Set 101	5.68	4.373	5.735	5.735	4.9
102	3.81	2.670	8.576	5.921	4.8
103	3.15	7.157	7.308	4.089	6.9
104	2.97	4.632	9.072	5.571	7.6
101/102 combined	nd	6.122	3.693	6.463	4.9
101/103 combined	nd	6.701	5.872	4.897	6.5
103/104 combined	nd	5.583	9.041	4.512	7.4
102/104 combined	nd	4.200	9.060	5.739	7.2
All-data	3.73	5.201	7.787	5.287	7.0
11:12:15 Set 101/102 combined	4.92	7.594	4.752	4.003	4.2
103/104 combined	nd	3.554	9.585	5.906	8.8
All-data	4.14	4.531	8.017	5.803	8.0

(E) Regression analyses and determinations of the standard error of the estimate using TRAP on I.B.M.650 Computer.

Since the selection of sampling times on the basis of the high correlation of their production data with the total production data and of minimum intercorrelations was not entirely successful in selecting the best combination of sampling times to use for prediction, the opportunity of a limited availability of time on an I.B.M.650 Computer was used to try a modified Tape Regression Analysis Programme on the data. A much less selective approach on data used thus became possible, although methods of limiting the extent of this "head on" approach were seriously considered. This was the reason for the "t" test rejection system.

(a) Selecting sampling times to be examined and use of "t" test for rejection.

Because of limited capacity in computer storage it was possibly only to examine a limited group of sampling times in any one analysis. The contribution of sampling times to the prediction of production was examined by testing each sampling time against a "t" value after each regression analysis and only those having a "t" test greater than 2 were retained and used in the next regression analysis. In this way the number of sampling times contributing significantly to a regression equation was ultimately derived and it is only this final group, in each case, that is given in the tables in Appendix (E (a).

The sampling times selected were identified by a group number. Group 1 represented the sampling

times considered likely to be significant on the basis of the correlation data and was extended to include sampling time 4 to give a wider coverage of the lactation period. Group 2 was all the odd sampling times and Group 3 was all the even sampling times. Group 4 included all those sampling times found significant in the analyses of Groups 2 and 3, and these were examined set by set. In Group 5, those sampling times retained as contributing significantly to the regression equation for set 101 in Group 4 were used for set 102 and vice versa, and similarly those retained in Group 4 for set 103 were used for set 104 and vice versa. Other groups were examined but did not add further information.

The frequency with which individual sampling times were retained on the basis of the "t" test when the data from all groups were plotted is shown in Fig. 3. Sampling time 9 was never selected and only sampling times 4,7,11,12,15 and 16 were selected for every set of data but not necessarily in every grouping.

This would appear to suggest that independent of breed, protein production when measured at sampling times 4,7,11,12,15 and 16 could be expected to give the best indication of total production.

The results summarised in Table E (a),illustrate the problem that arises with this form of analysis and rejection. Examination of the sampling times selected indicates their wide diversity and, as a consequence, it is impossible to select a common core of sampling times which contributed significantly to the prediction

of production using all four sets of data. Even in Group 1, in which the original group of sampling times contained those considered to be significantly related to total production, the common core, except for sampling time 4, was not apparent, although there was a greater degree of uniformity. It is interesting to note that the sampling times selected in Group 4 for sets 101 and 102 gave standard errors of estimate identical with those selected in Group 1, despite the fact that for individual sets the majority of the sampling times selected were different. These sampling times, however, differed by only one up or down and this suggests that sampling times adjacent to that selected may, in general, be used to give a reasonably accurate prediction. There is, however, always a limiting factor, if a low standard error of estimate is to be retained then a new set of coefficients of regression, often of appreciably different value, are required whenever such a change in sampling times is made.

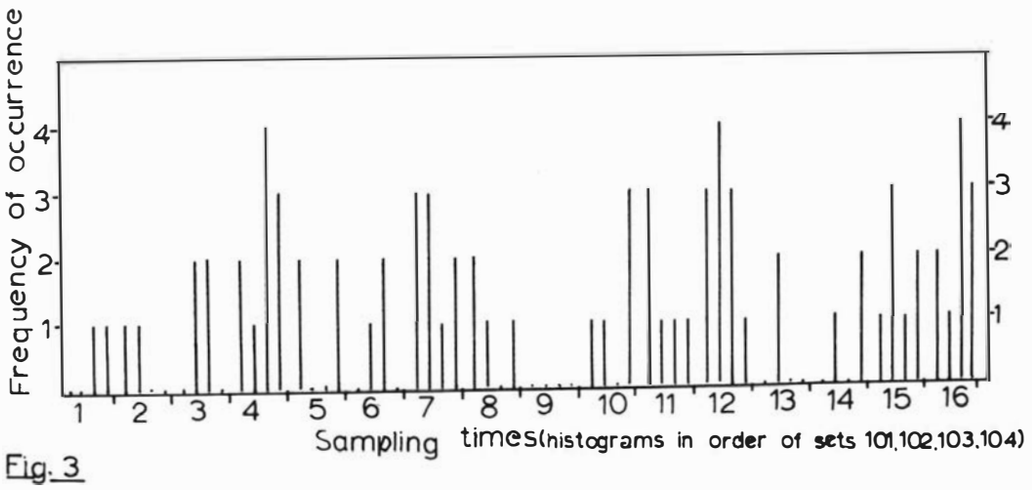

Fig. 3

Frequency of occurrence of significant sampling times in TRAP procedure.

TABLE E (a)

Sampling times and their standard errors of estimate, retained as contributing significantly to the prediction of production when pre-selected data was processed by TRAP procedure

Group	Set No.	Sampling times retained	S.E.of Estimate
1	101	4:7:11:16	3.8
	102	4:6:7:12:14	2.9
	103	4:6:12:16	5.6
	104	4:7:10:14:16	5.4
	101/102	4:6:7:11:14:16	4.3
	103/104	4:6:7:12:14:16	5.9
2	101	5:7:11:15	4.7
	102	3:7:11:13:15	3.3
	103	3:7:15	5.3
	104	1:5:11:15	6.2
3	101	4:8:10:12:16	4.7
	102	2:4:12:16	3.4
	103	4:6:12:16	5.6
	104	4:8:10:14:16	5.6
4	101	5:8:11:12	3.8
	102	3:7:12:13:15	2.9
	103	3:4:12:16	4.7
	104	5:10:16	5.8
5	101	2:7:11:12	4.8
	102	8:10:12:15	4.0
	103	1:4:11:16	6.0
	104	4:7:12:15	6.4

(E) (b) An examination of the use of the "t" test for discarding variables in regression analyses.

The basis on which the "t" test rejects variables in the TRAP procedure was examined to try to establish a basis for pre-selection of data so that the time on the computer could be reduced. It was considered that the major factor contributing to a rejection would be a high intercorrelation between the variables, but it was not possible to determine unequivocally just why the rejected variable gave a low "t" value. This aspect is further discussed in Appendix E (b) (1).

The relationship between the "t" values of variables and the standard error of the estimate using these variables was also examined and is discussed, in detail, in Appendix E (b) (2). In general, the substitution of sampling times with low "t" values for those with higher "t" values resulted in increasing standard errors of estimate, but interaction of the sampling times did at times upset this trend.

Despite the lack of strict compliance with these generally observed trends between the variables (sampling times) and their associated "t" values, the use of the value "t" for selecting variables that can contribute significantly to the computer derived regression equation has been justified and this study has indicated that probably the most economical way of determining which group of sampling times will give the lowest standard error of

estimate is through a process of elimination based on the "t" test of the variables used in each regression equation. The programme should be further modified to reject on the basis of the lowest "t" value and to test not against the "t" value, but against a pre-determined number (say, 3 or 4) of residual variables (i.e. selected sampling times). This would give a more uniform collection of data to further investigate.

A disadvantage of this approach is that it is very much more difficult to select sampling times common to several groups of original data and having minimal standard errors for all data. Such information is not generated in the "t" selection programme.

(E) (c) Examination of all permutations of four sampling times from a pre-selected group of sampling times.

Since the selection of sampling times by the "t" basis produced groups of sampling times that had little in common between the various sets of data, it was impossible to establish a comparative evaluation of the predicting potential of different groups of sampling times. All combinations of four sampling times out of the group 4,6,7,10,11,12,14,15,16 were, therefore, examined using the TRAP procedure. Combinations of four sampling times were used instead of three to partially compensate for the absence of a value corresponding to "a" derived in the manual method.

From the data, so derived, it was possible to list the various groups of sampling times in increasing order of their standard error of estimate (Tables E (c) (1) and (c) (2) on p.104 and 105.)

Examination of these tables shows that all the sampling times used in the permutations are to be found in the first 26 groups of sampling times when ranked in order of their increasing standard error of estimate. The frequency of their occurrence is shown in Fig.4, in which the total frequency of occurrence of the various sampling periods is plotted with the Jersey data frequency indicated by the bar on the histogram.

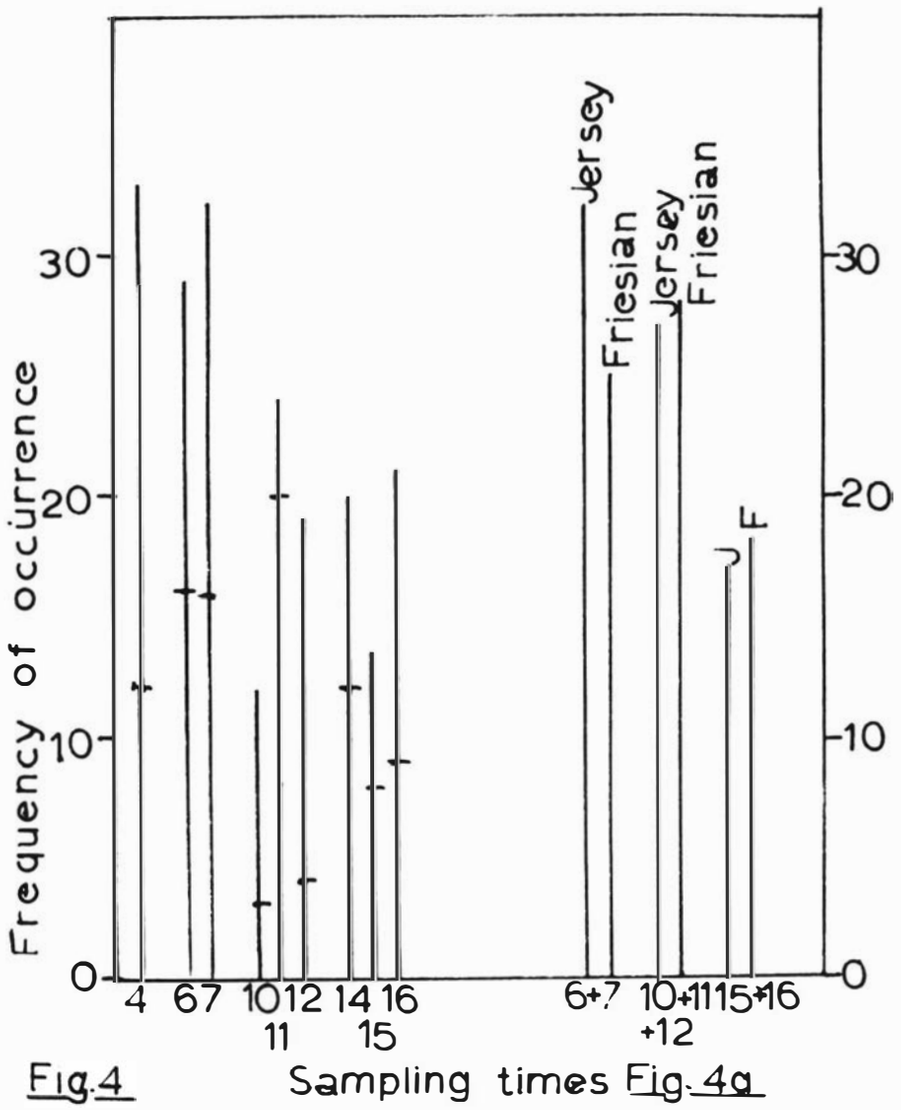

Fig.4 Fig.4a

Total frequency of occurrence of significant sampling times in TRAP procedure.

The frequency of occurrence of sampling times 4 and 6 may be biased since not all possible permutations of the group of sampling times selected were examined with the combined data for the Friesian cows. Only those groups containing sampling time 4 and sampling time 6 with the other sampling times could be examined because of the lack of further time on the computer. Bearing the above limitations in mind, protein production in sampling times 7, 11 and 16 can obviously be used to effectively predict total protein production. When the frequency of occurrence for the Jersey cows and the Friesian cows is examined separately, it is seen that sampling time 7 occurs with equal frequency in both. With sampling time 11, however, it occurred with much greater frequency in the Jersey data and this was balanced by contributions from both sampling times 10 and 12, with the Friesian data. This is illustrated in Fig. 4 (a) where the frequency of occurrence for sampling times 6 and 7; 10, 11 and 12; 15 and 16 are bulked separately for the Jersey data and the Friesian data. When these frequencies are bulked in this way a very uniform frequency of occurrence is observed between the Jersey data and the Friesian data.

In view of this uniformity it was necessary to decide whether further work should be concentrated on these groups of sampling which were also those selected on the basis of the high correlation of protein production at individual sampling times with total production.

Sampling time 14 has already been discussed and discarded as being unsuitable because of its consistent negative regression coefficients when included in groups of three sampling times examined by the manual procedure.

The table below shows that the correlations of production in sampling time 4 with total production (4:17) were low and the inclusion of data from sampling time 4 in the groups selected was primarily because of its low intercorrelation with other sampling times, particularly in set 103.

	Correlation $\bar{r}$				
Sampling time relationship	Set 101	Set 102	Set 103	Set 104	Mean
4:17 (total)	0.66	0.70	0.27	0.84	0.62
4: 6	0.61	0.63	0.29	0.80	0.58
: 7	0.60	0.39	0.11	0.67	0.44
:11	0.56	0.69	0.01	0.76	0.51
:12	0.59	0.58	0.10	0.73	0.50
:15	0.51	0.48	0.01	0.63	0.41
:16	0.40	0.36	0.21	0.63	0.40

Sampling time 4 was rejected on the basis of this low and very variable correlation with the total production.

Sampling time 10 was examined in a similar way since it was the sampling time having the lowest overall frequency of occurrence and its inclusion may not have been warranted.

	Correlation $\bar{r}$				
Sampling time relationship	Set 101	Set 102	Set 103	Set 104	Mean
10:17 (total)	0.81	0.85	0.64	0.91	0.80
6:10	0.58	0.77	0.69	0.79	0.71
7:10	0.66	0.82	0.77	0.68	0.73
10:11	0.86	0.87	0.51	0.90	0.79
:12	0.68	0.77	0.56	0.85	0.72
:15	0.57	0.58	0.46	0.82	0.61
:16	0.57	0.49	0.59	0.79	0.61

The correlation of production in sampling time 10 with total production was high and comparable with that of the other periods selected, e.g. table below.

	Correlation $\bar{r}$				
Sampling time relationship	Set 101	Set 102	Set 103	Set 104	Mean
6:17	0.77	0.69	0.77	0.82	0.76
7:17	0.79	0.80	0.77	0.80	0.79
11:17	0.92	0.92	0.65	0.92	0.85
12:17	0.87	0.92	0.78	0.86	0.86
15:17	0.78	0.86	0.67	0.86	0.79
16:17	0.80	0.75	0.69	0.84	0.77
Mean	0.82	0.82	0.72	0.85	-

Sampling time 10 could, therefore, be included and furthermore it can conveniently be associated with sampling times 11 and 12.

TABLE E (c)

(1) Standard errors of estimate of permutations of four sampling times listed in order of increasing error. Data used was the combined data for Jersey cows. (Sets 101 and 102 combined)

All combinations of four out of sampling times 4,6,7,10,11,12,14,15,16 (i.e. Group 1) were examined using "t" values greater than two for retention.

Number of analyses made 156. Only the first 26 groups have been listed in rank order in terms of increasing standard error of the estimate.

Ranking Order	Sampling times	S.E. of Estimate %
1	6:7:11:14	4.647
2	4:7:11:14	4.743
3	6:7:11:15	4.791
4=	6:7:11:16 4:7:11:15	4.887
6	4:7:11:16	4.940
7=	4:6:7:16 4:6:11:14	5.078
9=	4:7:10:16 6:11:12:14 6:11:14:15	5.222
12=	6:11:14:16 7:11:14:15 4:6: 7:11 4:6: 11:16 4:6: 11:15	5.270
17=	6:10:11:14 6: -:11:14 4: 7:12:16	5.318
20=	6: 7:10:16 4: 7:10:15 4: 6: 7:15	5.366
23=	7:11:12:14 7:11:- :14 7:11:14:16 6:11:12:15	5.416

TABLE E (c)

(2) Standard errors of estimate of permutations of four sampling times listed in order of increasing error. Data used was the combined data for Friesian comb. (Sets 103 and 104 combined)

Combinations of four out of sampling times 4,6,7,10,11,12,14,15,16 (i.e. Group 1) were examined using "t" values greater than two for retention. Number of regression analyses 100. (Only these combinations containing sampling time 4 and then 6 with all other sampling times were examined because of limitations on computer time). This limitation may have biased the frequency with which sampling times 4 and 6 appear in the list. Only the first 26 groups have been listed in rank order.

Ranking Order	Sampling times	S.E. of Estimate
1	4:7:12:14	6.353
2	4:7:12:16	6.388
3	4:7:11:16	6.635
4	4:7:12:15	6.670
5=	4:6:12:14 4:6:11:16	6.705
7	4:6:12:16	6.776
8	4:7:14:16	6.811
9	4:7:10:12	6.847
10=	6:7:12:16 4:6:14:16 4:6: 7:16	6.917
13=	6:7:12:14 4:7:10:16	6.952
15=	4:7:11:12 4:6:7:12	6.997
17=	6:7 :12:15) 4:10:12:14)	7.058
19=	4:7:10:14 4:6:12:15	7.164
21=	6:10:12:14) 4:10:11:16) 4: 6:10:15)	7.23
24=	6:10:12:16) 4: 7:15:16) 4: 7:10:15)	7.305

(E) (d) Comparison of the standard errors of estimate for the manually calculated and computer calculated regression equations based on data from the same groups of sampling times.

The groups of sampling times listed in Tables E (d) (1) and (2) were the only ones that were common to both the manually and computer generated regression data.

None of the other groups of three sampling times listed in Table D (a) and (b) for manually calculated regression equations were calculated in the computer programme, since one of the sampling times was rejected before the final analysis on the basis that its contribution to the regression analysis was not significant. It should be noted that the data used in the manual calculation were the corrected sums of squares and the regression equation so derived included a value for the constant "a", whereas the computer calculated value was based on the uncorrected sums of squares and the regression equation was "forced through the zero" (i.e. "a" was arbitarily taken as zero).

Despite this difference in method of computation, it is interesting to note with the Jersey data that of those sampling times that were analysed by both processes, sampling times 7:11:15 gave the lowest S.E. of estimate by both procedures. The inclusion of sampling time 12 in the group did not reduce the standard error and the substitution of sampling time 16 for 15 only marginally increased the error.

Although the rankings of the Jersey sampling times were not well correlated (Spearman's rank correlation coefficient 0.1), the standard errors of estimate were not widely varied in either the manual or computer series. Both methods of computation thus appear to be equally satisfactory for determining the significance of a group of sampling times to predict protein production.

TABLE E (d)

(1) Jersey 1957/1958 and 1958/1959 (Sets 101 and 102) combined.

	S.E. of Estimate $\frac{p}{p}$		Rank based on S.E.	
Sampling times	Manual	Computer	Manual	Computer
7:11:15	3.4	5.6	1	1
11:12:16	4.1	7.0	2	9
11:12:15	4.2	6.5	3	5
7:11:16	4.3	6.0	4	2
10:12:15	4.4	6.7	5	8
7:11:12	4.6	6.4	6	4
10:11:15	4.7	6.6	7	6=
7:12:15	4.9	6.6	8=	6=
7:10:15	4.9	6.3	8=	3
7:11:12:15	3.6	5.6	1	1
7:11:12:16	4.1	5.9	2	3
4: 7:11:16	4.2	4.9	3	2

(2) Friesian 1957/1958 and 1958/1959 (Sets 103 and 104) combined.

	S.E. of Estimate $\frac{p}{p}$		Rank based on S.E.	
Sampling times	Manual	Computer	Manual	Computer
6:12:14	7.1	7.3	1	1
6: 7:12	7.4	7.5	2=	2
6:11:16	7.4	7.7	2=	3
6:10:12:14	4.3	7.2	1	1
6: 7:10:12	7.3	7.3	2	2

(F) Selection of the partial regression coefficients for use with the protein production data from the sampling times selected.

The problem of the selection of the partial regression coefficients to be used arises because, for each group of sampling times and for each set of data, there is a characteristic set of partial regression coefficients which differ appreciably from set to set or between sampling time groupings.

This difficulty, which was mentioned in Section D (b), is illustrated and discussed in Section F (a). Following this, two different methods for deriving a generally applicable set of partial regression coefficients is considered.

(F) (a) <u>A comparison of partial regression coefficients obtained when data for the same sampling times but from different breeds was used</u>.

The following Table F (a) p.112 is a summary of the regression coefficients derived by the TRAP procedure for six of the groups of sampling times ranked reasonably highly, on the basis of the extent of their standard error of estimate, for both the Jersey data and the Friesian data.

It will be seen that the regression coefficients may vary appreciably:-

(a) for any particular sampling time within a breed classification.

(b) between breeds for any particular combination of sampling times.

These results suggest that it would be desirable to use the appropriate breed controlled regression coefficients for any particular combination of sampling times if maximum accuracy of prediction were to be obtained and further that indiscriminate selection of data from sampling times 6 or 7; or 10,11 or 12; or 15 or 16 is not possible, if only one set of the calculated partial regression coefficients were to be applied to this data to obtain a prediction of total production. The group of sampling times 4,7,10 and 16 was the only group in which the partial regression coefficients were sufficiently similar for both breeds for a common set of coefficients to be used.

The group of sampling times 4,7,10 and 16, however, are not an entirely satisfactory grouping to use.

Sampling time 4 has already been rejected as being unsatisfactory and the use of sampling times 7, 10 and 16 alone is considered too restrictive in that it requires very close control over the sampling programme for each cow in the herd.

It has been shown that sampling times 6 and 7; 10, 11 and 12; 15 and 16 group effectively to produce a uniform pattern of prediction of reasonable accuracy. If it were possible to derive a set of partial regression coefficients applicable to data obtained in any sampling time, within each of the sections in this larger grouping of sampling times, and capable of giving reasonably accurate prediction, flexibility in the sampling programme could be introduced.

TABLE E (a)

	Partial regression coefficients				S.E.of Estimate
Sampling times	4	7	11	16	
Data used for regression analyses					
Set 101/102	3.67	4.73	6.19	5.08	4.9
103/104	4.09	4.47	5.09	7.03	6.6
	4	7	12	16	
Set 101/102	4.38	5.29	3.91	6.48	5.3
103/104	3.31	5.23	6.98	5.36	6.4
	4	6	11	16	
Set 101/102	2.73	3.93	7.86	5.25	5.3
103/104	3.21	4.70	5.34	7.12	6.7
	4	6	7	16	
Set 101/102	3.32	3.67	5.44	7.05	5.1
103/104	3.72	3.77	3.94	9.08	6.9
	4	7	10	16	
Set 101/102	4.20	4.91	3.53	7.34	5.2
103/104	4.10	4.78	4.32	7.57	7.0
	4	7	10	15	
Set 101/102	3.45	4.72	3.60	8.19	5.4
103/104	3.68	4.47	5.91	6.31	7.3

(F) (b) Examination of the effect on the standard error of estimate of combining data from several contiguous sampling times, namely 6 and 7; 10, 11 and 12; 15 and 16, and using this combined data to determine partial regression coefficients for use with data obtained in any combination of the sampling times taken from each of the sections aggregated.

The regression equation derived by this procedure is called the mean-data regression equation and this is compared later with the mean regression equation which has been derived by determining the mean of the regression equations calculated for each possible grouping of sampling times within the sections of the contiguous sampling times selected.

All the partial regression coefficients used in this and subsequent parts of the study have been manually derived using corrected sums of squares in the calculation of the regression equation.

The use of a mean-data regression equation requires samples to be taken in each of the contiguous sampling times and then to be bulked appropriately before their analysis.

To derive the mean-data regression equation the total corrected sums of squares and sums of products based on all the data for each of the sampling times used were found, the means of these totals were then calculated and these mean values were used to derive the partial regression coefficients. The procedure used to derive these mean values is detailed in Appendix F (b) (1).

Mean-Data Partial Regression Coefficients for sampling times grouped 6 and 7; 10,11 and 12; 15 and 16.

	Partial Regression Coefficients				S.E.of Estimate
	a	byl:mn	bym:ln	byn:lm	%
Using all Jersey data (Sets 101/102)	4.80	3.401	6.661	6.223	5.0
Using all Friesian data (Sets 103/104)	3.96	5.659	5.822	6.678	7.8
Using all-data	3.90	5.286	5.834	6.694	7.3

The effect on the standard error of estimate as contiguous groups of sampling times are progressively used for calculating mean-data regression equations is shown in the following table along with the standard errors of estimate of the regression equations based on simple groups of sampling times.

		Standard error of estimate		
	Partial regression coefficients used	Mean-data Sets 101/102	Mean-data Sets 103/104	Mean All-data
Sampling time		%	%	%
6:11:15		4.5	8.0	--
6:11:16		4.4	7.4	--
6:12:15		5.1	5.5	--
6:12:16		5.1	7.2	--
7:11:15		3.4	8.4	7.5
7:11:16		4.3	7.9	--
7:12:15		4.9	7.4	7.0
7:12:16		5.3	7.9	--
7:11 and 12:15		--	--	7.2
7:11 and 12:15 and 16		--	--	7.8
6 and 7; 11 and 12; 15 and 16		4.7	3.4	3.4
6 and 7; 10,11 and 12; 15 and 16		5.0	7.8	7.3

The progressive inclusion of the data from more sampling times did not make a great deal of difference to the standard error of the estimate when the means of all data were used for calculating the regression equation. Nor was there much difference between the standard errors of estimate for sets 101/102 combined and for sets 103/104 combined, irrespective of whether the partial regression coefficients characteristic of any particular group of sampling times or characteristic of the meaned data for the aggregated sampling times for each of the combined sets was used.

The grouping of 6 and 7; 11 and 12; 15 and 16 had a most pronounced effect on the accuracy of prediction when the Friesian data was used for the computation and this effect of the Friesian data was also equally obvious in the low standard error when the means of all-data were used in the computation.

From this examination, it appears that the use of meaned data for deriving a regression equation will not cause any serious diminution in the efficiency of the equation for prediction and, in fact, it may bring about an appreciable improvement in prediction.

The effect on the standard error of estimate of applying partial regression coefficients, derived from data from a specific set, was also examined and a typical table of results is given below.

Using data from sampling times 7:11:15 in the various sets, the effect of substitution of the partial regression coefficients on the standard error of the

estimate is shown. The value underlined is the one characteristic of the particular regression equation being used.

Regression equation used		Set of Estimate			
		Set 101	Set 102	Set 103	Set 104
7:11:15	Set 101	3.5	2.9	7.1	7.5
	102	2.5	4.3	7.5	12.2
	103	5.7	3.6	7.4	7.2
	104	5.2	4.8	8.0	8.1
	101/102	2.2	4.0	7.3	8.3
	101/103	5.2	4.3	7.3	8.8
	103/104	3.2	3.1	7.0	8.9
	102/104	4.7	4.0	5.8	7.8
	All-data	3.2	3.4	7.1	9.2
7:11 and 12:15	Mean-all-data	2.2	2.5	7.4	9.6
7:11 and 12:15 and 16	Mean-all data	4.7	4.6	7.5	9.8
6 and 7:11 and 12: 15 and 16	Mean-all-data	1.1	1.4	5.4	8.5

It is interesting to note that with the exception of set 104, the substitution of the regression coefficients for sampling times 7:11:15 based on all the data resulted in lower standard errors of estimate than was obtained when the regression coefficients based on data specific to each set were used. (The use of the regression coefficients for 7:11 and 12:15; and for 6 and 7; 11 and 12; 15 and 16 gave even lower standard errors of estimate).

Even with the wide range of regression coefficients used, the maximum standard error of estimate did not exceed 6.0% for the Jersey data and 10% for the Friesian data, with the exception of that based on data from 7:11:15, set 102.

It thus seems possible to apply partial regression coefficients, characteristic of selected data, to other data derived at comparable sampling times from the same herd in different years or derived from a different breed in the same or in different years, without seriously upsetting the accuracy of the prediction.

The general applicability of a regression equation based on mean-data was further examined by applying the mean-data partial regression coefficients to data from each of the possible combinations of sampling times used to obtain the mean-data regression equation. Thus with the grouping of sampling times 6 and 7; 10,11 and 12:15 and 16, there are twelve possible combinations of sampling times. The following table shows the maximum standard error of estimate that occured when all the combinations of sampling times were examined set by set.

Mean-data regression equations used	Maximum observed S.E. of Estimate %			
	Set 101	Set 102	Set 103	Set 104
6 and 7;10,11 and 12;15 and 16				
Set 101/102	4.7	6.4	--	--
Set 103/104	--	--	6.7	9.6
All-data	4.2	5.2	7.2	9.6

The use of common partial regression coefficients on data from twelve different groupings of sampling times did not introduce excessive increases in the standard error of the estimate.

Whereas it had been observed earlier that the grouping 6 and 7; 11 and 12; 15 and 16 had much lower standard errors for the regression equations based on the mean Friesian data or the mean all-data, when these equations were used on all combinations of sampling times, the maximum standard errors of estimate generated, with the exception of mean-all-data on set 101, were only slightly less than those generated by the regression equation for 6 and 7; 10,11 and 12; 15 and 16 used in similar conditions. The advantage of this aggregation of sampling times was thus not maintained. The maximum observed standard errors of estimate for the grouping 6 and 7; 11 and 12; 15 and 16 were as follows. Eight combinations were possible.

Mean-data regression equations used	Maximum observed S.E. of Estimate %			
	Set 101	Set 102	Set 103	Set 104
6 and 7;11 and 12; 15 and 16				
Set 101/102	4.1	6.1	-	-
Set 103/104	--	--	6.8	9.2
All-data	1.8	4.2	7.0	9.5

A final assessment of the general applicability of the concept of using common partial regression coefficients was done by using the mean-data partial regression coefficients for predicting protein production using the set by set data for each of the possible combinations of sampling times. The results of this calculation are listed in Appendix F (b) (2) and are depicted in Fig.5 for Jersey data and Fig. 6 for Friesian data. The difference in pounds between protein production predicted, and protein production as measured, is plotted for each set of data and for each combination of sampling times using either the mean-all-data partial regression coefficients for prediction or the partial regression coefficients based on mean-data for the Jersey cows to predict production using the Jersey data (sets 101 and 102) and the partial regression coefficients based on mean-data for the Friesian cows to predict production using the Friesian data (sets 103 and 104). Fig.7 and 8 are plots similar to those in Figs. 5 and 6, except that the percentage difference between predicted protein production and actual production is plotted.

Fig.5 illustrates clearly the very close way in which Jersey data from the two different years follows the same trend in the different combinations of sampling times. This close relationship between years with the Jersey data is also apparent in Fig.7, except for set 101 data where, because no account is taken of whether the percentage difference of the predicted production is over or under actual production values, trends into negative values will be depicted as peaks in the plot.

On the other hand, the two sets of Friesian data

(Fig.6), although exhibiting the same overall trend as the Jersey data, are not nearly as similar in their own variations except in the form of a mean trend.

Reference to Figs. 5 and 6, and examination of the plots for which the mean-data Jersey partial regression coefficients were used for prediction only on Jersey data and the mean-data Friesian partial regression coefficients were used for prediction only on the Friesian data, shows that there is a very close relationship between data derived in year 1 (set 101 and 103) for both breeds. This is not so apparent in year 2 (set 102 and 104). In view of the fairly uniform relationship between related sets of data shown in these figures, and in view of earlier favourable evidence, the use of mean data partial regression coefficients based on data from several groups of sampling times seems to have been justified.

Because of this favourable evaluation of this approach to extending the time during which samples could be taken but bearing in mind the limitation that a composite sample, based on samples taken in each of the sampling times within a group, should be used for analysis, the effect on prediction of production of taking the mean of all the partial regression coefficients for each of the possible combination of sampling times in each set was examined and this is discussed in the following sections.

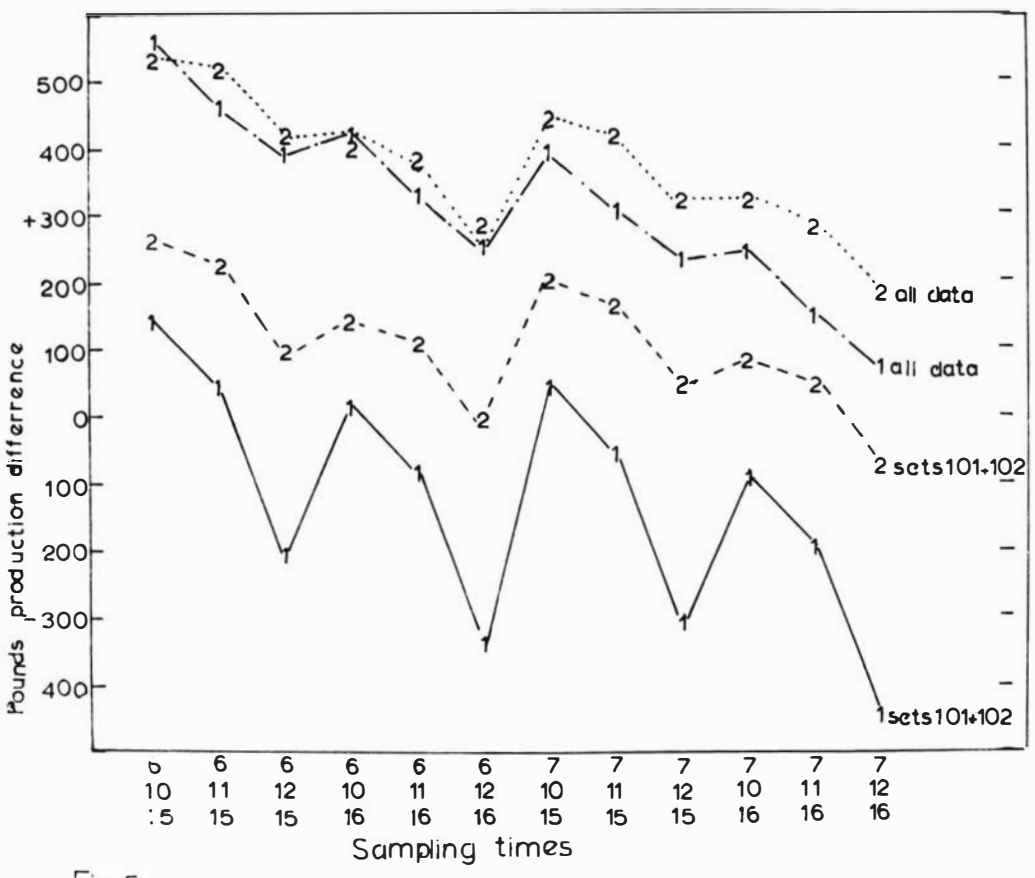

Fig.5

Difference, in pounds of protein, between actual production and production predicted by using mean-data partial regression coefficients.

1 = set 101 (Jersey) 2 = set 102 (Friesian)

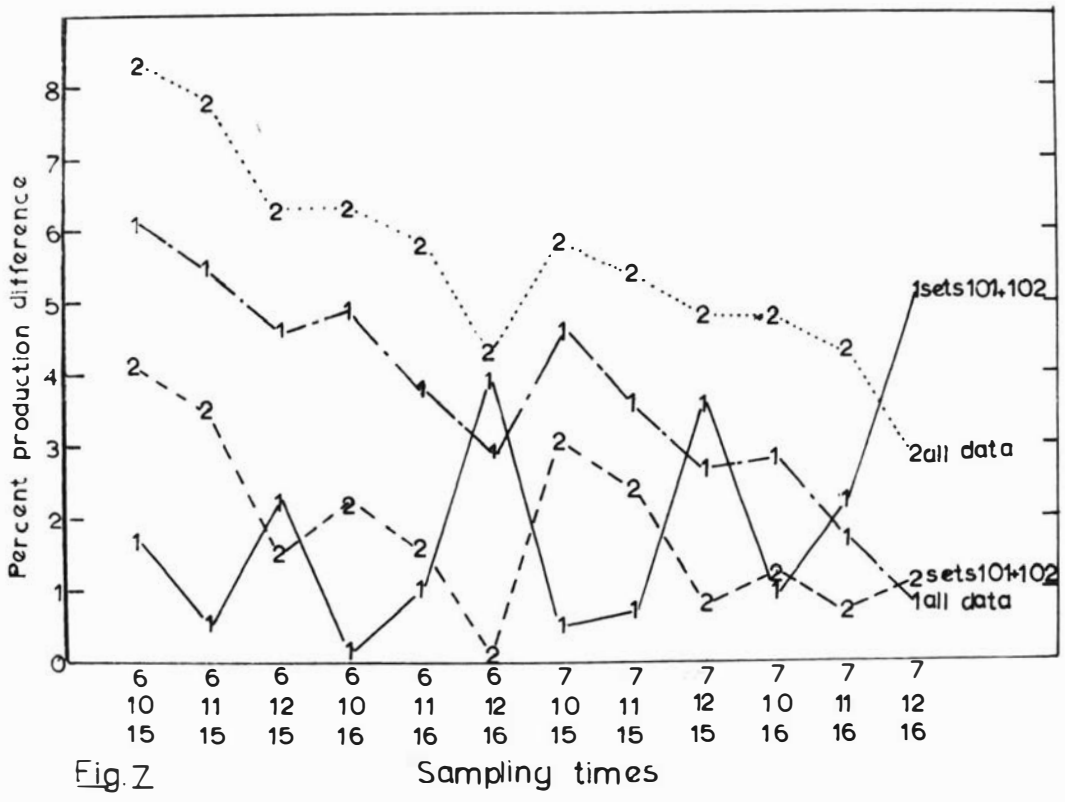

Fig. 7

Difference, in percent protein, between actual production and production predicted by using mean-data partial regression coefficients.

1 = set 101 (Jersey) 2 = set 102 (Friesian)

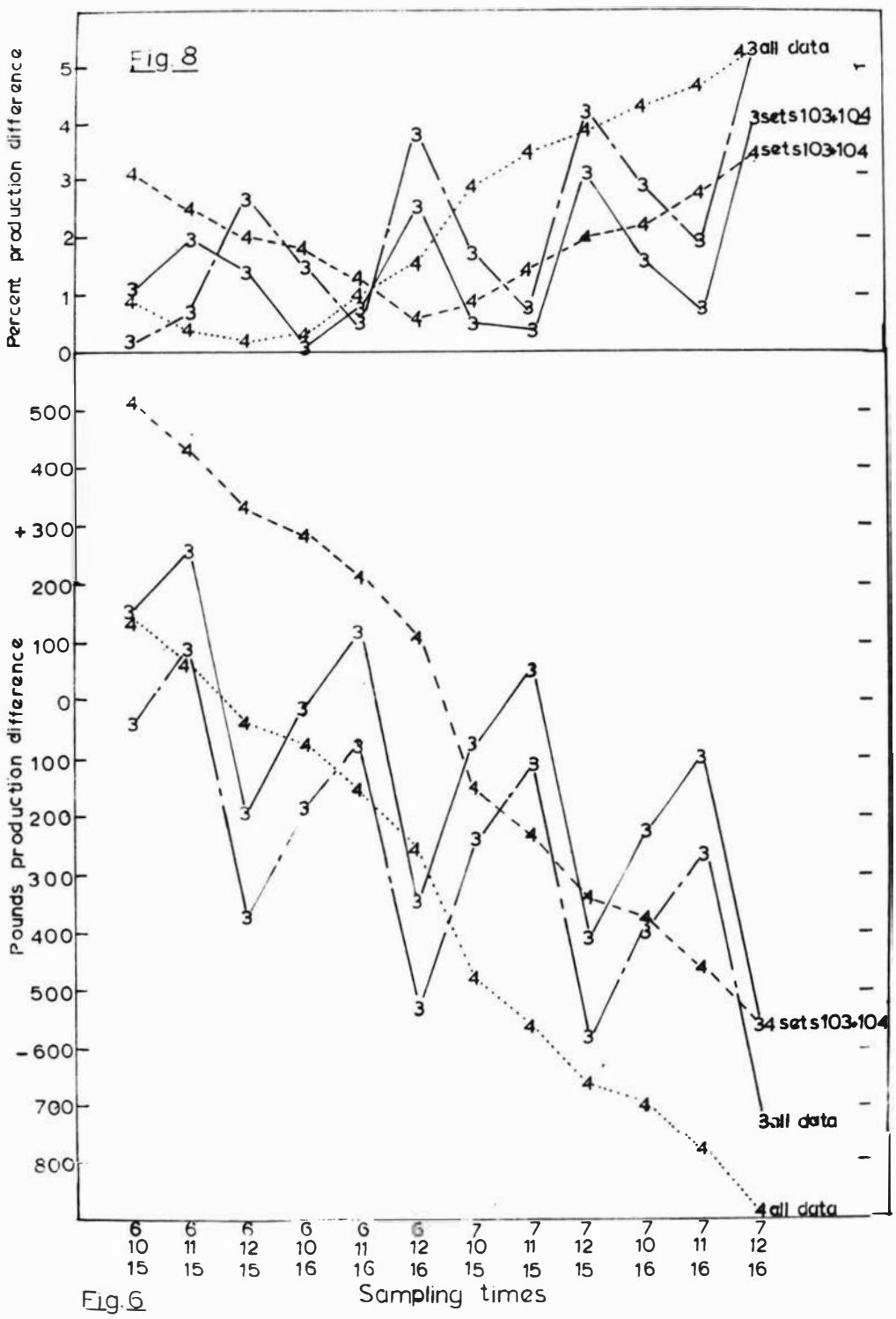

Fig.6

Fig.6 : Difference, in pounds of protein, between actual production and production predicted by using mean-data partial regression coefficients.

Fig.8 : Difference, in percent protein, between actual production and production predicted by using mean-data partial regression coefficients.

3 = set 103 (Friesian) 4 = set 104 (Friesian)

(F) (c) Study of the effect of various combining procedures on the mean values of several sets of regression coefficients and on the prediction of production.

Before the use of mean regression coefficients could be examined, an evaluation of the various ways in which a mean regression coefficient could be derived was undertaken. Three methods of deriving a mean regression equation were examined, namely:-

(1) simple arithmetic mean of corresponding partial regression coefficients.

(2) use of meaned data for corresponding sampling times, i.e. the same regression coefficients as derived in the manual computations for sets 101/102 combined, etc.

(3) arithmetic mean after weighting the individual partial regression coefficients by the number of samples used in their derivation.

The various partial regression coefficients and their mean values derived, as indicated, are shown in the following table.

Data used		Partial regression coefficients			
		a	by 7:11:15	by 11:7:15	by 15:7:11
7:11:15	Set 101	3.453	5.940	3.888	10.580
	102	3.250	2.203	8.638	6.606
	Arithmetic mean 101/102	3.332	3.072	6.263	8.593
	Set 101/102 combined	3.698	2.300	8.987	5.995
	Weighted mean 101/102	3.363	3.172	5.989	8.822
	Set 103	5.537	9.388	-1.651	9.240
	104	6.135	3.550	1.400	12.570
	Arithmetic mean 103/104	5.836	6.469	-0.126	11.905
	Set 103/104 combined	--	5.979	5.456	6.039
	Weighted mean 103/104	5.863	6.204	0.013	11.056
	Arithmetic mean all-data	4.584	4.770	3.006	10.249
	All-data combined	4.327	5.526	5.929	5.902
	Weighted mean all-data	4.855	4.982	2.422	10.155

The effect these different mean partial regression coefficients had on the accuracy of prediction was examined using test data from sampling times 7:11:15, set 101. The results were as follows:-

Partial regression coefficients used		Protein production			
		Actual	Predicted	Difference lbs.	%
7:11:15	Set 101	616.99 lbs.	616.98 lbs.	nil	nil
	102		595.55	-21.44	3.5
	Arithmetic mean 101/102		606.30	-10.69	1.7
	Set 101/102 combined		607.29	- 9.70	1.6
	Weighted mean 101/102		607.50	- 9.44	1.5
	Weighted mean all-data		639.04	+22.05	3.6

A test was also done using test data from sampling times 7:11:15, set 102. The results were as follows:-

Partial regression coefficients used		Protein production			
		Actual	Predicted	Difference lbs.	%
7:11:15	Set 101	468.40 lbs.	478.92 lbs.	+10.52	2.2
	102		468.39	nil	nil
	Arithmetic mean 101/102		473.68	+ 5.28	1.1
	Set 101/102 combined		478.10	+ 9.20	2.1
	Weighted mean 101/102		474.26	+ 5.86	1.3
	Weighted mean all-data		494.82	+26.42	5.6

The results indicate that weighting of the regression coefficients by the number of observations on which they are based prior to taking their means has only a minor effect on the predicted results giving a marginally improved prediction of

0.2 - 0.4% in set 101 and a marginal increase in prediction error of 0.2 - 0.3% in set 102. There thus seemed little to be gained from the use of weighted means. The use of means of the regression coefficients derived for individual sets gave a slightly better prediction than the regression coefficients derived using bulked data (sets 101/102 combined).

A similar trial using data from sampling times 7:11:12 gave similar results.

Simple arithmetic means of the partial regression coefficients were used in subsequent investigations.

(F) (d) Examination of the effect on the standard error of estimate of using mean partial regression coefficients applicable to grouped sampling times.

The regression coefficients and standard error of the estimate given in the following tables were derived as follows:-

(1) The characteristic partial regression coefficients were determined for each combination of three sampling times in the series 6 and 7; 10, 11 and 12; 15 and 16 for each set of data. These results represent the "actual" data and with the standard error of the estimate they are listed in Tables in Appendix D (c).

(2) All the individual regression coefficients on a "within breed" basis determined in (1) were bulked as by_1, by_2 and by_3; where by_1 includes sampling times 6 and 7; by_2 10,11 and 12; and by_3 15 and 16, and the mean value was then determined. Thus within each breed, 24 sets of regression coefficients were averaged to give the "mean regression coefficients." In set 103, sampling times 10 and 11 occasionally gave slightly negative regression coefficients. However, the mean coefficients for 10 and 11 were both positive and so these sampling times were retained in the system. These mean values for the regression equations were then applied to the various combinations of data in the individual sets on a "within breed" basis to obtain the "predicted" standard error of the estimate for sets 101/102 and sets 103/104 respectively.

(This was also done in the determination of the difference in protein production by prediction with each individual set (i.e. 101, 102, etc.).

(3) The mean regression coefficients for each breed were then bulked and the "mean all-data regression coefficients" were established and used to obtain the "all-data predicted" standard error of the estimate and the "all-data" difference in protein production by prediction with the specific set of data.

Mean values for regression coefficients and standard error of the estimate for sampling times 6 or 7; 10,11 or 12; 15 or 16.

Data used	Partial regression coefficients				S.E. of Estimate
	a	by_1	by_2	by_3	
Set 101/102	4.36	3.162	7.297	6.040	4.67
103/104	3.62	5.944	6.767	5.475	7.15
All-data	3.99	4.553	7.032	5.757	6.24

Although these mean regression coefficients and the mean-data regression coefficients listed in section F (b) have been obtained by very different procedures, their values do not differ greatly and their application to data obtained over this relatively broad spectrum of sampling times would not appear to be likely to result in grossly unfair weighting of any specific group of sampling times. The mean regression coefficients have a slightly smaller standard error of estimate than the

mean-data regression coefficients, and since the mean regression coefficients can predict using fewer samples their use has advantages both in practice and in accuracy.

Comparison of regression coefficients
for sampling times 6 or 7; 10,11 or 12; 15 or 16.

Data used	Partial regression coefficients				S.E. of Estimate
	a	by_1	by_2	by_3	$s_{\hat{g}}$
Set 101/102					
Mean regression coefficients	4.36	3.162	7.297	6.040	4.7
Mean-data regression coefficients	4.80	3.401	6.661	6.223	5.0
Set 103/104					
Mean regression coefficients	3.62	5.944	6.767	5.475	7.2
Mean-data regression coefficients	3.96	5.699	5.822	6.678	7.8
All-data					
Mean regression coefficients	3.99	4.553	7.052	5.757	6.2
Mean-data regression coefficients	3.90	5.286	5.834	6.694	7.3

The various standard errors of the estimate based on the use of these mean regression coefficients are tabulated for the various sampling time combinations in Appendix F (d). These analyses indicate that in using "mean regression coefficients" on a "within breed" basis, the maximum standard error of the estimate for the Jersey breed was 6.2% and for the Friesian breed 9.5%. If the "all-data" mean regression coefficients were used, these maximum standard errors of the estimate increased to 10.5% for both breeds. These maximum standard errors of the estimate should be compared with the maximum and minimum values for the "actual" standard

errors of the estimate in the following table, based on the characteristic regression equations for any three of the sampling times used in this section (i.e. 6 and 7; 10,11 and 12; 15 and 16.

Standard errors of the estimate

Regression coefficients used	Mean all-data	Mean 101/102	Mean 103/104	Individual sampling time combinations (ACTUAL) Set 101	Set 102	Set 103	Set 104
Maximum	10.5	6.2	9.5	5.6	5.3	9.1	7.7
Minimum	-	-	-	3.5	4.2	4.0	6.2

The minimum possible standard errors which did not appear in the same group of sampling times from year 1 to year 2 within or between breeds have thus been roughly doubled to obtain a more flexible sampling pattern. As the minimum values were not obtained in a consistent pattern it would not be possible to exploit these low values consistently, and hence the loss of accuracy will not be as great as the results given above may at first indicate.

The use of the mean partial regression coefficients based on all-data would be of considerable convenience, but their use in place of the mean partial regression coefficients based on the within breed data cannot be justified in view of the considerable increase in the maximum standard error of estimate such an action would introduce, and this is particularly so with the mean partial regression coefficients for the Jersey breed (i.e. those based on data from sets 101 and 102).

Tests of significance between the mean standard errors of the estimate calculated by different methods, and the mean "actual" standard error were determined. The mean "actual" standard error of the estimate was based on the standard errors associated with the characteristic regression equation for each selected group of sampling times in each set.

The following partial regression coefficients were used to determine the standard errors of the estimate:-

(1) Mean partial regression coefficients for set 101 and set 102 combined for sampling times 6 and 7; 10,11 and 12; 15 and 16 (i.e. mean of 24 sets of coefficients). (Mean regression coefficients for sets 101 and 102 combined).

(2) As for (1) but for set 103 and set 104 combined. (Mean regression coefficients for sets 103 and 104 combined).

(3) As for (1) but the mean partial regression coefficients based on "all-data" for these sampling times (i.e. mean of 48 sets of coefficients). (Mean regression coefficients for all-data).

(4) Regression coefficients derived after production data for sets 101/102 for sampling times 6 and 7; 10,11 and 12; 15 and 16 had been meaned (i.e. only one regression analysis involved). (Mean-data regression coefficients for sets 101 and 102 combined).

(5) As for (4) but for sets 103/104. (Mean-data regression coefficients for sets 103 and 104 combined).

(6) As for (4) but combining data from all four sets before taking the mean values and thence deriving the regression coefficients. (Mean-data regression coefficients for all-data).

The results are given in Table F (d).

The differences between the "calculated" and "actual" mean standard errors of the estimate are significant when individual sets of data are examined, but this difference disappears when the estimates were based on both sets of data within a breed. This is not surprising but it confirms that the concept of taking the mean value of a whole series of regression equations will not give a biased result. An interesting sidelight to this concept is that basing a regression coefficient on data that has been averaged rather than on the average of many regression coefficients calculated from this data also gives mean standard errors of the estimate which do not differ significantly from the actual means. These conclusions apply only on a "within breed" basis; the use of coefficients based on all the available data gave significantly different results in all but one of the systems tested.

The "actual" means obtained with individual sets of data within breeds (i.e. sets 101 and 102, or sets 103 and 104) were extremely close and did not differ significantly, although the standard deviations varied considerably. This suggests that although there were considerable differences in the data used, the regression coefficients obtained gave overall predictions that did not differ significantly within a breed from year to year and could, therefore, be bulked and averaged with confidence.

The use of mean regression coefficients on a within breed basis will give a mean standard error of the estimate, when all possible sampling combinations are examined that does not differ significantly from the mean "actual" standard error of the estimate for these same sampling combinations. These mean values were 4.6% for the Jersey data and 7.5% for the Friesian data, and their corresponding maximum values were 6.2 and 9.5%.

TABLE F (d)

Mean standard errors of estimate based on the use of various partial regression coefficients

Partial regression coefficients used	Mean standard error of estimate					
	Set 101 (n =12)	Set 102 (n =12)	Set 101/102 (n = 24)	Set 103 (n =12)	Set 104 (n =12)	Set 103/104 (n = 24)
"Actual" (S.D. in brackets)	4.80 ± (-0.64)	4.68 ± (-0.43)	4.63 ± (-0.56)	6.93 ± (-1.19)	7.07 ± (-0.16)	7.07 ± (-0.90)
"t" test on difference between sets 101 and 102 and sets 103 and 104 (i.e. between breeds).		0.51			0.39	
Mean regression coefficients based on sets 101/102 combined or sets 103/104 combined	3.91	5.38	4.61	5.61	8.75	7.50
"t" test on difference from "Actual"	>3	>3	0.886	>3	>3	0.959
Mean regression coefficients based on all-data	8.86	4.02	7.17	6.54	9.90	8.95
"t" test on difference from "Actual"	>3	>3	>3	>3	>3	>3
Mean-data regression coefficients based on sets 101/102 combined, or sets 103/104 combined.	5.32	5.13	4.72	6.08	8.63	7.35
"t" test on difference from "Actual"	2.450	>3	0.18	2.336	>3	1.865
mean regression coefficients based on all-data	3.32	3.82	3.57	6.38	8.98	7.68
"t" test on difference from "Actual"	>3	>3	>3	1.532	>3	>3
Probability limits for "t" values α=0.50	0.697	0.697	0.685	0.697	0.697	0.685
α=0.25	1.215	1.215	1.180	1.215	1.215	1.180
α=0.05	3.497	3.497	2.069	3.497	3.497	2.069

(F) (e) Examination of the effect on prediction of production of applying mean regression coefficients to individual sets.

The effect on prediction of production when mean regression coefficients are applied to data from any of the twenty-four possible combinations of sampling times within the selected groupings showed that the maximum error was 6.3% (range 0.3 - 6.3% mean 1.98% S.D. ±1.67) with the Jersey data (sets 101 and 102), and 3.5% (range 0.1 - 3.5% mean 1.91% S.D. ± 0.93) with the Friesian data (sets 103 and 104) when mean regression coefficients on a within breed basis were used. When the mean regression coefficients based on all-data were used, the errors in prediction of production using the available data increased and ranged as follows:- Jersey (sets 101 and 102); (range 0.5 - 7.0%, mean 3.33% S.D. ± 1.85). Friesian (sets 103 and 104): (range 0.9 - 6.8%, mean 3.80% S.D. ± 1.87).

Thus on a within breed basis the mean variations of the predicted production from actual production are relatively minor, although the standard deviation about the mean is relatively large and consequently occasional combinations are likely to give considerably more divergent results (6-7% have been demonstrated using the data available).

These results have been listed in Appendix F (d) and are plotted in terms of the percentage production in Figs. 10 and 12, and in terms of difference in pounds of protein in Figs.9 and 11. The plots are very similar in their trends to those obtained using partial regression equations based on mean-data and the comments made on Figs. 5 to 8 apply equally well to Figs.9 to 12.

Both methods of establishing a regression equation applicable to data from any of several sampling times within an aggregated group of sampling times have been shown to give predictions of protein production having similar trends and similar accuracies. In view of the simpler sampling system possible when mean partial regression coefficients are used and in view of the improved accuracy when the within breed mean coefficients are used, further work has been limited to the use of the mean partial regression coefficients for sets 101 and 102 combined for use with Jersey data and for sets 103 and 104 combined for use with Friesian data.

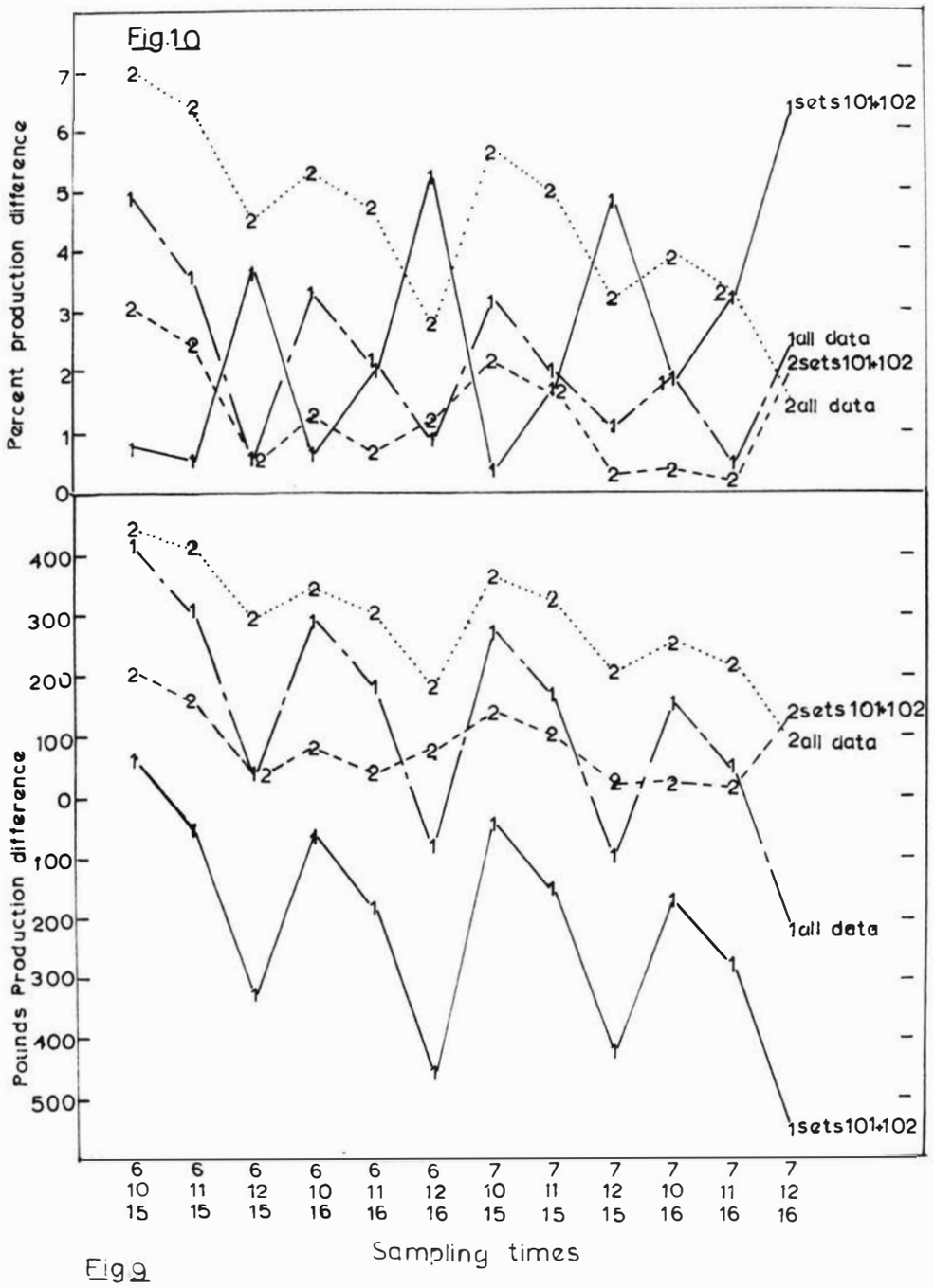

Fig 9

Fig.9 : Difference, in pounds of protein, between actual production and production predicted by using mean partial regression coefficients.

Fig.10 : Difference, in percent protein, between actual production and production predicted by using mean partial regression coefficients.

1 = set 101 (Jersey) 2 = set 102 (Friesian)

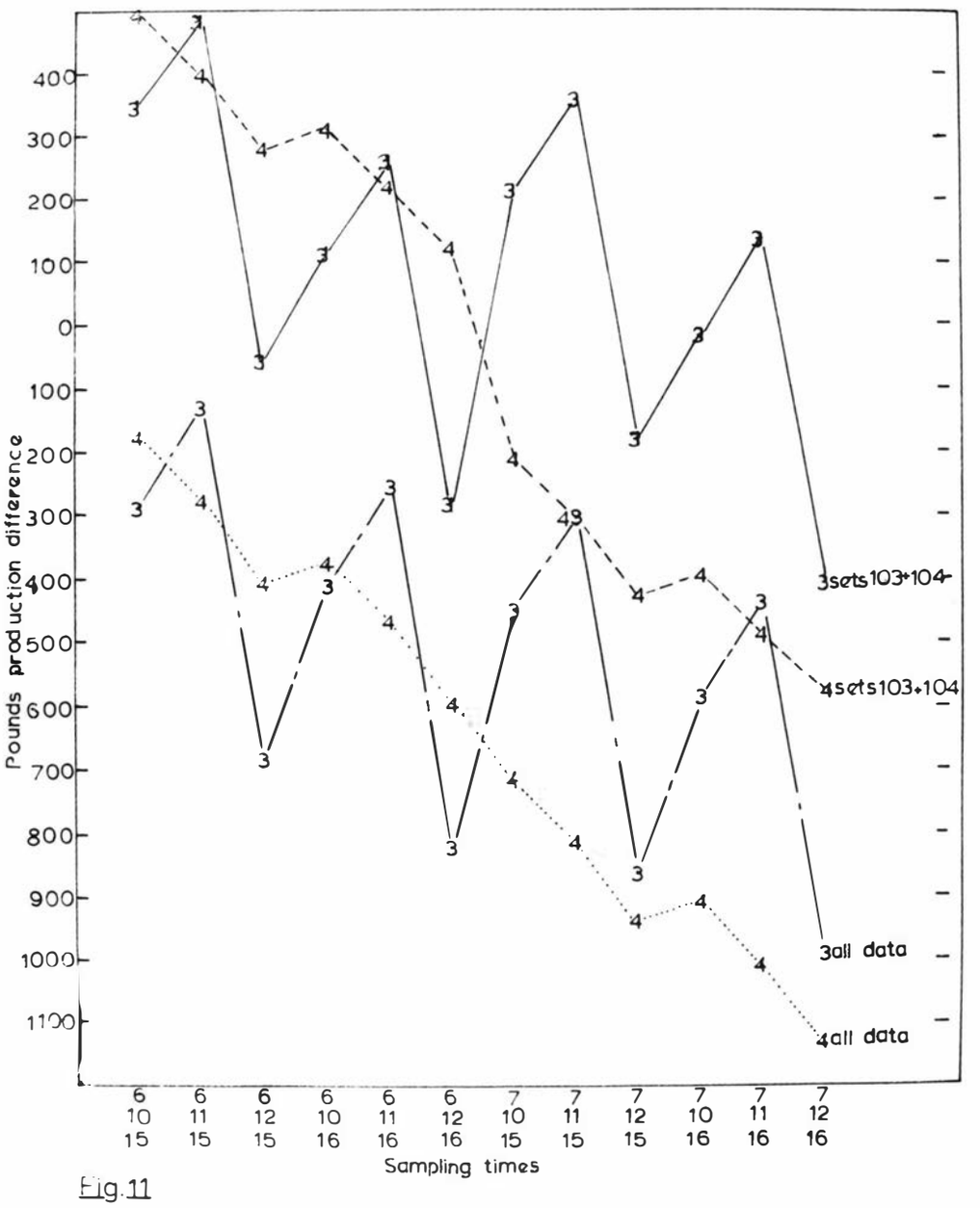

Fig.11

Difference, in pounds of protein, between actual production and production predicted by using mean partial regression coefficients.

3 = set 103 (Friesian) 4 = set 104 (Friesian)

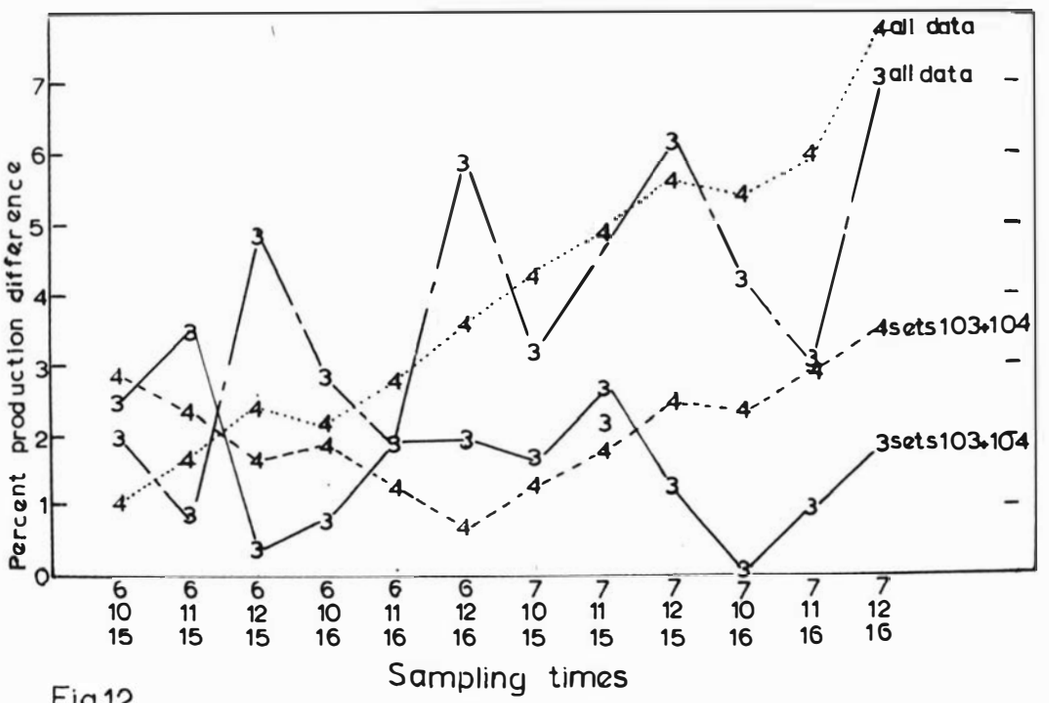

Fig.12

Difference, in percent protein, between actual production predicted by using mean partial regression coefficients.

3 = set 103 (Friesian) 4 = set 104 (Friesian)

(F) (f) Accepted accuracy of prediction of production.

Few authors have committed their thoughts on this to paper but a review of the literature on recording can give some ideas. Thus, Alexander and Yapp observed that variations from their reference method of the following orders were insufficient to reject their modified sampling procedure for fat yield.

	% Cows varying from actual records				Estimated S.D. %
Extent of variation %	0-2.0	2.1-5.0	5.1-10.0	10.1	
Holstein	49	27	18	6	±3.9
Jersey	37	29	26	9	±5.0

Davey and Alexander considered a 10% error not too serious and Czaké et al (1963) considered a range of error of +2 to -5% in estimating the fat yield as being not significant, whereas they did consider an average deviation of around 8% as of significance. Erb and Ashworth's data on repeatability is referred to in Section G (d). They commented that their repeatabilities were higher than generally observed, yet their standard errors of the estimate were around 17 to 18%.

It is interesting to note that Witt and Walter discussing three- or four-weekly testing in relation to breeding programmes observed that the standard deviation of their estimate of butterfat production increased from ± 3.99% for a three-weekly sampling interval to ± 4.64% for a four-weekly sampling interval, but they concluded that despite this increase in the standard deviation the heritability was altered

only slightly and they recommended, on economic grounds, that a change to a four-weekly sampling interval be made. Campbell discussing the level of errors present in systems of herd testing current in 1946, compared with daily sampling and testing, showed that the standard deviation in the estimation of fat yield by the Herd Improvement Association testing procedure was ± 6.51% and by the Official Herd Test procedure was ± 6.35%. (Appendix F (f)).

It thus seems that a standard deviation of around 5% is an acceptable level of accuracy and in New Zealand 6.5% is generally accepted as satisfactory for butterfat yield recording purposes. Herold and Veress quoted the average correlation between the lactational yield of milk protein based on monthly samples and that based on daily samples as +0.82. For comparison with the data presented in this study, their value has been recalculated to $R^2 = 0.67$. Only two sampling time combinations out of the 48 used in this study for determining the mean regression coefficients fell below this level. Both combinations occurred in the same year in the Friesian data. Thus on this basis the three times a lactation sampling was equally as effective as monthly sampling for prediction. As has also been mentioned earlier in the discussion on Methods, Senft stated monthly sampling for protein was as satisfactory as sampling once every ten days. It is worthy of note that Herold and Veress observed a slightly higher average correlation (+0.838) between daily sampling for protein production and sampling twice in consecutive months mid-lactation than they found with monthly sampling.

It is also of interest, at this stage, to review the inherent limitations of the standard methods of sampling and analysis, as these contribute to the errors of prediction as well as being present in the basic data used for comparison purposes. This has been discussed in an earlier paper by Wallace (a). Carré et al. list the sources and extent of physical errors in testing for fat yield as follows:-

(1) Weighing, about 2.5% (may be up to 4% if quantity of milk is small);

(2) Compositing, about 0.5%;

(3) Measuring for Gerber, about 1.0%;

(4) Reading Gerber, about 1.25%.

These errors do not allow for human errors in sampling and technique.

Edwards and Simpson mention that the probable deviation from the true mean when sampling from a weigh tank for S.N.F. is between 1.7 to 2.3% of the S.N.F. estimate. It is likely that the same order of variation will be present when sampling for protein.

The standard deviation of the Kjeldahl method of protein analysis using single samples is about 0.05% protein, i.e. about 1.5% of the component measured.

Thus with these known errors, the error in the measurement of the protein content could possibly reach 10% in extreme cases.

In the light of the above discussion, the standard errors of estimate using a mean regression equation for sampling times 6 and 7; 10,11 and 12; 15 and 16 based on all the Jersey data will predict protein production of individual Jersey cows with a standard error of estimate of 4.7% which is an acceptable accuracy, and likewise total production of the herd to within 2% of actual. The corresponding regression equation for predicting the production of individual Friesian cows has an average standard error of estimate of 7.2% which is in excess of the acceptable 6.5% but probably is still acceptable for many purposes, and it estimates total protein production of the herd within 2% of actual.

The prediction of production of protein by a herd or by individual cows using a restricted frequency of sampling and a range of possible sampling times is, therefore, quite practicable.

(F) (g) Extension to data based on different methods of analysis.

The values for the protein content of the milk used in this study were based on the Kjeldahl method. The use of alternative methods of protein analysis would introduce further errors as indicated below:-

Methods used on milk from individual cows	Standard deviation from Kjeldahl value	
	Protein %	% of protein content
Macro amido black (p.34)	± 0.06	± 2.2
Formol titration (Have and Mulder)	± 0.13	± 4.4
Kofranyi alkaline steam distillation (Have and Mulder)	± 0.06	± 2.06

Thus the macro amido black or the Kofranyi alkaline steam distillation techniques will increase the standard error of the estimate by between 2 and 3 per cent and if one accepts a 10% error in prediction as not unreasonable, these techniques could be used with a restricted frequency of sampling.

(F) (h) Extension of data to use with other herds.

Fritz et al. discussing the environmental influences on regression factors for estimating 305-day production from part lactation data observed that variation due to herd differences was significant only for the first month of production and suggested that herd differences are not an important factor influencing part to whole lactation relationships. They concluded that it may not be necessary or practical to derive extension factors on an intra-herd basis to achieve maximum accuracy in extending on complete records to a 305-day basis. In view of the greater variability in fat yield when compared with protein yield (Politiek), these observations should, therefore, be applicable to this protein prediction study and suggest that the regression equations derived here may have a general application on a within breed basis.

In New Zealand, the Dairy Board (1958, 1962) is using within herd and within season comparisons extensively for sire survey, AB testing and merit cow testing, in an attempt to separate environmental and management influences from genetic effects. Therefore, as the regression equations derived in this study do not appear to be unduly influenced by environmental and management influences as pointed out in the comparison of the mean "actual" standard errors of the estimate, they should be applicable for these same purposes.

(G) Examination of the effect of predicted protein values on ranking of cows.

(a) Preliminary examination of the application of the prediction equations to the ranking of cows for protein production.

In herd improvement it is often sufficient to be able to rank animals in order of their productions so that the animals possessing various levels of production can be adequately selected for the purpose desired, whether it be culling of low producers or breeding from high producers.

A preliminary examination of the effect of using a prediction equation on the ranking of cows in a herd was undertaken.

Using sampling times 4,7,11,16* and their appropriate computer derived partial regression coefficients, the predicted protein production for individual cows was derived; the extent of ranking differences was examined using the Jersey herd data (set 101) and the Friesian herd data (set 103). The individual cows were separately ranked in order of their production as estimated by prediction and as measured on the fortnightly sampling basis, the rank list is given in Appendix G (a). This simple ranking procedure was not entirely an adequate basis of comparison, since it tended to exaggerate small

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* Sampling times 4,7,11,16 were selected since they were the group that together gave the lowest standard errors of estimate for both the Jersey data (4.9%) and the Friesian data (6.6%).

differences in production. Therefore, within this rank, cows were grouped on the basis of an arbitary scale having increments of 5% of mean protein production of the herd. This procedure reduced the number of ranks from 29 to 11 for the Jersey herd and from 33 to 10 for the Friesian herd. With the Jerseys, in no case was there a greater difference than 2 ranks and with only three cows was the ranking different by 2 between the actual and the predicted rank. With the Friesians, one cow only differed by 3 ranks, and six cows differed by 2 ranks. In both cases, all other cows ranked equally or differed by only 1 rank. It thus seems quite possible and adequate to use the predicted data for cow selection purposes.

Using these sampling times and regression coefficients, the predicted protein production for these herds was determined and the difference from that based on the fortnightly analyses were:-

Jersey	Friesian
0.99% less	1.07% less

(G) (b) Use of a common sampling date for all cows within a herd.

One disadvantage of the use of individual sampling times is that the milk from all cows in the herd can not be sampled for examination at any one time since the cows normally calve down over an extended period of time and this will entail repeated visits to the herd to obtain samples within the required periods of the lactation for the individual cows.

To examine the effect of using a common sampling date rather than having to sample at repeated intervals, the Jersey herd data (set 101) was used to predict production using sampling times 4:7:11:16 as the basis, but the date on which the maximum number of cows were first tested (i.e. sampling time 4) was used as the datum and all cows were then tested on the same day irrespective of the relationship this day had to their lactational progression. Ten cows out of the 29 were in this way examined on the correct basis, all other cows being one or more fortnightly periods out of line. The same regression coefficients were used as in the preliminary study. The rank list is given in Appendix G (a).

The effect of this procedure was to increase the standard error of the predicted value.

In ranking	1 cow	was	5 ranks out of place (approx.20% error in prediction).
	3 cows	were	3 ranks out of place (approx. 13% error in prediction).
	8 cows	were	2 ranks out of place (approx. 9% error in prediction).

This may still be adequately accurate for selection purposes.

An interesting sidelight was the effect of this modification on the prediction of herd production in that it gave an error of only 0.74%, even less than that of the previous method. Thus this particular example showed little effect in estimating the herd production of protein but did lead to the expected increase in the standard error of the estimate of production for individual cows and also increased, as would be expected, the differences in ranking. However, depending on the purpose for which the prediction is required, the increase in the standard error of the estimate and in the differences in ranking introduced by this procedure may not be sufficient to warrant the more rigorous sampling approach.

This concept has been anticipated in earlier discussion by using regression equations for groups of contiguous sampling times.

(G) (c) Study of Ranking Procedures

The decision to rank in 5% incremental steps was examined to determine the effect it would have in determining rank differences and on rank correlation.

(1) Comparison of ranking of cows which were common to both sets 101 and 102. Ranking in terms of actual production.

Ranking of cows in sets 101 and 102 (Jersey year 1 and Jersey year 2) was examined using actual production values. All the cows in both sets were not available for comparative ranking. There were eleven cows used in set 101 (1,29,30,38,50,28,16,3,69,72,39) which were not used in set 102, and there were five cows used in set 102 (17,24,63,112,114) which were not used in set 101. The reasons for changes in cows used in the different sets within breed are given in Appendix Methods (a).

The limits for the sums of squares of rank differences were used to evaluate the significance of the rank correlations. The cows identified by their herd test numbers are listed in rank order in Appendix G (c).

Comparison of sums of squares of rank differences and of Spearman's rank correlation coefficient for estimating significance in ranking when absolute ranking and 5% incremental ranking procedures were used based on actual productions for year 1 and year 2 for Jersey data.

		Absolute ranking	5% Incremental ranking
N (number of ranks)		18	10
$\leq \Delta^2$ (sums of squares of rank differences)		286	80
Value of $\leq \Delta^2$ for probability limits indicated	$\alpha = 0.05$		72
	$\alpha = 0.025$		58
	$\alpha = 0.005$	363	
r_s (Spearman's rank correlation coefficient)		0.705	0.516

In terms of absolute ranking and using the sums of squares of rank differences as the criterion of similarity, the two sets of ranked data were very significantly similar. However, the probability of the two rankings being different has shifted from 0.005 or less in the absolute series to greater than 0.05 in the 5% incremental grouping, and so the test for significance using 5% incremental ranks is more rigorous than absolute rankings. Furthermore, besides being easier to apply, the 5% incremental grouping detects variations in rank correlation which would not

be obvious with absolute ranking procedures, since the sums of the squares of absolute rank differences were in almost all cases considerably less than figures given in the probability tables for $\alpha = 0.005$, whereas these differences were detectable because of the reduction in the number of ranks involved when 5% incremental ranks were used.

From a production point of view, a 5% change in production is a minimally significant change on which a selection should be based in view of the vagaries of sampling and testing.

This procedure of ranking in 5% increments was examined further with the following results:-

Comparison of ranking of cows which were common to both sets 103 and 104. Ranking in terms of actual production.

Ranking of cows in sets 103 and 104 (Friesian year 1 and Friesian year 2) was examined using actual production values. All cows in both sets were not available for comparative ranking. There were nine cows (128, 147, 187, 162, 168, 155, 171 159, 136) used in set 103 which were not used in set 104 and there were sixteen cows (132, 133, 134, 135, 144, 146, 149, 152, 154, 157, 163, 165, 170, 172, 185, 190) used in set 104 but not used in set 103. The ranked sequences are given in Appendix G (c) (1).

Comparison of ranking procedures comparing actual production in year 1 and year 2, using Friesian data.

		Absolute ranking	5% Incremental ranking
N		26	13
$\leq \Delta^2$		779	155
Value of $\leq \Delta^2$ for probability limits indicated	α=0.025	-	158
	α=0.01	-	119
	α=0.005	1418	-
r_s		0.734	0.571

Here again the uncritical character of the probability limits for sums of squares of rank differences in evaluating the absolute rankings against the 5% incremental rankings is obvious.

Using the 5% incremental ranking, the actual rank displacement of cows from year 1 to year 2 were:-

	Set 101/102	Set 103/104
0 ranks	5	4
1	5	8
2	4	8
3	2	2
4	1	3
5	1	-
6	-	-
7	-	1

(2) Extension of the comparison study to examining the effect of ranking procedures on the relationship between the actual ranking and the predicted ranking of the same cows in the same year.

The mean-all-data partial regression coefficients for sampling times 6 and 7; 10,11 and 12; 15 and 16 were used to predict individual cow production on the basis of data from sampling times 6:11:15, set 101, and the cows were ranked in order of their protein production. The ranking in absolute and 5% incremental steps were compared using "actual" and "predicted" production values. A further ranking comparison was made,as follows:- Whereas in the 5% incremental ranking procedure the difference in rank was determined as the difference between the incremental group rank number, i.e. only the groups have been assigned rank numbers, in the modified group procedure note was taken of the number of cows occurring in each group and all cows in the group were given a mean rank number which was dependant on the number of cows in the group. This latter procedure did not reduce the number of absolute ranks but introduced a system where several cows could have the same rank number without reducing the total number of ranks as happened in the simple grouping in 5% increments.

Set 102 was examined also, using mean-data regression coefficients for sampling times 6 and 7; 10,11 and 12; 15 and 16 based on sets 101/102 and applied to data from sampling times 6:11:15.

The results from both these studies were as follows:-

Comparison of ranking procedures comparing actual and predicted ranks

		Set 101			Set 102	
		Absolute ranking	Ranking in 5% increments but using mean rank number	Ranking in 5% increments	Absolute ranking	Ranking in 5% increments
N		29	29	10	23	11
$\leq \Delta^2$		344	346	69	138	38
Value of $\leq \Delta^2$ for probability limits indicated	$\alpha = 0.05$	-	-	72	-	-
	$\alpha = 0.025$	-	-	58	-	-
	$\alpha = 0.005$	2083	2083	-	912	40
r_s		0.915	0.915	0.582	0.929	0.805

Both studies illustrate the greater rigour of the 5% incremental group procedure and show how relatively useless is testing on the basis of absolute ranking, except to indicate an extremely high probability that the two ranks are strongly correlated. The use of mean rank numbers in the 5% incremental system had no advantage over the absolute ranking procedure.

Spearman's rank correlation coefficient was introduced subsequently so that comparison could be made with published data.

Using Spearman's rank correlation coefficient, the rigour of the 5% incremental ranking procedure becomes more obvious. The following table summarises the data on the rank correlation coefficient.

	Spearman's rank correlation	
	Absolute ranking	5% Incremental ranking
Set 101 on set 102 Actual data	0.705	0.516
Set 103 on set 104 Actual data	0.734	0.571
Set 101 Predicted on actual	0.915	0.582
Set 102 Predicted on actual	0.929	0.805

In view of the limited data, it is difficult to determine the value of the rank correlation coefficient using 5% incremental ranking which is of equivalent merit to, say, a rank correlation of 0.9 in absolute rank correlation. The mean absolute rank correlation was 0.821 and the mean 5% increment rank correlation was 0.619, and so it would appear that the 5% increment rank correlation would have values about three-quarters of their corresponding absolute rank correlation values.

In all subsequent studies, the 5% incremental ranking procedure has been used because of its greater rigour and because of its greater simplicity of application.

(G) (d) **Examination of the effect on production and ranking, of applying the regression coefficients for those groups of three sampling times selected on the basis of giving the lowest standard error of the estimate when all-data within a breed had been used for the computation.**

(1) **Effect on prediction of production and ranking of applying regression coefficients for 7:11:15 (Set 101/102) on set 101 and 102 data for sampling times 7:11:15.**

Difference in production	Set 101	Set 102
Total actual production	8637.86 lbs.	6557.60 lbs.
Total predicted production	8516.76 lbs.	6578.04 lbs.
Difference from actual	-121.10 lbs.	+20.44 lbs.
Difference % of actual	1.40%	0.31%
Standard error of the estimating equation 3.4%		

Difference in ranking, using 5% increments

		Set 101	Set 102
N		9	12
$\leq \Delta^2$		41	37
Value of $\leq \Delta^2$ for probability limits indicated	$\alpha = 0.05$	48	-
	$\alpha = 0.025$	38	-
	$\alpha = 0.005$	-	63
r_s		0.658	0.871

(2) Effect on prediction of production and ranking of applying regression coefficients for 6:12:16 (Set 103/104) on set 103 and 104 data for sampling times 6:12:16.

Difference in production	Set 103	Set 104
Total actual production	13846.98 lbs.	16697.66 lbs.
Total predicted production	13688.50 lbs.	16872.10 lbs.
Difference from actual	-158.58 lbs.	+174.44 lbs.
Difference % of actual	1.14%	1.04%
Standard error of the estimating equation 7.2%		

Difference in ranking, using 5% increments

		Set 103	Set 104
N		14	19
$\leq \Delta^2$		59	114
Value of $\leq \Delta^2$ for probability limits indicated	$\alpha = 0.005$	125	447
r_s		0.870	0.900

Using these specific regression coefficients, the ability to predict total production and to rank cows within the herd is high, e.g. error in prediction of total production is less than 1.50% and with sets 102, 103 and 104 the sums of squares of rank differences was well inside the limits set for $\alpha = 0.005$, and even with set 101 it was only a little over the $\alpha = 0.025$ value.

Despite this high predictability, the actual rank displacement of cows were:-

	Set 101	Set 102	Set 103	Set 104
0 ranks	9	8	10	9
1	13	11	17	24
2	7	2	6	5
3	-	2	2	2
4	-	-	-	1
5	-	-	-	-
6	-	-	-	1

A rank difference of 1 cannot be considered as pertinent in the examination of the practicability of this predicting procedure for a minimum difference of only 0.28 lbs. of protein could be involved, although the maximum difference which would still give only 1 rank difference was 27.72 lbs. protein in sets 101/102 and 38.92 lbs. protein in sets 103/104; this is because an arbitary fixed scale increasing by 5% increments was used to rank the appropriate data. If one accepts this concept of ignoring 1 rank difference, then the percentages of cows incorrectly ranked were:

Set 101	Set 102	Set 103	Set 104
24.1%	17.4%	22.9%	21.4%

For comparison, the percentage of cows differing in year 2 by two or more ranks from their position in year 1 based on actual production was:-

Set 101/102	Set 103/104
50%	66.7%

Hence, these displacements resulting from errors in the prediction equation in terms of ranking were only between one-half and one-third of those arising from year to year variability using actual data.

At this stage, it is interesting to compare the repeatabilities of the data used here with repeatability figures given by other authors. Using Spearman's rank correlation coefficient as a measure of repeatability, the following values were obtained in this study for protein yield based on actual production values and the absolute ranking procedure.

r_s (101 : 102) 0.705

r_s (103 : 104) 0.734

Castle and Searle (1957) state that with butterfat yield repeatability for individual herds, ignoring the year effect, ranged from 0.05 to 0.76 with a pooled value of 0.49. When the year effect was eliminated, repeatability values ranged from 0.16 to 0.94 with a pooled estimate of 0.61.

Erb and Ashworth, comparing individual cow records from one lactation to the next, found correlations of the following order:-

	r	S.E. of Estimate
Milk	0.82	16.5%
Fat yield	0.80	18.0%
Protein yield	0.80	16.6%

They commented that these correlations (or estimate of repeatability) were higher than generally observed. The extent of their standard error of estimate was, therefore, surprising.

Van Vleck and Henderson (1961) (b)) compared several systems for estimating fat yield and their effect on the correlation between yields in succeeding complete lactations and observed that correlations of 0.52 to 0.57 were obtained using sequential or cumulative bimonthly and trimonthly tests for predicting succeeding lactations. Using a complete lactation record, a correlation of only 0.55 was obtained, and when any single test in the fourth to sixth month of lactation was used there was a correlation of 0.50.

The repeatability, as measured by Spearman's rank correlation coefficient, of the actual data used in the investigation discussed herein is thus relatively high and uniform for both the Jersey data and the Friesian data.

(G) (e) Ranking by prediction using regression equations based on mean-data for sampling times 6 and 7; 10, 11 and 12; 15 and 16, and examining effect on rank displacement on sets 101 and 103.

Using the sets 101/102 regression equation based on mean-data and testing on set 101, up to 25% of the cows were ranked out of order by prediction, although in some combinations of sampling times less than 10% of the cows were incorrectly ranked. The maximum displacement in rank was 5 and this same cow was four places out of rank in three of the other combinations of sampling times tested, and three places out of rank in various combinations. The major discrepancies in ranking in this test herd was thus limited to four cows or 15% of the herd.

Using the all-data coefficients based on meaned data for sampling times 6 and 7; 10,11 and 12; 15 and 16, and again testing on set 101, the discrepancies in ranking were more frequent; eight cows out of the herd being displaced 3 or 4 ranks in some combination of sampling times. Comparing the effect on ranking when using these different regression coefficients, in only two cases were the cows which were displaced in rank the same, thus the problem of incorrect ranking whilst undoubtedly contributed to by consistent individual non-conformity with the general pattern is also contributed to by a non-conformity at a specific sampling time by a specific individual. These results are tabulated in Appendix G (e) (1).

With the set 101 data (Jersey), the average actual protein production per cow was 298 lbs. and the cows displaced in rank by prediction produced as follows:-

Cow No.	lbs.protein produced	No.of times displaced using mean-data regression equations based on	
		Set 101/102	All-data
2	359	-	1+
39	311	-	3+
18	307	-	2+
1	307	-	1+
33	306	-	3+
69	301	5-	3-
48	294	-	5+
21	291	1-	-
43	238	3-	-
30	222	3+	3+

When the mean-all-data regression equation was used for prediction for ranking, the level of production did not indicate any clear trends in rank displacement, either in number of cows or in number of times displaced. With the set 101/102, mean-data regression equation, there was an obvious trend towards displacement of the lower than average producers.

The partial regression coefficients used were:-

	(a)	b 6 or 7	b 10,11 or 12	b 15 or 16
Mean-data Set 101/102	4.80	3.40	6.66	6.22
Mean-all-data	3.90	5.29	5.83	6.69

As a consequence of the difference in the partial regression coefficients in the two regression equations, the emphasis on individual sampling times varied appreciably in the two sets of rankings. With the test on set 103, however, the partial regression coefficients were:-

	(a)	$b_{6 \text{ or } 7}$	$b_{10,11 \text{ or } 12}$	$b_{15 \text{ or } 16}$
Mean-data Set 103/104	3.96	5.66	5.82	6.68
Mean-all-data	3.90	5.29	5.83	6.69

and in this series it was only sampling times 6 and 7 which directly contributed to the differences in ranking, detailed in Appendix G (e) (2).

As a consequence of the similarity in regression coefficients, nine cows were excessively displaced in rank using either set of regression coefficients; three of these cows (150,159,180) were completely out of line with the rest of the herd of 39. The average actual production of protein by this herd was 355 lbs. and it is interesting to observe that most of the cows listed were above average producers of protein.

Cow No.	Lbs. protein produced	No. of times displaced using mean-data regression equations based on	
		Set 101/102	All-data
140	540	-	4-
179	470	2-	4-
159	468	12-	12-
136	468	3-	7-
178	450	-	1-
150	443	10+	8+
180	443	4-	5-
139	421	1-	2-
168	417	1-	3-
147	389	2-	3-
169	384	2+	2+
		2-	2-
148	347	12+	8+
161	346	2+	-

Those combinations of sampling times which appear to give fewest displacement in rank were:

Partial regression coefficients used	Set 101	Set 103
Mean-data Set 101/102	7:10:15, 7:12:15, 7:10:16 7:11:15, 6:10:16, 6:11:16, 6:12:15	
Mean-all-data	7:12:16, 6:12:16, 7:11:16 7:12:15, 7:10:16	6:11:16, 6:12:15, 7:11:15
Mean-data Set 103/104		6:12:15, 7:12:15 7:10:15, 6:11:16

This selection of group combinations within the whole group negates to some extent the advantages of a broad spread of sampling times, but in doing this no more than five cows would be displaced, 2 or more ranks in the set 101 (Jersey) data, nor more than eleven cows would be similarly displaced

in the set 103 (Friesian) data. This applies, however, to this set using this data and would probably not apply to other data tested in this way even for the same breed. The effect of difference in breed on the selection of sampling time combinations for this purpose is illustrated in the above table using mean-all-data coefficients on sets 101 and 103, where there has been no group of periods common to both selected.

A final assessment of the effect of displacement of rank on say the culling out or selection for breeding programme can be assessed best by listing the top or bottom 10% of the cows as ranked by both procedures, or those cows not reaching a preset production limit. Using the all-data regression equation Table G (e) (3) was prepared. The six lowest producers ranked as such 67 times out of a possible 72 or on 93% of the occasions and thus despite the differences in ranking by prediction the chance of unfair culling was not high.

TABLE 6 (e)

(3) Predicted ranking for all cows producing less than 280 lbs. protein using mean-all--data regression coefficients on Set 101

Rank	Actual Rank Order	RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN											
		6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	30	43	30	30	43	43	30	43	43	30	43	43	30
2	43	50	43	50	30	30	96	50	28	28	50	38	43
3	38	30	50	43	50	96	50	30	50	50	30	30	28
4	96	96	96	28	96	38	43	28	38	43	96	28	50
5	50	38	38	96	38	50	69	38	30	38	28	50	38
6	28	28	28	69	16	28	38	96	96	32	16	96	96
7	32	-	-	38	-	-	16	32	32	16	32	32	16
8	16	-	-	16	-	-	28	-	-	21	38	21	69
9	-	-	-	-	-	-	-	-	-	69	3	-	32
10	-	-	-	-	-	-	-	-	-	96	-	-	21

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	Chance of rejection on weight of protein produced %
1	30	5	2	4	-	1						100
2	43	7	2	1	2	-						100
3	38	-	1	-	2	6	1	1	1			100
4	96	-	1	1	4	1	4	-	-	-	1	100
5	50	-	4	5	1	2	-	-	-	-	-	100
6	28	-	2	1	3	1	3	-	1	-	-	92
7	32	-	-	-	-	-	1	4	-	1	-	50
8	16	-	-	-	-	-	2	3	1	-	-	50
9	22	-	-	-	-	-	-	-	-	-	-	Nil
10	3	-	-	-	-	-	-	-	-	1	-	8
11	74	-	-	-	-	-	-	-	-	-	-	Nil
12	21	-	-	-	-	-	-	-	2	-	1	25
13	48	-	-	-	-	-	-	-	-	-	-	Nil
14	69	-	-	-	-	1	1	-	1	1	-	33

Using the set 101/102 mean-data regression equation for predicting the ranking of cows in set 101, the six lowest producers ranked, as such, 69 times out of a possible of 72. (Table G (e) (4)). These results could be examined in a slightly different way.

Chance of culling lowest producer/s

No. of cows to be culled	Set 101		Set 103	
	101/102 Mean-data coefficients	Mean-all-data coefficients	103/104 Mean-data coefficients	Mean-all-data coefficients
	%	%	%	%
1	16.7	41.7	Nil	Nil
2	50.0	66.7	75.0	79.2
3	50.0	61.1	69.4	72.2
4	72.9	66.7	87.5	85.4
5	88.3	86.7	90.0	88.3
6	95.8	93.1	85.9	83.3
7	86.9	91.3	80.3	73.8
8	88.4	94.2	79.7	73.1
9	94.2	92.1	82.3	76.3
10	96.4	92.3	86.1	79.6

Thus provided one wished to cull at least five of the lowest producing cows in these herds, the chance was over 85% that the cows that should be culled would be the ones selected. This was in agreement with the observed occasional displacement of up to 5 ranks.

If culling practice was based on discarding those cows producing less than a predetermined production, e.g. 280 lbs. protein in the case of the Jersey data (set 101), then the

chances of an incorrect decision are listed in the table below.

Cow No. in rank order giving actual production less than 200 lbs.	Actual Production	Chance of being discarded by prediction using: 101/102 Mean-data coefficients	Chance of being discarded by prediction using: Mean-all-data coefficients
		%	%
30	222 lbs.	100	100
43	236 lbs.	100	100
38	238 lbs.	100	100
96	238 lbs.	100	100
50	240 lbs.	100	100
28	259 lbs.	100	92
32	269 lbs.	83	50
16	272 lbs.	83	50

The chance of a marginal low producer not being detected was 4% using set 101/102 mean-data coefficients and 14% using mean-all-data coefficients. The following cows could have been unjustly discarded by prediction on the listed percent of occasions in set 101.

Cow No. in actual rank order	Actual Production	Chance of being discarded by prediction using: 101/102 Mean-data coefficients	Chance of being discarded by prediction using: Mean-all-data coefficients
		%	%
22	285 lbs.	17	Nil
3	289 lbs.	83	8
74	289 lbs.	25	Nil
21	291 lbs.	58	25
48	296 lbs.	Nil	Nil
69	301 lbs.	67	33

Thus whilst all the lowest producers were definitely discarded, some of the other marginal cows may have been discarded by the same criterion.

TABLE 6 (e)

(4) Predicted ranking using Set 101/102 mean-data coefficients on Set 101. All cows producing less than 280 lbs. protein

		RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN											
Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	30	43	43	30	43	43	96	43	43	28	43	43	30
2	43	50	96	50	96	96	30	50	28	30	96	38	96
3	38	96	30	96	30	30	50	96	38	50	50	96	43
4	96	30	50	28	50	38	69	30	96	43	38	30	28
5	50	38	38	43	38	50	43	28	50	96	30	28	38
6	28	28	28	69	16	28	38	38	30	38	28	50	50
7	32	16	3	38	28	69	28	32	21	69	16	21	69
8	16	3	21	16	-	3	16	16	32	16	32	32	16
9	-	-	-	32	21	-	21	21	3	3	32	-	69	21
10	-	-	-	69	32	-	32	32	-	69	3	-	3	32
11	-	-	-	-	3	-	16	3	-	-	-	-	16	74
12	-	-	-	-	-	-	-	22	-	-	-	-	74	3
13	-	-	-	-	-	-	-	74	-	-	-	-	-	22

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	11	12	13	Chance of rejection on weight of protein produced %
1	30	2	2	3	3	1	1								100
2	43	8	-	1	1	2	-								100
3	38	-	1	1	2	4	3	1							100
4	96	1	5	4	1	1	-	-							100
5	50	-	3	3	2	2	2	-							100
6	28	1	1	-	2	2	4	2							100
7	32	-	-	-	-	-	-	1	3	2	4				83
8	16	-	-	-	-	-	1	2	5	-	-	2			83
9	22	-	-	-	-	-	-	-	-	-	-	-	1	1	17
10	3	-	-	-	-	-	-	1	2	2	2	2	1	-	83
11	74	-	-	-	-	-	-	-	-	-	-	1	1	1	25
12	21	-	-	-	-	-	-	2	1	4	-	-	-	-	58
13	48	-	-	-	-	-	-	-	-	-	-	-	-	-	Nil
14	69	-	-	-	1	-	1	3	-	1	2	-	-	-	67

When the set 101/102 mean-data coefficients were used for ranking, the cows in set 102 and those cows producing less than 280 lbs. of protein were listed (Table G (e) (5)), the six lowest producers ranked as such 66 times out of 72. Of the marginal low producers, all were detected but not on all occasions.

In summary, the following cows would have been correctly discarded by prediction on the listed percent of occasions in set 102.

Cow No. in actual rank order	Actual Production	Chance of being discarded by prediction using 101/102 Mean-data coefficients
		%
5	223 lbs.	100
74	226 lbs.	100
112	233 lbs.	100
22	239 lbs.	100
43	246 lbs.	100
17	247 lbs.	100
96	257 lbs.	100
63	259 lbs.	100
48	269 lbs.	67
18	270 lbs.	17
53	270 lbs.	100
75	271 lbs.	50

The following cows could have been unjustly discarded by prediction on the listed percent of occasions in set 102.

Cow no. in actual rank order	Actual Production	Chance of being discarded by prediction using 101/102 Mean-data coefficients
		%
21	289 lbs.	Nil
114	290 lbs.	Nil
32	294 lbs.	25

TABLE 6 (c)

(5) Rank order frequency table for all cows producing less than 280 lbs. protein using 101/102 sean-data coefficients on Set 102

Rank Actual	Predicted	1	2	3	4	5	6	7	8	9	10	11	12	13	Chance of rejection on weight of protein produced
	Cow No.														%
1	5	12	-	-	-	-	-								100
2	74	-	2	4	5	-	1								100
3	112	-	6	3	-	2	1								100
4	22	-	4	-	1	5	-	1	1						100
5	43	-	-	4	2	3	1	-	2						100
6	17	-	-	1	2	2	5	2	-						100
7	96	-	-	-	2	-	2	2	2	1	2	1			100
8	63	-	-	-	-	-	1	5	4	2	-	-			100
9	48	-	-	-	-	-	-	-	2	2	-	1	2	1	67
10	18	-	-	-	-	-	-	-	-	-	2	-	-	-	17
11	33	-	-	-	-	-	1	2	1	1	5	2	-	-	100
12	75	-	-	-	-	-	-	-	-	4	1	1	-	-	50
13	21	-	-	-	-	-	-	-	-	-	-	-	-	-	Nil
14	114	-	-	-	-	-	-	-	-	-	-	-	-	-	Nil
15	32	-	-	-	-	-	-	-	-	2	-	1	-	-	25

Examining set 103 data in this same fashion using 350 lbs. protein production as a minimum, gave the rank order listed in Table G (e) (6) for all-data coefficients.

The six lowest producers rank as such 60 times out of 72 (83%). Of the marginal low producers, one was not detected, one was detected on only 8% of occasions.

The predicted ranking for all cows in set 103 producing less than 350 lbs. of protein, based on set 103/104 mean-data coefficients, is given in Table G (e) (7).

The six lowest producers ranked as such 61 times out of 71. Of the marginal low producers, two were not detected, and of the other two, one was detected on 50% of tests and the other on 75% of tests.

TABLE 6 (c)

(6) Predicted ranking for all cows producing less than 350 lbs. protein using mean-all-data coefficients on Set 103

Rank	Actual Rank Order	RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN											
		6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	160	128	128	129	128	128	128	128	129	128	129	128	128
2	128	129	129	160	129	129	160	160	160	160	160	160	160
3	142	160	160	128	169	160	129	129	157	142	169	142	142
4	129	142	157	142	160	142	143	143	142	129	142	157	129
5	157	143	142	157	142	157	142	142	129	158	127	129	157
6	158	138	138	138	138	138	157	157	158	157	147	143	147
7	161	169	143	158	127	143	158	157	143	143	129	147	158
8	148	157	-	143	157	-	-	158	138	138	143	138	138
9	-	-	-	-	-	143	-	-	-	161	-	157	-	127
10	-	-	-	-	-	-	-	-	-	-	-	-	-	143

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	Chance of rejection on weight protein produced %
1	160	-	8	3	1							100
2	128	11	-	1	-							100
3	142	-	-	3	5	4						100
4	129	1	4	2	2	2	-	1				100
5	157	-	-	1	2	3	2	1	2	1		100
6	158	-	-	-	-	-	5	-	4	-		75
7	161	-	-	-	-	-	-	-	-	1		8
8	148	-	-	-	-	-	-	-	-	-		Nil
9	127	-	-	-	-	1	1	1	-	1		33
10	158	-	-	-	-	1	1	3	1	-		90
11	143	-	-	-	2	1	1	4	2	1	1	100
12	169	-	-	2	-	-	-	1	-	-	-	25
13	138	-	-	-	-	-	-	-	-	-	-	Nil
14	156	-	-	-	-	-	-	-	-	-	-	Nil
15	147	-	-	-	-	-	2	1	-	-	-	25

TABLE 5 (c)

(7) Predicted ranking for all cows producing less than 390 lbs. protein using Set 103/104 mean-data coefficients on Set 103

RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN

Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	160	128	129	129	128	128	129	128	128	128	128	128	128
2	128	129	128	160	129	129	160	160	160	160	160	160	160
3	142	160	160	128	169	160	128	129	129	129	169	142	142
4	129	142	137	142	160	142	142	143	137	142	142	138	129
5	137	143	142	137	142	137	137	142	142	158	127	129	137
6	138	-	138	138	138	138	138	127	158	137	129	143	147
7	161	-	143	-	-	-	-	-	-	143	147	147	158
8	148	-	-	-	-	-	-	-	-	-	143	138	137

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	Chance of rejection on weight of protein produced
										%
1	160	-	8	3	1					100
2	128	9	1	2	-					100
3	142	-	-	2	6	4				100
4	129	3	3	3	1	1	1			100
5	137	-	-	-	3	4	1	-	1	75
6	138	-	-	-	-	-	3	-	1	50
7	161	-	-	-	-	-	-	-	-	Nil
8	148	-	-	-	-	-	-	-	-	Nil
9	127	-	-	-	-	1	1	-	-	17
10	158	-	-	-	-	1	1	1	-	25
11	143	-	-	-	1	1	1	2	1	50
12	169	-	-	2	-	-	-	-	-	17
13	130	-	-	-	-	-	-	-	-	Nil
14	156	-	-	-	-	-	-	-	-	Nil
15	147	-	-	-	-	-	1	2	-	25

In summary, using set 103, the following cows would have been correctly discarded by prediction on the listed percent of occasions.

Cow No.in actual rank order	Actual Production	Chance of being discarded by prediction using 103/104 Mean-data coefficients	Chance of being discarded by prediction using Mean-all-data coefficients
		%	%
160	276 lbs.	100	100
128	299 lbs.	100	100
142	307 lbs.	100	100
129	317 lbs.	100	100
137	318 lbs.	75	100
138	340 lbs.	50	75
161	346 lbs.	Nil	8
148	347 lbs.	Nil	Nil

The following cows could have been unjustly discarded by prediction on the listed percent of occasions in set 103.

Cow No.in actual rank order	Actual Production	Chance of being discarded by prediction using 103/104 Mean-data coefficients	Chance of being discarded by prediction using Mean-all-data coefficients
		%	%
127	355 lbs.	17	58
150	366 lbs.	25	50
143	368 lbs.	50	100
149	384 lbs.	17	25
130	386 lbs.	Nil	Nil
156	388 lbs.	Nil	Nil
147	389 lbs.	25	25

An assessment of the chance of being correctly selected by prediction for culling was as follows:-

Chance of culling lowest producer/s

	Set 101		Set 102	Set 103	
No.of cows to be culled	101/102 Mean-data coefficients	Mean-all-data coefficients	101/102 Mean-data coefficients	103/104 Mean-data coefficients	Mean-all-data coefficients
	%	%	%	%	%
1	17	42	100	Nil	Nil
2	50	67	58	75	79
3	50	61	75	69	72
4	73	67	77	88	85
5	88	87	88	90	88
6	96	93	90	86	83
7	87	91	93	80	76
8	88	94	93	80	73
9	94	92	88	82	76
10	96	92	86	86	80

(G) (f) Ranking by prediction using mean regression equations for sampling times 6 and 7; 10, 11 and 12; 15 and 16, and its effect on rank displacements and rank correlation.

Table G (f) (1) (p. 180) gives the frequency distribution of rank displacements for sets 101 and 102 when the mean regression coefficients for set 101/102 were used for prediction.

In set 102, all the rankings by prediction were highly significantly correlated with the actual rankings and even with the worst combinations of sampling time in set 101 (6:12:16 and 7:12:16), the ranking was probably significantly correlated with the actual, especially in view of the rigorous character of the sums of squares of rank differences test on the ranking procedure adopted. With set 102, there was no consistently favourable combination of sampling times which ranked significantly better than the others (7:11:15 maybe, but the ranking differences were not great). With set 101, inclusion of sampling time 12 consistently increased the number and extent of the ranking differences but still without too serious consequences.

Table G (f) (2) gives the frequency distribution of rank displacements for sets 103 and 104 when the mean regression coefficients for set 103/104 were used for prediction.

In both sets the ranking by prediction was significantly correlated with the actual ranks and in neither did either any individual or group of sampling

times have any major effect on the rank displacements.

Appendix G (f) lists the cows and sampling time combinations for which the predicted ranking differed from the actual ranking by three or more places.

In set 101, only two cows (6.9% of herd) were displaced more than 2 ranks in any sampling time combination and as these were consistently displaced their production data was atypical of the herd. In set 102, three different cows (13.0% of herd) were involved in major displacements but only on one occasion for each.

It is probably significant that cows No. 43 and 69 in set 101 were also found to be displaced excessively in rank when mean-data coefficients were used (Section G (e)), again indicating that they were atypical.

In sets 103 and 104, as in 101 and 102, the same cow was not displaced more than 2 ranks in both sets. In set 103, five cows (14.3% of herd) accounted for 42 out of the 54 displacements (13 cows) listed, and similarly in set 104, six different cows (13.6% of herd) accounted for 37 out of the 48 displacements (12 cows) listed.

Comparing the cows excessively displaced in set 103, as listed in Appendix G (f) with the list given in Appendix G (e) (2), eleven out of the thirteen cows were common to both groups. It would appear that regression coefficients based on mean-data or on the mean of individually determined coefficients are both affected to about the same extent by atypical data.

Discussing the use of bimonthly records for ranking cows within a herd and using Spearman's rank correlation coefficient, Castle and Searle (1961) observed that all values in their study exceeded 0.81 with 70% of them exceeding 0.90, and on this basis concluded that "cows within a herd are ranked similarly by bimonthly records and by monthly records, and the culling of low producers on bimonthly records removes from the herd almost exactly the same cows as would be removed by culling on monthly records."

As the rank in this study has been based on 5% increments and not on absolute ranks, a direct comparison with Castle and Searle's work is not possible, but if the observations made earlier that the 5% increment rank correlation coefficients may be about three-quarters of the value of their absolute rank correlation coefficient equivalents, then the results listed above would indicate, using this criterion, that the predicted ranking order was entirely adequate for the culling of low producers.

The probability that the ranks do not differ is a better means of evaluating these ranking procedures. With the Jersey data (sets 101 and 102), in only four cases was the probability that the ranks did not differ greater than 1%, and of these four one was less than 2.5%, and with the other three the probability was less than 5%. With the Friesian data (sets 103 and 104), in only three cases was this probability greater than 1% and in those three it was less than 2.5%. The regression equations used thus rank both sets of data with equal merit and with a very satisfactory accuracy.

The New Zealand Dairy Board, 1962, reported that a Production Ranking Test was being introduced as an alternative to the Alternate Monthly Test and Group Herd Test. This Production Ranking Test is based on only two tests during the season. The production ranking test will be used for culling purposes only.

TABLE 8 (f)

(1)

Frequency distribution of rank displacements for sets 101 and 102 using mean regression coefficients for Set 101/102.

Rank displacement extent	Number of rank displacements and extent for sampling times and sets indicated																							
	6 10 15		6 11 15		6 12 15		6 10 16		6 11 16		6 12 16		7 10 15		7 11 15		7 12 15		7 10 16		7 11 16		7 12 16	
	101	102	101	102	101	102	101	102	101	102	101	102	101	102	101	102	101	102	101	102	101	102	101	102
2	4	3	5	3	4	4	3	3	2	2	7	2	1	4	4	1	7	4	4	3	5	2	9	3
3	1	1	-	-	-	-	1	1	1	-	-	-	1	-	-	-	-	-	-	1	1	-	-	-
4	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-
No. of Ranks	10	11																						
No. of Cows	29	23																						
Sums of squares of rank differences	36	34	32	24	49	26	36	31	31	16	71	17	31	28	28	18	56	25	41	32	37	23	73	22
Probability that these ranks do not differ was	< 0.01				- < 0.025		- < 0.01				< 0.05						< 0.05		< 0.01		< 0.01		< 0.05	
	< 0.005 except where indicated																							
r_s	0.782	.845	.805	.891	.703	.882	.782	.859	.812	.927	.570	.923	.812	.873	.830	.914	.661	.886	.752	.855	.776	.895	.560	.900

TABLE 8 (f)

(2)

Frequency distribution of rank displacements for sets 103 and 104 using mean regression coefficients for Set 103/104.

Rank displacement extent	Number of rank displacements and extent for sampling times and sets indicated																							
	6 10 15		6 11 15		6 12 15		6 10 16		6 11 16		6 12 16		7 10 15		7 11 15		7 12 15		7 10 16		7 11 16		7 12 16	
	103	104	103	104	103	104	103	104	103	104	103	104	103	104	103	104	103	104	103	104	103	104	103	104
2	8	9	10	7	4	9	10	10	10	8	8	5	8	8	6	5	6	8	10	1	7	5	5	7
3	1	5	2	4	2	4	1	2	3	4	2	2	1	1	3	1	3	1	3	3	3	1	5	1
4	3	-	1	-	1	-	3	1	1	-	-	-	3	3	1	2	1	1	3	3	1	3	-	3
5	-	-	1	-	-	-	-	1	-	-	-	1	-	-	1	1	-	-	-	-	-	1	-	1
6	-	-	-	1	-	1	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-
No.of Ranks	13	13																						
No.of Cows	35	44																						
Sums of squares of rank differences	103	132	98	126	67	113	112	119	99	117	67	118	107	106	117	107	82	122	118	96	107	120	91	123
Probability that these ranks		< 0.025		< 0.025	< 0.005						< 0.005						< 0.005	< 0.025				< 0.025	< 0.005	< 0.025
do not differ was between	0.01 and 0.005, except where indicated																							
r_s	0.717	.637	.751	.653	.816	.690	.691	.673	.728	.679	.916	.676	.705	.709	.679	.706	.775	.665	.676	.736	.705	.670	.790	.662

(G) (g) <u>The effect of rank displacement on selection procedures using regression equations based on mean regression equations</u>.

Tables G (g) (1) and G (g) (2) give the ranking order frequency for sets 101 and 102 for all cows producing less than 280 lbs. of protein when the mean regression coefficients for set 101/102 were used for prediction.

In set 101, all of the "actual" low producers (i.e. all cows above the line in the rank order frequency table) would have been detected on 83% or more of the combinations of sampling times. However, as indicated in the table, other cows could also have been rejected two on 8 occasions, one on 7 occasions, one on 3 occasions, one on 2 occasions and one on 1 occasion.

The mean production in set 101 was 298 lbs. protein, whereas in set 102 it was 285 lbs. If the 280 lbs. limit used in set 101 was used in set 102, then the number of non-complying cows would represent a greater percentage of the total herd. The equivalent limit in set 102 was 268 lbs. Using the 280 lb. limit for set 102, ten of the twelve low producing cows would have been detected on 75% or more occasions and the other two on 50-25% of occasions. Two other cows would have been incorrectly selected on 17 and 25% of occasions. If the 268 lb. limit was used, i.e. all cows ranked above the line, then the results should be more comparable with those of set 101 and it is seen that of the eight low producing cows using this criterion, all would have been selected on 92% or more occasions with four cows being unjustly included on 25, 17, 17 and 8% of the occasions.

Thus the selection of low producing animals is reasonably satisfactory with the lowest producers always being selected and the marginal ones sometimes excluded, sometimes unjustly included. However, the injustice is not serious when one considers the inherent variability of production from year 1 to year 2 as indicated in the ranking tables using "actual" data (Appendix G (c)) where the cows above the line would have been discarded on the 280 and 268 lb. limits.

Tables G (g) (3) and G (g) (4) give the ranking order and rank order frequency for set 103 and 104, respectively for all cows producing less than a predetermined quantity of protein when the mean regression coefficients for set 103/104 were used for prediction.

In sets 103 and 104, the limit of production selected was proportionately the same as for sets 101 and 102.

In set 103, twelve cows under-produced and prediction selected eight of them on 75% or more occasions with the others being detected on 58, 33, 25% of the occasions. One low producing cow was not detected at all and eight cows were unfairly selected on 50, 17, 33, 25, 8, 8, 8, 8% of the occasions.

Of the seventeen cows under-producing in set 104, fifteen were detected on 75% or more occasions and the other two were detected on 58 and 42% of occasions. Five cows would have been unfairly selected by prediction on 92, 50, 17, 17 and 8% of occasions.

TABLE 9 (g)

(1) Predicted ranking for all cows producing less than 280 lbs. protein using mean regression coefficients for Set 101/102 on Set 101.

RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN

Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	7 10 15	7 11 15	7 12 15	6 10 16	6 11 16	6 12 16	7 10 16	7 11 16	7 12 16
1	30	43	43	30	43	43	28	43	43	30	43	43	30
2	43	90	30	28	90	38	90	90	96	96	90	38	43
3	38	30	96	90	30	28	43	30	30	90	30	96	90
4	96	96	90	43	96	90	30	96	38	69	96	30	28
5	90	38	38	96	28	30	38	38	90	43	38	38	38
6	28	28	28	69	38	32	69	16	28	38	16	28	69
7	32	16	3	38	32	21	96	28	69	28	28	32	16
8	16	3	32	16	16	3	16	3	3	16	32	69	96
9	-	32	69	21	3	69	32	-	32	32	3	21	32
10	-	-	21	-	-	74	21	-	16	21	-	3	21
11	-	-	-	-	-	96	18	-	-	18	-	16	18
12	-	-	-	-	-	-	-	-	-	-	-	74	22

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	11	12	Chance on rejection on weight of protein produced %
1	30	3	1	5	2	1								100
2	43	8	1	1	1	1								100
3	38	-	2	-	1	6	2	1						100
4	96	-	2	2	4	1	-	1	1	-	-	1		100
5	90	-	5	3	2	2	-	-	-	-	-	-		100
6	28	1	1	1	1	1	4	3	-	-	-	-		100
7	32	-	-	-	-	-	1	2	2	5	-	-		83
8	16	-	-	-	-	-	2	2	4	-	1	1		83
9	22	-	-	-	-	-	-	-	-	-	-	-	1	8
10	3	-	-	-	-	-	-	1	4	2	1	-	-	67
11	74	-	-	-	-	-	-	-	-	-	1	-	1	17
12	21	-	-	-	-	-	-	1	-	2	4	-	-	58
13	69	-	-	-	1	-	3	1	1	2	-	-	-	67
14	18	-	-	-	-	-	-	-	-	-	-	3	-	25

TABLE G (g)

(2) Predicted ranking for all cows producing less than 280 lbs. protein using mean regression coefficients for Set 101/102 on Set 102.

Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
		RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN											
1	5	5	5	5	5	5	5	5	5	5	5	5	5
2	74	112	74	22	112	112	22	112	74	22	112	112	22
3	112	43	112	43	43	74	112	74	112	74	74	74	17
4	22	74	43	74	74	43	43	43	22	43	96	96	74
5	43	22	22	112	17	22	17	22	43	17	43	22	112
6	17	33	63	17	33	96	74	33	63	112	17	63	96
7	96	17	17	63	96	17	96	17	96	63	33	17	43
8	63	63	48	48	22	63	63	63	17	96	22	43	63
9	48	75	75	75	63	32	75	96	48	48	63	32	33
10	18	96	33	33	75	33	33	18	33	33	18	33	48
11	33	-	96	96	-	75	48	-	75	75	-	-	21
12	75	-	-	-	-	-	21	-	-	-	-	-	75
13	-	-	-	-	-	-	-	-	-	-	-	-	18
14	-	-	-	-	-	-	-	-	-	-	-	-	32

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Chance on rejection on weight of protein produced %
1	5	12														100
2	74	-	2	5	4	-	1									100
3	112	-	6	3	-	2	1									100
4	22	-	4	-	1	5	-									100
5	43	-	-	3	5	2	-	1	1							100
6	17	-	-	1	-	3	2	(4)5	1							(92)100
7	96	-	-	-	2	-	2	3	1	1	(0)1	(0)2				100
8	63	-	-	-	-	-	3	2	(4)5	2	-	-				(92)100
9	48	-	-	-	-	-	-	-	(1)2	(1)2	(0)1	(0)1				(17) 50
10	18	-	-	-	-	-	-	-	-	-	(0)2	-	-	(0)1		(NIL)25
11	33	-	-	-	-	-	3	1	-	(0)1	(0)7	-	-	-		(25)100
12	75	-	-	-	-	-	-	-	-	(0)4	(0)1	(0)3	(0)1	-		(8) 75
13	21	-	-	-	-	-	-	-	-	-	-	(0)1	(0)1	-		(NIL)17
14	32	-	-	-	-	-	-	-	-	2	-	-	-	-	(0)1	(17) 25

The figures in parenthesis represent the frequencies using 268 lbs. as the limiting level.

TABLE 8 (a)

(3) Predicted ranking for all cows producing less than 372 lbs. protein using mean regression coefficients for Set 103/104 on Set 103

RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN

Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	160	129	129	129	128	128	129	128	128	128	128	128	128
2	128	128	128	160	129	129	160	160	160	160	169	160	160
3	142	160	160	128	169	160	128	169	137	129	160	142	142
4	129	169	137	142	160	142	142	129	142	158	127	137	129
5	137	142	142	137	142	137	138	142	129	142	142	129	137
6	138	138	138	138	138	141	137	127	143	137	129	143	158
7	161	143	143	158	127	138	169	143	158	143	143	138	138
8	148	127	126	143	137	143	158	158	138	138	137	158	127
9	126	-	158	126	143	-	141	137	187	127	138	141	169
10	127	-	161	161	-	-	143	187	126	126	158	180	143
11	158	-	-	141	-	-	127	138	-	-	130	-	139
12	143	-	-	168	-	-	161	130	-	-	187	-	131

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	11	12	Chance of rejection on weight of protein produced %
1	160	-	7	4	1									100
2	128	8	2	2	-									100
3	142	-	-	2	4	6								100
4	129	4	2	1	2	2	1							100
5	137	-	-	1	2	3	2	-	2	1				92
6	138	-	-	-	-	1	4	3	2	1	-	1		100
7	161	-	-	-	-	-	-	-	-	-	2	1		25
8	148	-	-	-	-	-	-	-	-	-	-	-		Nil
9	126	-	-	-	-	-	-	-	1	1	2	-		33
10	127	-	-	-	1	-	1	1	2	1	-	1		58
11	158	-	-	-	1	-	1	2	3	1	1	-		75
12	143	-	-	-	-	-	2	5	2	1	2	-		100
13	169	-	1	2	1	-	-	1	-	1	-	-		50
14	130	-	-	-	-	-	-	-	-	-	-	1	1	17
15	156	-	-	-	-	-	-	-	-	-	-	-	-	Nil
16	147	-	-	-	-	-	-	-	-	-	-	-	-	Nil
17	187	-	-	-	-	-	-	-	-	1	1	-	1	25
18	141	-	-	-	-	-	1	-	-	2	-	1	-	33
19	139	-	-	-	-	-	-	-	-	-	-	1	-	8
20	180	-	-	-	-	-	-	-	-	-	1	-	-	8
21	160	-	-	-	-	-	-	-	-	-	-	1	-	8
22	131	-	-	-	-	-	-	-	-	-	-	-	1	8

TABLE 6 (g)

(4) Predicted ranking for all cows producing less than 374 lbs. protein using mean regression coefficients for Set 103/104 on Set 104

RANK ORDER FOR PREDICTED PRODUCTION BASED ON SAMPLING TIME GROUPINGS SHOWN

Rank	Actual Rank Order	6 10 15	6 11 15	6 12 15	6 10 16	6 11 16	6 12 16	7 10 15	7 11 15	7 12 15	7 10 16	7 11 16	7 12 16
1	137	152	152	152	152	152	152	134	134	134	134	134	134
2	152	137	129	134	137	165	165	152	152	152	129	152	129
3	129	134	134	137	129	129	134	129	129	129	152	129	152
4	134	127	137	129	134	134	137	157	165	157	157	165	165
5	170	132	165	165	127	137	129	132	157	165	138	138	157
6	172	129	132	132	165	163	170	127	132	132	132	157	137
7	127	163	127	127	132	127	163	137	169	169	127	170	138
8	157	165	163	163	170	132	127	126	137	137	137	137	132
9	138	170	169	170	163	170	132	165	127	170	170	132	170
10	146	126	170	169	141	172	172	138	138	146	141	127	172
11	126	169	172	172	144	141	141	149	170	127	172	172	169
12	165	141	146	141	172	169	146	169	172	149	144	169	127
13	132	157	141	-	146	146	169	170	126	172	149	141	141
14	169	146	157	-	138	138	138	141	148	138	126	163	146
15	141	146	-	-	157	144	144	172	149	126	146	146	149
16	144	172	-	-	169	-	142	144	141	141	169	144	163
17	148	-	-	-	-	-	-	146	146	148	148	148	144
18	-	-	-	-	-	-	-	148	163	163	-	149	126
19	-	-	-	-	-	-	-	163	144	144	-	150	161
20	-	-	-	-	-	-	-	-	150	-	-	161	-
21	-	-	-	-	-	-	-	-	-	-	-	126	-

RANK ORDER FREQUENCY TABLE

Rank Actual	Predicted Cow No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Chance of rejection on weight of protei production %
1	137	-	2	1	2	1	1	1	4										100
2	152	6	4	2	-	-	-	-	-										100
3	129	-	3	6	1	1	1	-	-										100
4	134	6	1	3	2	-	-	-	-										100
5	170	-	-	-	-	-	1	1	1	6	1	1	-	1					100
6	172	-	-	-	-	-	-	-	-	-	3	4	2	1	-	1	1		100
7	127	-	-	-	1	1	1	4	1	1	1	1	1	-	-	-	-		100
8	157	-	-	-	3	2	1	-	-	-	-	-	-	1	1	1	-		75
9	138	-	-	-	-	2	-	1	-	-	2	-	-	-	4	-	-		75
10	146	-	-	-	-	-	-	-	-	-	1	-	2	2	1	3	-	2	92
11	126	-	-	-	-	-	-	-	1	-	1	-	-	1	1	1	-	2	58
12	165	-	2	-	3	3	1	-	1	1	-	-	-	-	-	-	-	-	92
13	132	-	-	-	2	5	1	2	2	-	-	-	-	-	-	-	-	-	100
14	169	-	-	-	-	-	2	-	1	1	2	3	1	-	-	2	-	-	100
15	141	-	-	-	-	-	-	-	-	2	2	2	3	1	-	2	-	-	100
16	144	-	-	-	-	-	-	-	-	-	1	1	-	1	2	2	3	-	83
17	148	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4	42
18	142	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	8
21	149	-	-	-	-	-	-	-	-	-	-	1	1	1	-	2	-	1	50
27	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	17
34	163	-	-	-	1	2	2	1	-	-	-	-	-	-	1	-	1	3	92
20	161	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	17

Thus with the Friesian herds, as with the Jersey herds, a reasonably satisfactory selection is possible by prediction. This data is summarised in the following table:-

	No.of cows to be culled based on actual production.	No.of times selected by prediction.		No.of cows unfairly selected.	
		>75% of occasions	<75%	>25% of occasions	<25%
Set 101	8	8	Nil	3	3
Set 102	8	8	Nil	Nil	4
Set 103	12	8	4	2	6
Set 104	17	15	2	2	3

The effect of variations in rank order can also be measured by determining the chances of the variously lowly ranked cows being selected for culling.

No. of cows to be culled	Chance of culling lowest producers using any combination of sampling times and the appropriate regression coefficients.			
	Set 101	Set 102	Set 103	Set 104
1	25%	100%	Nil %	Nil %
2	54	58	71	50
3	58	78	69	67
4	69	77	81	81
5	90	90	88	68
6	78	86	86	63
7	87	89	76	64
8	88	94	71	69
9	83	90	68	70
10	87	84	71	70
No. of animals to be culled to reach a culling rate of:-				
10% of herd	3	3	4	4
20% of herd	6	5	7	8

As with the ranking using regression equations based on mean-data, the chance of picking the lowest producer using these prediction equations is not necessarily high, e.g. in this series the chance ranges from nil to 100%, but as the number to be culled increases up to five or six animals, so does the probability that the lowest producers will be the ones selected. However, above this number, an increase in the number to be culled does not result in further improvement in the chances of selecting the correct cows for culling. In fact the chances regress in set 103. With the Jersey mean regression equation applied to the Jersey data and when five or more cows are to be selected for culling this can be done with a better than 85% chance of the correct cows being chosen by prediction. With the Friesians using the Friesian mean regression coefficients the correct selection is not as strong - the chances being that the selection will be 70% correct.

These probability figures of correct selection must, however, be considered in relation to the natural errors inherent in ranking cows for selection purposes. This was discussed earlier when it was shown that, using actual production data, 50% of the Jersey cows and 67% of the Friesian cows would have been two or more ranks displaced from year 1 to year 2, or expressed in the same terms as used earlier in this section.

No.of cows to be culled	Chance of culling the lowest producer in year 2 based on data from year 1 within breeds and using actual data.	
	Set 101/102	Set 103/104
1	Nil %	Nil %
2	Nil	Nil
3	Nil	33
4	25 (20% culling level)	50
5	40	60 (20% culling level)
6	33	50
7	43	56
8	50	75
9	56	67
10	60	80

Thus using actual data from year 1 and applying it to year 2, would not correctly select the lowest producing animals with any degree of certainty, unless eight or more animals were to be culled and even in this case the Jersey selection would only be 50-60% correct, despite the fact that over 50% of the cows were being culled. With the Friesian data the selection was slightly more satisfactory, but even here a culling rate of 42% had to be achieved to reach an 80% correct decision.

A ranking and culling procedure for protein production of about the same merit as that currently used for herd improvement based on butterfat yield is thus possible, using a set of mean regression coefficients on a within breed basis when samples are taken three times during a normal lactation within fairly widely specified sampling times.

CONCLUSION

This investigation has shown that it is possible to predict protein yield with considerable precision when a regression equation based on a specific set of data is used, e.g.

(a) (manually computed regression equations based on data from four or less sampling times).

	Sampling times used to establish regression equation	S.E. of Estimate
With Jersey data		
Year 1	7:11:15	3.5%
Year 2	6:10:15)) 6:12:16)) 7:10:15)	4.2%
	6:11:15) 7:11:15)	4.3%
Year 1 and 2 combined	7:11:15	3.4%
With Friesian data		
Year 1	6:12:16	4.0%
Year 2	6:10:16)) 7:10:16)	4.2%
Year 1 and 2 combined	6:12:16	7.2%

(b) (computer generated regression equations based on data from four or less sampling times).

	Sampling times used to establish regression equations	S.E. of Estimate
With Jersey data		
Year 1	5:8:11:12)	3.8%
	)	
	4:7:11:16)	
Year 2	2:4:12:16	3.4%
Year 1 and 2 combined	6:7:11:14	4.6%
With Friesian data		
Year 1	3:4:12:16	4.7%
Year 2	5:10:16	5.8%
Year 1 and 2 combined	4:7:12:14)	4.4%
	)	
	4:7:12:16)	

It has also been shown that it is possible to apply the same partial regression coefficients to data from three samples taken over a reasonable spread of sampling times without increasing the error of the estimate too greatly, e.g. Data collected during the following stages of the lactation 84-111 days (by_1), 140-181 days (by_2) and 210-237 days (by_3) could be used to predict protein production during the whole lactation by using the following partial regression coefficients:-

	a	by_1	by_2	by_3	S.E. of Estimate
(1) for Jersey data	4.36	3.162	7.297	6.040	4.67%
(2) for Friesian data	3.62	5.944	6.767	5.475	7.15%

The maximum standard errors of the estimate of protein production of individual cows using this system were found to be:

Jersey	6.2%	(mean 4.61%)
Friesian	9.5%	(mean 7.50%)

The maximum errors in predicting the protein production of the whole herd were:

Jersey	6.3%	(mean + 1.98, S.D. ± 1.67)
Friesian	3.5%	(mean - 1.91, S.D. ± 0.93)

These errors have been considered in relation to the errors of sampling and estimating systems in current use and are considered not to differ greatly from the accepted variabilities, and so this sampling and predicting system is recommended as being practicable.

The interchangeability of sampling times mentioned in the introduction as being a desirable aim in a limited frequency of sampling system has been achieved by using a breadth of time during which each of the three samples needed can be taken. Thus there are 27 days in the first and last sampling periods and 41 days in the middle period. This should allow a flexibility in the sampling and testing operations sufficient to enable a reasonably efficient sampling and testing scheme to be operated.

The sampling programme envisaged and discussed here could be readily integrated into the Herd Improvement Association testing programme for fat yield since the same sampling officers can be used to take samples for protein analysis on

their regular visits at the appropriate times.

The protein production predicted by these regression equations is sufficiently accurate for normal ranking and culling purposes. The effect of prediction on ranking has been closely scrutinized and, although it has been shown that the chance of selecting the lowest producer in the herd by this procedure can vary from 0 to 100%, chances of detecting the lowest two cows are over 50%, three cows 60%, four cows 70%, at which stage chances level off but if one of the cows selected is not the next lowest, it is likely to be not greatly different in production from the next lowest, so the ranking and culling process can be operated using this data with as much confidence as any of the procedures currently in use.

The estimate of total protein production from a herd using the regression equations gave a mean difference of +2% with the Jersey data and -2% with the Friesian data. With the range in equivalent sampling times likely with the varied calving dates of the cows in a herd the tendency will be for this mean difference between predicted and actual production to be approached, rather than the extreme limits of 6.3% and 3.5% respectively mentioned. Bearing in mind sampling and analytical sources of variation, a prediction within 2% of the actual production should not give an unjust distribution of payments for protein production if paid for on a predicted basis.

REFERENCES

ALEXANDER, M.H., YAPP, W.W. (1949). Comparison of methods of estimating milk and fat production in dairy cows. J.Dairy Sci. 32 : 621.

ASHTON, E.D. (1956). Milk and butterfat recording - A world survey. Tech.Comm. No.3., Comm.Bureau Dairy Sci. Farnham Royal, Bucks., England.

BENNETT, C.A., FRANKLIN, N.L. (1954). Statistical analysis in chemistry and the chemical industry. John Wiley & Sons, New York.

CAMPBELL, I.L. (1946). The accuracy of various methods of estimating a dairy cow's production of milk and butterfat. Proc.N.Z.Soc.Anim. Prod., p.99.

CARRÉ, D., POLY, J., VISSAC, B. (1958). A study of methods of determining milking performance. Ann.Inst.Nat. Rech.agron.,Paris, Sér. D. (Ann.Zootech.) 7 :243.

CASTLE, OLIVE M., SEARLE, S.R. (1961). Use of bimonthly records in herd testing. J. Dairy Sci. 44 : 1335.

_____ (1957). Repeatability of dairy cow butterfat records in New Zealand. ibid 40 : 1277.

CIANCI, D. (1963). Accuracy of milk recording dairy cattle at different intervals. Dairy Sci.Abstr. 25 : No.22.

_____(1963). Determination of lactation yield from part lactation. Dairy Sci.Abstr. 25 : No.24.

CROXTON, F.E., COWDEN, D.J. (1955). Applied General Statistics, 2nd Edition, Sir Isaac Pitman and Sons, London.

CZAKÓ, J., CSUKAS, M. (1962). Data concerning the number of analyses required for the determination of the milk protein percentage characteristic of lactation. Hung.agric.Rev. 3 : 40 (No.321).

CZAKÓ, J., ENYEDI, S., NIKLAI, J. (1963). Studies into the simplication of milk recording. Dairy Sci.Abstr. 25 : No.1297.

DAVEY, GILLIAN P., ALEXANDER, G.I. (1954). The accuracy of estimation of a dairy cow's production of milk and butterfat. Qd.J.agric.Sci. 11 : 75.

DICK, I.D. (1950). Some results on the accuracy of milk sampling over a complete lactation and the daily variability in milk yields. N.Z.J.Sci.Tech. 32 : Sect.A (1) 25.

EDWARDS, R.A., SIMPSON, M.E. (1958). Milk sampling at weigh tanks. J.Soc.Dairy Tech. 11 : 30.

ERB, R.E., ASHWORTH, U.S. (1963). Within breed variations in milk composition and estimating the average composition of milk for the lactation from partial records. J.Dairy Sci. 46 : 1228.

FISTEAG, I., MIASNICOV, I., BANICA, P., PETRESCU, A., CIRSTEA, E. (1958). The optimum intervals for recording the milk production of cattle breeds in Roumania. Lucr.Sti.Inst.Cercet.Zooteh. 16 : 63.

FRITZ, G.R., McGILLIARD, L.D., MADDEN, D.E. (1960). Environmental influences on regression factors for estimating 305-day production from part lactation. J.Dairy Sci. 43 : 1108.

HAVE, A.J.v.d., MULDER, H. (1956). Some methods for the estimation of protein in milk. XIVth Int.Dairy Cong. Sect. III, 693.

HEROLD, I., VERESS, L. (1964). Studies to establish the individual milk protein producing capacity of Hungarian spotted cows. Dairy Sci.Abstr. 26 : No.3438.

HORN, A. Cited by Voigtländer and by Witt and Walter.

INTERNATIONAL DAIRY FEDERATION (1963). Quality payment for milk, Appendix D - sampling and preservation of milk samples. Annual Bulletin, Part II, p.63.

JOHANSSON, I. (1942). Investigations on methods of milk recording. Z.f.Tierz und Züchtungsbiol. 51 : 229.

LAMB, R.C., McGILLIARD, L.D. (1960). Variables affecting ratio factors for estimating 305-day production from part lactations. J.Dairy Sci. 43 : 519.

LEMVIGH, H. (1934). Hannover, Dissertation. Cited by Voigtländer.

McCARTHY, M.D., BOYLE, C. (1946). The accuracy of monthly, six-weekly and two-monthly weighings in estimating milk yields. Dairy Sci.Abstr. 9 : 258.

MADDEN, D.E., McGILLIARD, L.D., RALSTON, N.P. (1956). Relations between monthly test day milk production of Holstein-Friesian cows. J.Dairy Sci. 39 : 952.

_____ (1959). ibid 42 : 319.

NEW ZEALAND DAIRY PRODUCTION AND MARKETING BOARD 34th ANNUAL REPORT 1958.

_____ 35th Annual Report 1959
38th Annual Report 1962
39th Annual Report 1963
Sire Survey 1965

PATCHELL, M.R. (1954-55). Annu.Rep.Dairy Res.Inst. N.Z.,p.26.

POLITIEK, R.D. (1957). The influence of heredity and environment on the composition of the milk of Friesian cows in the province of Friesland and the practical possibilities of selection on the protein content. Doctorate Thesis. Wageningen.

PJANOVSKAYA, L.P. Cited by Voigtländer.

SENFT, B. (1960). Cited by Vanschoubrek and by Voigtländer. Dairy Sci.Abstr. 22 : No.799.

THOMPSON, N.R., STONE, W.K., GRAF, G.C., KRAMER, C.Y., FREUND, R.F. (1960). Errors in estimation of lactational yields of milk, fat and S.N.F. from individual cows. J.Dairy Sci. 43 : 951.

VANSCHOUBREK, F.X.E.J. (1963). Determination and variation of protein, fat and lactose in cow's milk and selection on these factors. Neth.Milk Dairy J. 17 : 12.

VAN VLECK, L.D., HENDERSON, C.R. (1961). (a) Use of part lactation records in sire evaluation. J.Dairy Sci. 44 : 1511.

_____ (1961). (b) Regression factors for predicting a succeeding lactation milk record from part lactation records. ibid 44 : 1322.

VOIGTLANDER, K.H. (1965). The number of tests necessary to assess the milk yield and its composition. Arch. Tierzucht, 8 : 113. (Translation included with thesis).

WALLACE, G.M. (1962). Application of the hydrometric method of analysis to the estimation of solids-not-fat production. J.Dairy Res. 29 : 11.

WITT, M., WALTER, E. (1959). Comparison of errors in three or four-weekly milk recording. Z.Tierz. U. Zuchtungsbiol. 73 : 221.

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Appendix : Introduction 1

SONDERDRUCK

aus dem Band A

des

XVII. Internationalen

Milchwirtschaftskongresses

1966

Prediction of Protein Production based on a Restricted Frequency of Sampling

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Low SNF levels in the liquid milk industry prompted an enquiry into methods of analysis and into the frequency of analysis necessary to predict the production of SNF or SNF components.

With regard to the prediction of production using a restricted frequency of sampling, it was considered that the following requirements must be met:

1. For herd improvement on an individual cow basis.
   (a) One should be able to select the top and bottom 10% with confidence - the main requirement is one of ranking rather than an accurate knowledge of individual performance.
   (b) If several groups of sampling times gave approximately equivalent estimates for the purposes of (a), this would be an advantage, as it would spread the sampling and analysis requirements. This would be an advantage both within and between breeds.
2. For estimating production on a whole herd basis.
   One should be able to give an estimate of total production of a named component - this would be of value as an experimental aid in assessing the effects of changes in husbandry on the production of components - a knowledge of the error of the estimate would assist in determining the significance of observed changes.
3. For determining monetary return to the farmer. One should be able to make a reliable estimate of milk components supplied during a dairying season on a whole herd basis so that a reasonably accurate calculation can be made of the value of the component supplied.

It was considered that the desirable frequency of sampling should be some combination of 2, 3 or 4 sampling times which give the best estimates of the total production for the individual animals and for the herd. It was considered desirable that the partial regression coefficients for the selected sampling times should not differ greatly from year to year and it would be useful as well if these coefficients did not differ greatly between breeds.

Analytical and production data, using standard methods of analyses, were accumulated over two dairying seasons from samples taken fortnightly from individual cows at the P. M. and A. M. milking of the cows comprising the main

Jersey and Friesian herds on the Massey University dairy farm. A composite P. M., A. M. sample was prepared on a proportionate basis for each of these cows. Data from the first 16 fortnightly samplings were used in the computations [1].

To examine this data two procedures were adopted.

1. Manual calculation was used to determine:
   (a) The correlation between the production for individual sampling times with the total production. These correlations were ranked for each set of data (namely production and analysis data of approximately 30 Jersey cows for year 1 and year 2, and likewise for approximately 50 Friesian cows) and for the combined data.
   (b) The intercorrelation between the most highly ranked sampling times.
   (c) The partial regression coefficients and the standard error of estimate for combinations of 2, 3 or 4 sampling times. The sampling times selected were those having high individual correlations with total production and low intercorrelations with one another, 45 analyses of the data were done this way, a constant term (a) regression coefficients (b_1, b_2 etc.) and a standard error of estimate being calculated for each group of data.

2. *Computer calculation*

A modified Trap (Regression Analysis) programme was used to calculate partial regression coefficients, etc. as above. This programme was designed to force the regression line through zero since this would eliminate the constant factor (a) which was very variable.

The programme was designed to discard variables which did not give a t-value greater than 2 when their corresponding partial regression coefficient was tested for significance.

Because of limits in computer capacity, variables were selected on the basis of (1) all odd sampling times, (2) all even sampling times, (3) sampling times not rejected from (1) and (2), (4) sampling times selected by (3) for each year and for each breed (5) all combinations of four sampling times selected from those not rejected from (1) and (2).

MANUALLY CALCULATED DATA

With the Jersey herd the lowest standard error of estimate was 3.4% ($R^2 = 0.94$) using three sampling times, namely sampling times 7, 11, 15. The regression

[1] The fortnightly period post parturition corresponding to the sampling times quoted may be calculated as follows:
Sampling time x 14 + 0 to 13
Thus sampling time 5 corresponds to the period 5 x 14 + 0 = 70 to 5 x 14 + 13 = 83 that is between 70 and 83 days post parturition.

coefficients were (a) 3.70 (b_7) 5.99 (b_{11}) 2.30 (b_{15}) 8.99. With the Friesian herd the lowest standard error of estimate was 4.4% ($R^2 = 0.95$) using three sampling times, 10, 12, 14, which were different from those for the Jersey herd. The regression coefficients were (a) 1.27 (b_{10}) 11.43 (b_{12}) 19.98 (b_{14})-11.92. With the combined herds the lowest standard error of estimate was 6.95 ($R^2 = 0.84$) and this occurred when samples were taken between the 98th - 111th day, the 168th - 182nd day, and 210th—223rd day of lactation. The regression coefficients were (a) 3.73 (b_7) 5.20 (b_{12}) 7.79 (b_{15}) 5.29.

Sampling times: 7, 12, 14; 7, 11, 15; 11, 12, 15; were the only ones common to both Friesian and Jersey data that gave reasonably low standard errors of the estimate (Table 1).

TABLE 1

Regression data for common sampling times, both years considered together

	Standard Error of Estimate percent	*(a)*	*(b7)*	*(b12)*	*(b14)*
Jersey	5.5	not determined	4.12	7.74	4.18
Friesian	6.3	not determined	5.95	19.57	—7.03
Combined Jersey Friesian	7.3	not determined	6.10	14.51	—3.79
		(a)	*(b7)*	*(b11)*	*(b15)*
Jersey	3.4	3.70	2.30	8.99	5.99
Friesian	8.4	4.65	5.98	5.46	6.04
		(a)	*(b11)*	*(b12)*	*(b15)*
Jersey	4.2	4.92	7.59	4.75	4.00
Friesian	8.8	6.77	3.55	9.58	5.90
Combined Jersey Friesian	8.0	4.14	4.53	8.02	5.80

The combination of sampling times 7, 12, 14 was rejected on the basis that all positive regression coefficients were desirable since in these circumstances they all made a positive contribution to the prediction of production.

It would be feasible therefore to use sampling times 7 or 12, 11 and 15 for estimating protein production. The dilemma arises, however, as to which value for constant and regression coefficient should be used since they vary considerably.

COMPUTER CALCULATED DATA

Using the regression analyses made on all combinations of 4 variables based on the 9 sampling times which had been selected for their significant contribution

to prediction it was determined that estimates of production based on the following sampling times gave a standard error of estimate as follows:

	S. E. of estimate	
	Jersey	*Friesian*
4, 7, 11, 16	4.9%	6.6%
4, 6, 7, 16	5.1	6.9
4, 6, 11, 16	5.3	6.7
4, 7, 12, 16	5.3	6.4

These results were based on the use of the combined (2 year) data for the Jersey group and similarly for the Friesian group.

The partial regression coefficients differed for each group and were typically as follows:

	4	*7*	*11*	*16*
Jersey	3.67	4.73	6.19	5.08
Friesian	4.09	4.47	5.09	7.03
	4	*7*	*12*	*16*
Jersey	4.38	5.29	3.91	6.48
Friesian	3.31	5.23	6.98	5.36

The sampling time groupings above were the ones which were both, common to the Jersey and Friesian groups and, amongst those most highly ranked for lowest standard errors of estimate in each group. Combined (2 year) data were used since the regression coefficients so determined would be more generally applicable than those determined for data from a single year of sampling, the consequence of this was the higher standard errors of the estimates.

Examination of the data for the individual groups studied showed that the following sampling times gave the lowest standard errors of estimate for protein production.

	Sampling Period	*Standard error of estimate*
Jersey, Year 1	5, 8, 11, 12	3.8%
Year 2	3, 7, 12, 13, 15	2.9%
both years	5, 7, 8, 11, 12, 16	4.0%
Friesian, Year 1	3, 4, 12, 16	4.7%
Year 2	4, 7, 10, 14, 16	5.4%
both years	3, 4, 7, 12, 15, 16	5.5%

With these small standard errors differences in ranking would not be expected to be serious.

Using sampling times 4, 7, 11, 16 and the partial regression coefficients listed above, the extent of the ranking differences was examined using the Jersey sample herd data and the Friesian sample herd data. The individual cows were

ranked in the order of their production as estimated on the prediction basis and on the fortnightly sample basis. This simple ranking procedure was not entirely an adequate basis of comparison, since it tended to exaggerate small differences in production. Therefore, within this rank cows were grouped on the basis of increments of 5% of mean production. This procedure reduced the number of ranks from 29 to 11 for the Jersey sample herd and from 33 to 10 for the Friesian sample herd. With the Jerseys, in no case was there a greater difference than 2 ranks and with only 3 cows was the ranking different by 2 between the two methods of ranking. With the Friesians one cow only differed by 3 ranks, and 6 cows differed by 2 ranks. In both cases, all the other cows ranked equally or differed by only 1 rank. It is thus quite possible and adequate to use the predicted data for cow selection purposes.

Similarly using these sampling times and regression coefficients the predicted protein production was determined and the differences from that based on the fortnightly analysis were:

Jersey	*Friesian*
0.99% less	1.07% less

CRITICISM

1. One criticism of this system is that the milk from not all cows in the herd can be sampled for examination at any one time since the cows normally calve down over an extended period of time and this will entail repeated visits to the herd to obtain samples within the required periods post parturition for the individual cows.

To examine the effect of using a common sampling date rather than having to sample at repeated intervals, the Jersey sample herd data was used to predict production but the date on which the maximum number of cows were first tested was used as the datum and all cows were then tested on the same day irrespective of the relationship this day had to their lactational progression. 10 cows out of the 29 were in this way examined on the correct basis, all other cows being 1 or more fortnightly periods out of line. The same regression coefficients were used. The effect of this procedure was to increase the standard error of the predicted value.

In ranking	1 cow was 5 ranks out of place	(approx. 20% error in prediction)
	3 cows were 3 ranks out of place	(approx. 13% error in prediction)
	8 cows were 2 ranks out of place	(approx. 9% error in prediction)

This may still be adequately accurate for selection purposes.

An interesting sidelight was the effect of this modification on the prediction of herd production in that it gave an error of only 0.74%, even less than that of the previous method.

2. The base against which the predicted value is compared is itself subject to a prediction error since the estimation of total production assumes only minor changes in milk composition and volume produced in any one fortnightly period.

3. The regression coefficients and the sampling periods selected to give best prediction varied with each of the four individual sets of data and with the grouped data, hence the ability to predict from data other than that on which the regression analyses were based may not be high. Nevertheless, the fact that both the grouped Friesian and grouped Jersey data indicated that the periods selected will enable a reasonably accurate prediction to be made, bearing in mind always that different regression coefficients are involved, suggests that production of protein during these periods post parturition is fairly closely correlated with total production of protein.

A major problem has been the pre-selection of data likely to give significantly useful results after computation of the partial regression coefficients. The Trap programme is a fairly lengthy one and each analysis has required an appreciable time. The first attempt at selection was based on using high correlations between total production and production at individual sampling times and low inter-correlations between the latter, but this was not an adequate basis. The use of the t - value in the Trap programme was then examined and although this also was not entirely consistent the indications are that it will give the most economical way of determining which selection of periods will give the lowest standard error of estimate. The method is limited at the moment in that no more than ten values can be built into the matrix at any one time because of computer capacity. The selection on the t - value is based on the elimination of variables for which the t - value does not reach a predetermined level. It is considered that the programme could be improved by modifying it to reject on the basis of the lowest t - value and to test not against the t - value but against a predetermined number (say 3 or 4) of residual variables (i. e. selected sampling times).

A disadvantage of the t - value selection approach as against the simple permutation series approach is that it is very much more difficult to select, as has been done in this study, sampling periods common to several groups of original data and having minimal standard errors for all data. Present indications are that progressive working through all possible permutations of, say, 3 or 4 variables, of the analytical data is still the most useful way of determining the best combination of sampling periods when comparison between different sets of data are required for the selection of common features.

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Appendix : Introduction 2

THE NUMBER OF TESTS NECESSARY TO ASSESS THE MILK YIELD AND ITS COMPOSITION

K.H.VOIGTLÄNDER : Archiv fur Tierzucht 8 : 113 (1965)
(Translated by Dr.R.Brooks).

The continually increasing interest in the constituents of cows' milk and also the economic importance of increasing the level of the concentrations of these constituents has resulted in an increasing consideration of these factors in relation to animal husbandry. Besides interest in major constituents and fat content, consideration has also been given to the other milk constituents, in particular S.N.F. To do this it is, therefore, necessary to carry out research on bulls and cows; in the case of bulls, into a determined number of female offspring, and with cows, into individual performances during a lactation. The precise lactation performance of a cow can only be determined by daily weighings of the milk and its analysis. Johansson (1942) describes this as the true performance, although subject to errors in weighing and analysis. The determination of true performance is, however, not possible under practical conditions because of the cost involved in the large number of milk samples that have to be investigated. In the absence of means of determining the precise values it is possible, however, to make use of samplings at regular time intervals or of individual tests at predetermined times. The calculated performance so obtained compared with the true performance gives a difference known as the sampling error. The sources of this error are to be found in the day-to-day variations in milk yield and composition, as well as variations in the conditions of lactation (Johansson, 1942). The conflict between (a) keeping the number of test samples during lactation to a minimum and (b) the reliability and comparative value of the results has stimulated research into the "sampling

error". The sampling error resulting from sampling at predetermined time intervals has been discussed in relation to work on milking performance.

Whereas at the end of the century it was considered that weekly samples were sufficient, under the compulsion of the increasing number of cows to be examined it is now considered that 14, 21 or even 28-day intervals or even longer sampling time intervals should be used (Bayley u.a., 1952; Breirem, 1933; Campo, 1952; Cianci, 1963; Czakó u.a., 1962; Deger, 1935; Erb u. Mitarb., 1952; Gärtner, 1921; Herweg, 1911; Johansson, 1942; Jordao u.a., 1948; Lauprecht, 1950; Meerpohl, 1941; Ringler, 1937; Thompson, 1962; Thompson u.a., 1960; Vogel, 1931; Wendt, 1913, u.a.). These views and opinions reached their climax in 1951 during the F.A.O. European Conference on the unification of methods of milk and fat testing in which it was stated that tests should be carried out at least at monthly intervals (Kruger, 1958). The working guide for performance testing states that the present day milk yield tests should be carried out at 21 - 28 day intervals (Schaaf, 1960). Earlier workers also suggested that the protein and S.N.F. could also be determined during these intervals (O'Connor u.Lipton, 1960; Moustgaard u. Neimann-Sørensen, 1962; Senft, 1958; Thompson, 1962; Thompson u.a. 1960). In contrast to these recommendations in the case of bull and progeny testing stations, shorter intervals are recommended (Brüggemann, et al. 1956, 1958). In such station tests the dams are tested for milk yield and fat content every day as well as for weekly tests of protein. The progeny testing station for bulls in Denmark take tests of milk yields four times a week and the fat content twice a week, whereas the protein content is tested every 14 days (Hansen, et al. 1960, 1949/50).

On the other hand the German progeny testing station at Loga in East Friesia tests milk yields and fat content twice a week and protein and S.N.F. once a week (Haring, et al. 1956, 1958).

A different method for control at definite time intervals is the spot test control. This consists of two to six predetermined sampling times during the lactation period. Their average value is said to be representative of the whole performance. Although this method was suggested many years ago (Lemvigh, 1934), it is only since the last war that many trials have been made of this method (Alexander u. Yapp, 1950; Czakó u. Csukas, 1961; Horn, zit. nach Witt u. Walter, 1959; Jarrige u. Rossetti, 1957; Masuda u. Higaki, 1953; Pjanovskaya, 1960; Senft, 1958). The disadvantage of rigid predetermined testing times has been avoided by the method of Kwizda, et al. 1957. This method consists of the use of a theoretical formula to calculate the actual value of lactation performance in relation to milk yield, based on spot tests taken at convenient intervals. This method has been tested by Krippl and Schumann (1959); Stahl, Rasch and Dorfling (1960) and von Vachál and Teslík (1963), and has been tested in relation to fat content by the first two pairs of authors. The importance of the spot test sampling lies in obtaining a selection base based on breadth of data from broad experience. The method is also sufficiently precise to be applied for progeny tests. From the many possibilities this paper has developed a specific method for this type of testing. Since the most accurate performance data based on daily samplings could not be obtained, use has been made of weekly tests. This time interval has been selected because shorter intervals can hardly be considered on economic grounds.

Furthermore, a significant volume of literature has shown that good agreement can be obtained between weekly and daily testing.

(1) Materials and Methods

As a test herd, 29 complete and normal lactations were studied from 14 days after calving for 17 German Friesian cows. This is based on investigations at the Institute of Animal Husbandry and Dairying at the Karl Marx University. Samples were taken at regular 7-day intervals for the determination of milk yields. Approximately 1200 samples were taken and these were also used to determine the concentration of the milk constituents.

Fat was determined by Gerber method and protein by the Kofranyi method. Ash by usual methods. Lactose determined as N.F.E. equivalent by difference and S.N.F. also by difference.

For the samplings at definite time intervals, sample times of 14, 21, 28, 42 and 56 days' duration were used. The sample days were taken in the middle of the respective time intervals. The milk yield of the total lactation period was calculated by taking the milk yield of the sample days multiplying them by the length of the sample period and the milk yields so obtained were added together. In order to determine the average content of a milk constituent, the percentage value from each of the samples was multiplied by the milk yield. The total values were then calculated for each full individual lactation period. For random tests, the following methods were available:-

(a) Samples at 2, 6 and 10 months by the Method of Czakó and Csukas, 1961.

(b) Samples at 3, 6 and 9 months by the Method of Horn (see Witt and Walter, 1959).

(c) Samples at 4, 5 and 6 months by the Method of Lemvig 1934.

(d) Samples at 2, 5 and 8 months by the Method of Pjanovskaya 1960.

(e) Samples at 50, 150, 250 days by the Method of Senft 1958.

The previously mentioned method of Kwizda et al.1957 was not applied to these investigations in its broader application to fat content but was used only for the 300 or 305 day performance. Here, however, the performances during the actual duration of lactation should be compared. The method selected was one which gave the average daily performance from milk yields of the three testing days. The average percentage of the milk constituents were calculated from the content on the three test days. These figures were obtained by addition and division by the average milk yield performance.

For methods of a - d above, the test days lay in the middle of the corresponding months.

Lactation performances obtained by the different control methods were compared with the performance determined at 7-day intervals. These differences are an expression of the sampling error, the absolute and relative values for which are given below. (The absolute sampling error is expressed as a percentage of the values obtained by the weekly

sampling procedure). These comparative data were obtained for all 29 lactations and could be compared (by the method of Czakó and Csukas (a) above) with only 20 lactations containing 7-day samplings. The other 9 lactations could not be compared because they had ended before the middle of the 10th month.

(2) Performances by different sampling methods in relation to the methods used.

In the application of the chosen sampling methods, lactation performances were calculated and compared with the average of the methods used. The results are shown in Table 1 and show that in general there is an increase of sampling error with increasing length of the sampling period. This tendency, however, is not evident in all cases since low sampling errors were also found at long time intervals. In general, relative sampling errors are under 1% for time intervals up to 28 days, whereas in tests of periods of 42 and 56 days this value is often exceeded. Comparison of the values of these errors with those obtained by other authors is not possible because these were taken under vastly different testing conditions, namely daily samplings, monthly samplings, or 305-day samplings. The results of random sampling in general show greater sampling errors. This is particularly true with Lemvigh's method ((c) above), probably because during the tests in the middle of the lactation period (4,5 and 6 months) the lactation trends of the individual components were not considered sufficiently. The best values of the lactation performances are given obviously by the method of Czakó and Csukas (method (a) above) (2,6 and 10 months) and also that of Senft ((e) above) at 50,150,250-day intervals. Both of these methods have the

disadvantage that in the case of shorter but otherwise normal lactations no evaluation can be made. In this case the random samples should be taken from the middle of the lactation period prior to the beginning of the 10th month, because otherwise it is necessary to extrapolate to the 10th month in these short lactations. The other two methods have values of the sampling error lying intermediate between the rest. It is concluded after comparisons of the average values of all the methods that when tests are carried out at definite intervals a sampling time length of not more than 28 days is desirable. If investigations are carried out on very large populations by means of random sampling, the method of Senft with controls at 50, 150 and 250 days appears to have advantages. All these methods do not, however, tell us much about how the individual lactations can be evaluated by the different sampling methods since they are merely the average values of a number of lactations. It is, therefore, necessary to further investigate the sampling errors arising in this manner so that they may be applied to the classification of an individual animal, e.g. to the dam of a bull.

(3) Calculated performance of individual lactations obtained by different sampling methods.

The individual lactations were evaluated by the different sampling methods in the same way as the average performances for the total material had been determined. The value of the individual sampling errors were determined. The determining of the absolute sampling errors was in this case omitted since some methods give either over or under evaluation of these errors. The sampling errors were considered in relation to their standard deviations. The sampling errors using individual lactation data show somewhat higher results

than with the bulked data, but in most cases the relative errors still lie under 1% for 28-day intervals, although the random sampling methods on the whole show higher errors. The relatively small sampling errors obtained by the methods of Czaké, Csukas and Senft do not show up to the same extent as previously. In this connection the standard deviation is a reflection of the fluctuation of the individual sampling errors and is particularly interesting. With increasing sampling time intervals the standard deviation was usually greater - this shows that when there is a long period between tests the results lose certainty. When sampling times are less than 28 days, the standard deviations for the milk constituent values lies under 2%. It may further be observed that in contrast to the fat content the errors obtained with the protein estimates show considerably smaller scatter. The random sampling methods show a significantly greater scatter than those obtained using definite sampling intervals. With such random sampling methods the question of error must be taken into consideration much more frequently than when fixed interval tests are used. This statement underlines the sampling errors shown for the individual components and demonstrates the very evident truth that in none of these methods are low values to be found for all components together, and so the extent of the standard deviation must be allowed for. The fluctuations in the sampling error which have been shown to be a function of the method used raises the interesting questions as to what effect the sampling error has on individual components. For the investigation of this problem the distribution can be determined, which under normal conditions would apply to 99.73% of the cases. (The values will be between $\bar{x} \pm 3S$ where $\bar{x}$ is average value and S = standard deviation). By using the average values for the

sampling errors these limiting values were calculated for the above methods. These have been worked out for the particularly interesting data of milk yield, fat and protein, and are shown in Figure 1. The other milk constituents gave a different picture and have been left out. Even a casual glance will show that with tests taken at fixed intervals the control errors are low. The range of these errors increases in relation to milk yield and fat content with increasing intervals between sampling times. Such differences are less apparent in the case of protein content, where the span of error is less. If one accepts a maximum error range of ± 10% this will not be exceeded in the case of milk yield determinations for sampling time intervals up to 28 days and will not be exceeded for fat content determinations up to sampling time intervals of 42 days and will not be exceeded in the case of protein up to 56 days.

In the case of random sampling there are considerable variations from the average value of the sampling error. A maximum range of error of ± 10% is in all cases exceeded, though in the case of protein only on the negative side. In the field of animal husbandry it is often of importance to obtain an idea of the quality of the animal and to classify animals in ranked order and hence separate good beasts from those of lower quality. In so far as this was possible with the different sampling methods, it was applied to the above material. The performances for milk yields and milk constituents determined by weekly samples were used to obtain a ranking of the lactations. These ranks were used to compare the sampling methods. In order to obtain the numerical value for the agreement between the ranking of weekly samples the rank correlation coefficients were determined. These are

shown in Table 3 and show that samples taken at definite time intervals give a better indication of the animals behaviour than random sampling methods. Also the uncertainty of the ranking is increased as the control time interval is increased. It is further noteworthy that in the case of the coefficients for milk yields and the more important constituents, fat and protein, these coefficients always lie above +0.9 and, therefore, give a relatively good means of classifying the individual performances. Also the rank correlation coefficients of the S.N.F. are relatively high, whereas those for Lactose and Ash become smaller. Sampling intervals up to 28 days show furthermore that they are satisfactory in particular for the fat and protein content. The somewhat smaller coefficients for random sampling methods signify that these methods frequently give deviations from the correct rank order and hence a false evaluation can be attached for individual lactations.

It has not yet been possible to establish a "best" method for high coefficients for all components.

In the ranking of individual lactations a significant factor has been established. It was shown that in the case of samples taken at definite intervals the best and the worst lactations were found mostly at the top and bottom of the ranked series. In the range of the middle ranks there were often frequent deviations from the correct rank order. In the case of random sampling methods this phenomenon was less obvious, although errors in classification also occurred here in the top and bottom ranks, e.g. All these observations are shown in Table 4 where ranking in order of fat content is given. It can clearly be seen that in the case of tests taken at

predetermined intervals it is possible to determine the extremes more satisfactorily than by utilisation of random sampling controls. The relatively good results obtained for the fat content of 2, 6 and 10 month intervals by the method of Czakó and Csukas, do not show up in the same way for the other components.

The testing of individual sampling methods in relation to individual lactations has again established the already observed facts that control intervals of up to 28 days give reliable results. Longer intervals and also the use of random sampling methods lead, however, more frequently to errors which produce unreliable results in the classification of performances for selection work. In the case of random sampling methods it was not possible to determine any one method which gave better results than any other for the components which were investigated.

Conclusions

For the selection purposes it is of very great importance to establish the performance of the most important domestic animals such as bulls and their dams. The basis of such determinations are tests in which it is desired to obtain precise performance data at the lowest possible cost. There is, therefore, a general tendency to reduce as far as possible the number of samplings. This can be done by increasing the interval between sampling where a method of fixed interval samplings is used. Alternatively random sampling methods may be used at definite time intervals during a lactation. In this latter case it is important that the reliability of the data shall not be unduly in question.

In these investigations it has been shown that in the

determination of milk yields and milk composition, tests at intervals of 28 days produce the most reliable results. Longer sampling intervals and also the random sampling methods in their different combinations lead frequently to errors. Their application is, therefore, somewhat limited. The use of these methods has been in the field of the evaluation of animals in animal husbandry (daughter-mother regression,etc.) and in population genetics. These methods have also been used by other authors (Ashton, 1956; Bayley u.a., 1952; Carrèu u.a., 1959; Castle u.Searle, 1961; O'Connor u.Lipton, 1960; Erb u.a., 1956; u.a.). By these means it is possible to compare the expected deviations and the actual errors. For this problem, however, further investigations are necessary. For the testing of bulls and progeny at stations these methods do not provide quite the quality of results which are claimed to be required. In such cases sampling intervals of not more than 28 days must be used. They then allow a significantly accurate evaluation of performance to be made for a cross section of animals as well as for a relatively exact evaluation of individual performances. When testing individual bulls, not only must the average performance data be recorded but also the persistence and course of the lactation curves, and also the correlations, and then shorter time intervals are preferred. For 28-day intervals only 10-11 controls for a normal lactation are used and, as a consequence, no outstanding accuracy is claimed for these investigations. For the determination of an average for a group of animals a greater number of tests gives a higher accuracy so that in this case 28-day intervals are sufficient.

In relation to samples taken at definite time intervals

it is important, particularly in the case of milk yield tests, to take care that the sample day lies in the middle of the corresponding sample period (Campo, 1952; Erb, u.a. 1952 u. 1956; Johansson, 1942; Laben u.a. 1956; Vogel, 1932; Wendt, 1913, u.a.). Transformation to fewer days does not thereby affect the results (Dick, 1950; Thompson & Mitarb, 1960). Ashton 1956 has suggested that the length of the sample day should be 24 hours for milk yield tests. Increasing the sampling time to 48 hours improves the precision of the results to only a slight degree or not at all. (Ashton, 1956; Camp, 1952; Nagy, 1963; Vogel, 1931). Furthermore, a sampling time of 96 hours reduces the error by less than 1% for milk yields and for fat content by less than 2% (Erb et al. 1952). On this basis it does not appear necessary to make the testing day greater than 24 hours. A frequently discussed problem now remains, that is the timing of the beginning and end of the tests in relation to the complete lactation period. This problem and these questions are to be further investigated in a later study.

TABLE 1

	n	Milk yield/lactation			Milk yield/day			Fat %			Protein %			Sugar %			Ash %			S.N.F.%		
		Sampling error			Sampling error			Sampling error			Sampling error			Sampling error			Sampling error			Sampling error		
		$\bar{x}$ (kg)	abs. (kg)	relat. (%)	$\bar{x}$ (kg)	abs. (kg)	relat. (%)	$\bar{x}$	abs. (F.%)	relat. (%)	$\bar{x}$	abs. (P.%)	relat. (%)	$\bar{x}$	abs. (S.%)	relat. (%)	$\bar{x}$	abs. (A.%)	relat. (%)	$\bar{x}$	abs. (SNF %)	relat. (%)
7-Day-Interval	29	4669,7			15.3			3.83			3.51			4.74			0.77			9.00		
14-Day-Interval	29	4670,0	-0.3	±0	15.3	±0	±0	3.83	±0	±0	3.52	+0.01	+0.28	4.74	±0	±0	0.77	±0	±0	9.01	+0.01	+0.11
21-Day-Interval	29	4673,4	+3.7	+0.08	15.3	±0	±0	3.83	±0	±0	3.52	+0.01	+0.28	4.73	-0.01	-0.21	0.77	±0	±0	9.01	+0.01	+0.11
28-Day-Interval	29	4670,6	+0.9	+0.02	15.2	-0.1	-0.75	3.83	±0	±0	3.53	+0.02	+0.57	4.74	±0	±0	0.77	±0	±0	9.02	+0.02	+0.22
42-Day-Interval	29	4680,0	+10.3	+0.22	15.1	-0.2	-1.31	3.85	+0.02	+0.52	3.53	+0.02	+0.57	4.70	-0.04	-0.84	0.75	-0.22	-2.60	9.00	±0	±0
56-Day Interval	29	4686,5	+16.8	+0.36	15.2	-0.1	-0.75	3.83	±0	±0	3.57	+0.06	+1.71	4.75	+0.01	+0.21	0.76	-0.01	-1.30	9.06	+0.06	+0.67
2. + 6. + 10.Month	20				14.9	-0.1	-0.70	3.92	+0.03	+0.80	3.50	-0.01	-0.28	4.77	+0.02	+0.40	0.77	±0	±0	9.02	-0.03	-0.30
3. + 6. + 9.Month	29				14.6	-0.7	-4.98	3.79	-0.04	-1.04	3.47	-0.04	-1.14	4.77	+0.03	+0.63	0.76	-0.01	-1.30	9.01	+0.01	+0.11
4. + 5. + 6.Month	29				16.7	+1.4	+9.15	3.55	-0.28	-7.31	3.34	-0.17	-4.84	4.75	+0.01	+0.21	0.74	-0.03	-3.90	8.86	-0.14	-1.56
2. + 5. + 8.Month	29				16.7	+1.4	+9.15	3.75	-0.08	-2.09	3.41	-0.10	-2.85	4.78	+0.04	+0.84	0.76	-0.01	-1.30	8.95	-0.05	-0.56
50.+150.+ 250.Day	29				15.8	+0.5	+3.27	3.81	-0.02	-0.52	3.47	-0.04	-1.14	4.75	+0.01	+0.21	0.77	±0	±0	8.97	-0.03	-0.33

TABLE 2

Relative sampling errors using individual lactation data

	Milk yield kg/lactation		Milk yield kg/day		Fat %		Protein %		Sugar %		Ash %		S.N.F. %	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
14-Day-Interval	+ 0.08	1.36	- 0.34	1.63	+ 0.12	1.15	+ 0.17	0.85	+ 0.09	0.92	+ 0.42	1.45	- 0.02	0.74
21-Day-Interval	+ 0.08	1.60	- 0.28	1.71	+ 0.03	1.72	+ 0.33	0.89	- 0.14	1.57	+ 0.62	1.72	+ 0.12	0.80
28-Day-Interval	+ 0.17	2.09	- 0.34	3.29	+ 0.03	1.86	+ 0.77	1.45	+ 0.21	1.61	+ 0.97	1.60	+ 0.16	1.20
42-Day-Interval	+ 0.05	3.48	- 1.31	4.19	+ 0.62	2.50	+ 0.74	1.44	- 0.69	2.30	+ 0.72	2.35	+ 0.02	1.15
56-Day-Interval	+ 0.52	3.54	+ 0.31	5.62	+ 0.28	3.72	+ 1.63	1.40	+ 0.62	3.11	+ 0.03	2.14	+ 0.83	2.14
2. + 6. + 10. Month			- 0.75	11.98	+ 1.40	4.33	- 1.50	3.58	+ 0.00	4.53	± 0	5.53	+ 0.05	2.19
3. + 6. + 9. Month			- 3.10	12.28	- 1.10	7.15	- 1.17	3.49	+ 0.90	3.65	- 1.31	3.44	+ 0.21	1.86
4. + 5. + 6. Month			+ 9.31	9.20	- 6.34	7.38	- 5.48	4.86	+ 0.90	3.00	- 1.24	4.42	- 1.69	2.24
2. + 5. + 8. Month			+10.54	9.46	- 1.03	4.59	- 3.03	3.84	+ 1.45	4.11	+ 0.28	3.91	- 0.76	3.08
50. +150. + 250. Day			+ 3.80	12.16	- 0.07	4.79	- 0.97	3.57	+ 0.69	3.47	- 0.21	5.08	- 0.17	2.49

Appendix : Introduction (3)

Published observed data on the effect of non-daily short interval sampling on the accuracy of milk yield and butterfat yield estimates.

Maximum percentage difference between actual milk yield production and estimated production using periodic sampling (based in part on Carré et al.).

Author	No. of Lactations	Sampling frequency			
		7 days	15 days	21 days	30 days
Marcq & Lahaye*		3	5.93	9.75	
Martiny*		7.35			
Seidel*		14.68	17.93		
Gaertner*				18	23
Laplaud et al.*	10			8.1	
Niederhaein					17.1
Lauprecht*	252				+8.2 to -8.4
Perzco*	105	13.59	13.75	14.12	28.5
Ashton		1.47 to 3.9	2.5 to 9.5		4.69 to 9.95
Jordan*	400	-5 to +6	± 7	-11 to +7	-11 to +12
Davey	89	±10	±10		
Ashton	154	±4			
Zorn*	119			±10	-12 to +12.5
Johansson		±3.6	±5.4	±6.6	±7.8
Campbell	148	±3.3	±5.1		±11.2
McDowell*					8.3
McCarthy	350				± 9.3
Dick	52	±2.9	±3.6		± 7.2
Johansson Summary		±3.0 (N = 202)	±4.9 (N = 321)	±7.2 (N = 139)	± 7.5 (N = 188)
Cianci			±4.0		
Fisteag et al.			±8.7	±9.9	±12.0
Thompson et al.					± 7.2
Mean		6.3 (N = 12)	8.0 (N = 12)	10.5 (N = 9)	12.2 (N = 15)
S.D.		4.3	4.3	3.6	6.2

N = number of observations on which data were based

Maximum percentage difference between actual butterfat yield and estimated production using periodic sampling.

Author	No. of Lactations	Sampling frequency			
		7 days	15 days	21 days	30 days
Mareq & Labaye*		5.18	5.3		
Martiny*			9.5		
Lapland et al.*				11.7	9.9
Houston & Hale*	43	3.1		6.1	9.9
Farrington*					10.4
McDowell*					8.3
Lauprecht*	252				+14.1 to -19.5
Ashton		2.9 to 5.2	4.6 to 6.6		9.9 to 10.4
Zorn*	100				+12 to -11
Campbell	148	±8.0	±11.7		±19.4
Thompson					±9.6
Johansson		±5.1	± 6.6	± 8.4	±9.9
Mean		5.4 (N = 4)	8.3 (N = 4)	8.9 (N = 2)	12.0 (N = 9)
S.D.		2.0	2.9	4.0	4.3

* Cited by Carré et al.

Appendix : Methods (a)

Reasons for changes in cows used in different sets within breed.

The following cows used in Set 101 were not used in Set 102 for the reasons indicated:-

Cow No.	Age		
1	8 yrs.	Dried off early (3 months)	less than 16 consecutive tests available.
3	2 yrs.	Dried off early (6 months)	less than 16 consecutive tests available.
29	9 yrs.	Dried off early (3 months)	less than 16 consecutive tests available.
38	6 yrs.	Dried off early (4 months)	less than 16 consecutive tests available.
39	10 yrs.	Dried off early (7 months)	less than 16 consecutive tests available.
50	2 yrs.	Dried off early (7 months)	less than 16 consecutive tests available.
16	7 yrs.	Culled to work - persistent udder trouble.	
28	3 yrs.	Transferred to No. 2 Herd)	Production records indicate
30	2 yrs.	)	that these were not the lowest
68	8 yrs.	)	producers in the herd. Therefore,
72	8 yrs.	)	culling on production was not the prime reason for the transfer.
		)	

The following cows used in Set 102 were not available in Set 101 for the reasons indicated:-

Cow No.	Age	
17	3 yrs.	Not in the herd.
24	4 yrs.	Not in the herd.
63	3 yrs.	Not in the herd.
112	2 yrs.	Not in the herd.
114	2 yrs.	Not in the herd.

The following cows used in Set 103 were not used in Set 104 for the reasons indicated:-

Cow No.	Age	
128	3 yrs.	Dried off early (6 months) less than 16 consecutive tests available.
147	5 yrs.	Dried off early (5 months) less than 16 consecutive tests available.
155	5 yrs.	Dried off early (4 months) less than 16 consecutive tests available.
136	5 yrs.	Died.
162	4 yrs.	Died.
168	7 yrs.	Died.
159	4 yrs.	Autumn calver - less than 16 consecutive tests available.
171	3 yrs.	Autumn calver - less than 16 consecutive tests available.
187	6 yrs.	Empty.

The following cows used in Set 104 were not available in Set 103 for the reasons indicated:-

Cow No.	Age	
132	4 yrs.	Autumn calver - less than 16 consecutive tests available.
134	6 yrs.	Autumn calver - less than 16 consecutive tests available.
163	5 yrs.	Autumn calver - less than 16 consecutive tests available.
190	7 yrs.	Autumn calver - less than 16 consecutive tests available.
165	9 yrs.	late calver - less than 16 consecutive tests available.
149	3 yrs.	late calver - less than 16 consecutive tests available.
133	2 yrs.	Not in the herd.
135	2 yrs.	Not in the herd.
144	2 yrs.	Not in the herd.
146	2 yrs.	Not in the herd.
152	2 yrs.	Not in the herd.
154	2 yrs.	Not in the herd.
157	2 yrs.	Not in the herd.
170	2 yrs.	Not in the herd.
172	2 yrs.	Not in the herd.
185	7 yrs.	Carry over lactation (abnormal and too short).

Culling practices thus did not introduce the abnormal bias into the data used. Reference to the age distribution table shows a relatively uniform distribution of ages between Sets 101 and 102, and Sets 103 and 104, so that the age distribution did not unduly bias the data.

Age distribution of cows used in the study.

Set 101

2 yrs.	3 yrs.	4 yrs.	5-9 yrs.	10 yrs. and over	Total
5 (17%)	5 (17%)	3 (10%)	15 (52%)	1 (4%)	29

Set 102

2 yrs.	3 yrs.	4 yrs.	5-9 yrs.	10 yrs. and over	Total
3 (13%)	3 (13%)	5 (22%)	10 (43%)	2 (9%)	23

Set 103

2 yrs.	3 yrs.	4 yrs.	5-9 yrs.	10 yrs. and over	Total
9 (26%)	6 (17%)	7 (20%)	13 (37%)	nil	35

Set 104

2 yrs.	3 yrs.	4 yrs.	5-9 yrs.	10 yrs. and over	Total
9 (21%)	10 (24%)	4 (10%)	18 (43%)	1 (2%)	42

The data derived from these various sets were not adjusted to a mature cow equivalent.

Appendix D (a)

Regression coefficients and standard errors of estimate assembled in rank order using mean $\bar{r}$ (TOT) as basis of ranking. Data used were the combined data for Jersey cows. (Sets 101 and 102 combined).

Ranking of sampling times based on	Sampling times			S.E.of Estimate		Partial regression coefficients				
$\bar{r}$ (TOT)	l:m:n	$\bar{r}$ (TOT)	$\bar{r}$ (INT)	%	a	byl:mn	bym:ln	byn:lm	byp:lmn	R^2y:lmn
1:2:3	11:12:10	0.88	0.80	4.6	nd	1.408	7.763	5.903		0.89
4	:15	.88	.77	4.2	4.92	7.594	4.752	4.003		.91
5	: 7	.87	.77	4.6	nd	7.393	5.351	2.480		.89
6	:13	.87	.79	nd	-	-	-	-		-
7	:16	.87	.75	4.1	nd	7.957	4.877	3.411		.91
1:3:4	11:10:15	.86	.73	4.7	nd	2.423	7.775	5.976		.89
5	: 7	.85	.78	nd	-	-	-	-		-
6	:13	.85	.76	nd	-	-	-	-		-
7	:16	.84	.71	4.7	nd	2.257	8.729	4.964		.89
1:4:5	11:15: 7	.85	.69	3.4	3.70	8.987	5.995	2.300		.94
6	:13	.85	.77	nd	-	-	-	-		-
7	:16	.84	.71	4.7	nd	10.451	3.392	2.483		.89
8	: 6	.83	.66	4.5	nd	9.109	4.849	2.595		.90
1:5:6	11: 7:13	.84	.72	nd	-	-	-	-		-
7	:16	.83	.67	4.3	4.40	3.327	8.785	4.258		.91
1:6:7	11:13:16	.83	.73	nd	-	-	-	-		-
1:7:8	11:16: 6	.81	.63	4.4	nd	9.166	4.526	3.010		.90
2:3:4	12:10:15	.85	.68	4.4	nd	4.578	5.800	5.917		.90
5	: 7	.84	.74	nd	-	-	-	-		-
6	:13	.84	.71	nd	-	-	-	-		-
7	:16	.84	.65	5.1	nd	5.524	5.597	4.181		.87
2:4:5	12:15: 7	.84	.70	4.9	5.10	3.693	6.463	6.122		.88
6	:13	.84	.77	nd	-	-	-	-		-
7	:16	.83	.69	5.1	nd	8.888	5.822	1.193		.85
8	: 6	.81	.65	5.1	nd	7.417	6.039	2.958		.87
2:5:7	12: 7:16	.83	.66	5.3	5.57	6.820	4.181	4.773		.86
2:5:13	:14	.71	.64	5.5	nd	4.120	7.740	4.180		.85
2:7:8	12:16: 6	.81	.61	5.1	nd	7.475	5.298	3.608		.87
3:4:5	10:15: 7	.82	.64	4.9	4.84	3.433	4.946	7.918		.88
3:5:7	10: 7:16	.80	.60	5.6	nd	3.841	6.952	3.876		.84
4:5:7	15: 7:16	.80	.59	5.9	nd	6.403	6.407	3.473		.83
1:2:4:5	11:12:15: 7	.86	.73	3.6	4.24	1.707	6.851	4.648	3.476	.94
1:2:5:7	11:12: 7:16	.85	.71	4.1	nd	2.502	6.878	3.646	3.340	.92
1:5:7:13	11: 7:16: 4	.80	.59	4.2	3.23	6.490	3.500	4.210	2.500	.91

R^2y:lmnp - Coefficient of determination of estimated value about actual value.

$\bar{r}$ (TOT) - Mean correlation of production at individual sampling times with total.

$\bar{r}$ (INT) - Mean intercorrelation of production at individual sampling times.

Appendix D (b)

Regression coefficients and standard errors of estimate assembled in rank order using mean $\bar{P}$ (TOT) as basis of rank. Data used were the combined data for Friesian cows. (Sets 103 and 104 combined).

Ranking of sampling times based on	Sampling times			S.E. of Estimate		Partial regression coefficients				
$\bar{P}$ (TOT)	l:m:n	$\bar{P}$ (TOT)	$\bar{P}$ (INT)	%	a	byl:mn	bym:ln	bym:ln	byp:lmn	$R^2_{y:lmn}$
1:2:3	14:12:6	0.81	0.59	7.1	nd	-5.942	18.953	5.039		0.85
4	:7	.81	.61	6.3	nd	-7.033	19.568	5.947		.89
5	:10	.81	.72	4.4	1.27	-11.923	19.979	11.427		.95
6	:11	.81	.80	8.3	nd	-24.471	8.313	38.026		.84
2:3:4	12: 6: 7	.80	.68	7.4	nd	10.808	3.594	4.383		.84
2:3:7	12: 6:15	.80	.67	5.5	nd	19.321	3.291	-0.285		.90
2:3:8	12: 6:16	.80	.63	7.2	nd	7.192	5.901	6.125		.85
2:4:7	12: 7:15	.79	.66	7.4	nd	9.041	5.585	4.912		.84
2:4:8	12: 7:16	.79	.65	7.3	nd	8.829	5.829	4.820		.84
2:6:7	12:11:15	.79	.81	8.8	6.77	9.585	3.554	5.905		.78
3:6:7	6:11:15	.78	.67	8.0	nd	6.156	4.797	6.442		.81
3:6:8	6:11:16	.78	.62	7.4	nd	6.109	5.137	7.376		.84
4:6:7	7:11:15	.78	.69	8.4	4.49	5.979	5.456	6.039		.80
4:6:8	7:11:16	.78	.66	7.9	nd	5.742	6.026	6.804		.82
1:2:3:5	14:12:6:10	.81	.65	4.3	nd	-12.772	20.919	-1.076	12.400	.95
1:2:4:5	14:12:7:10	.80	.66	3.9	1.11	-10.732	18.359	2.534	9.386	.96
2:3:4:5	12: 6:7:10	.80	.70	7.3	nd	9.688	2.927	3.969	2.956	.85
2:4:6:7	12: 7:11:15	.79	.71	7.4	nd	8.370	5.447	1.425	4.017	.85
1	12	.82		10.1	nd	18.199				
2	14	.81		16.5	nd	7.821				
3	6	.80		11.1	nd	11.652				
4	7	.79		11.3	nd	11.902				
5	10	.79		11.2	nd	15.042				
6	11	.79		11.1	nd	16.125				
7	15	.77		10.8	nd	14.526				
8	16	.77		11.1	nd	15.396				

Appendix D (e)

Regression coefficients and standard errors of estimate for selected groups of sampling times. Data used were those of each set.

These results were determined primarily to have regression coefficients available for establishing average values for regression coefficients based on various combinations of sampling times. These results illustrate the variability between regression coefficients from year to year and with different combinations of sampling times and indicate clearly the problems involved in selecting a regression equation that is of universal application. In any one year it is possible for data from a single sampling time to be so highly correlated with the total production that the standard error of the estimate became extremely small, e.g. time 11 in set 101, but this same time in set 102, although just as highly correlated, gave a much bigger standard error of the estimate. Similarly no combination of the sampling times examined gave an obvious and consistent pattern of high prediction.

Jersey 1957/58 (Set 101)

Sampling times			S.E. of Estimate		Partial regression coefficients			
l:m:n	$\bar{r}$ (TOT)	$\bar{r}$ (TWT)	%	a	byl:mn	bym:ln	byn:lm	R^2y:lmn
6:10:15	0.79	0.59	5.0	4.94	4.481	5.175	9.884	0.81
6:11:15	.83	.67	4.2	4.88	3.435	9.288	2.735	.90
6:12:15	.81	.65	5.2	6.32	3.848	6.762	5.430	.84
6:10:16	.80	.59	4.8	4.86	4.068	5.133	7.089	.86
6:11:16	.83	.70	4.3	4.98	3.575	9.355	2.486	.89
6:12:16	.82	.69	5.3	6.34	3.250	6.572	5.912	.84
7:10:15	.79	.59	4.9	4.83	5.138	4.309	6.589	.86
7:11:15	.83	.67	3.5	3.45	3.940	3.889	10.579	.93
7:12:15	.81	.65	4.9	5.68	4.373	5.735	5.735	.86
7:10:16	.80	.59	4.4	4.39	5.127	4.046	7.945	.89
7:11:16	.84	.68	4.1	4.69	4.331	7.720	4.178	.90
7:12:16	.82	.66	4.8	5.26	5.039	4.704	6.872	.87
7:11:12	.86	.75	4.0	nd	2.694	8.185	3.701	.91
7:12	.83	.74	5.8	nd	5.032	8.208	-	.80
11	.92	-	2.8	nd	14.051	-	-	.95
12	.87	-	6.4	nd	11.546	-	-	.75

Jersey 1958/59 (Set 102)

6:10:15	0.80	0.62	4.2	3.98	0.013	7.647	9.854	0.92
6:11:15	.83	.64	4.3	2.74	1.903	9.094	6.921	.92
6:12:15	.83	.64	4.6	2.79	2.840	9.451	5.652	.91
6:10:16	.77	.53	5.3	4.70	2.249	7.171	6.855	.87
6:11:16	.79	.56	5.0	3.10	2.998	9.860	4.218	.89
6:12:16	.79	.52	4.2	2.63	3.475	10.137	4.417	.92
7:10:15	.84	.68	4.2	4.01	0.694	7.020	9.696	.92
7:11:15	.86	.72	4.3	3.25	2.203	8.638	6.606	.92
7:12:15	.86	.74	4.8	3.81	2.670	8.576	5.921	.90
7:10:16	.80	.62	5.5	5.31	1.855	7.577	6.250	.87
7:11:16	.82	.67	5.0	3.80	2.727	10.271	3.377	.89
7:12:16	.82	.65	4.9	3.94	2.459	10.703	3.751	.90
7:11:12	.88	.80	4.4	nd	1.100	7.815	8.126	.91
7:12	.86	.76	5.7	nd	2.724	13.152	-	.86
11	.92	-	6.0	nd	15.593	-	-	.84
12	.92	-	6.0	nd	16.176	-	-	.84

Friesian 1957/58 (Set 103)

Sampling times			S.E. of Estimate		Partial regression coefficients			
l:m:n	$\bar{r}$ (TOT)	$\bar{r}$ (INT)	%	a	byl:mn	bym:ln	byn:lm	R^2y:lmn
6:10:15	0.70	0.53	7.1	7.40	6.623	1.184	7.421	0.74
6:11:15	.70	.56	7 1	7.21	6.993	2.021	5.901	.74
6:12:15	.74	.58	6.5	5.00	5.821	7.334	3.858	.78
6:10:16	.71	.58	7.0	7.69	7.302	- 0.588	8.732	.75
6:11:16	.70	.49	6.6	6.00	6.393	3.400	6.387	.78
6:12:16	.75	.54	4.0	4.48	5.545	6.758	5.640	.81
7:10:15	.70	.55	7.2	8.42	9.133	-8.598	7.964	.73
7:11:15	.70	.60	7.4	5.54	9.388	-1.651	9.240	.72
7:12:15	.74	.58	6.5	3.15	7.157	7.308	4.059	.78
7:10:16	.71	.66	8.1	7.96	8.116	0.378	6.537	.66
7:11:16	.70	.59	9.1	10.47	12.100	-1.033	0.118	.91
7:12:16	.75	.60	6.6	3.27	6.100	9.349	3.268	.77
7:11:12	.73	.63	6.7	nd	7.180	-1.120	12.060	.77
12	.78	.-	8.7	nd	16.309	-	-	.61
16	.69	-	10.4	nd	12.565	-	-	.44

Friesian 1958/59 (Set 104)

6:10:15	0.86	0.78	7.2	1.25	3.330	10.908	5.457	0.89
6:11:15	.87	.79	7.3	0.30	3.200	12.285	5.049	.88
6:12:15	.85	.76	7.5	1.82	5.035	8.334	6.036	.87
6:10:16	.86	.74	6.2	-0.73	2.763	14.221	4.228	.91
6:11:16	.86	.75	7.2	0.05	4.358	10.795	5.676	.88
6:12:16	.84	.72	7.7	1.62	6.465	6.635	6.494	.87
7:10:15	.86	.74	6.7	1.28	3.928	12.132	3.887	.90
7:11:15	.86	.77	8.1	0.42	3.550	1.400	12.570	.85
7:12:15	.84	.74	7.6	2.97	4.632	9.072	5.571	.87
7:10:16	.85	.69	6.2	1.02	4.535	10.742	5.224	.91
7:11:16	.85	.72	6.7	0.46	4.353	11.778	4.925	.90
7:12:16	.83	.69	7.5	2.80	5.812	7.892	5.937	.88
7:11:12	.86	.75	6.3	nd	4.325	4.526	13.062	.91
12	.86	-	8.4	nd	21.965	-	-	.84
16	.84	-	11.4	nd	16.228	-	-	.71

Appendix D (d)

Regression coefficients and standard errors of estimate for selected groups of sampling times using data aggregated by years rather than by breeds.

Jersey and Friesian 1957/58 (Sets 101 and 103) combined.

Sampling times			S.E. of Estimate		Partial regression coefficients			
l:m:n	$\bar{r}$ (107)	$\bar{r}$ (1M7)	%	a	byl:mn	bym:ln	byn:lm	$R^2_{y:lmn}$
7:11:12	0.80	0.69	6.8	nd	6.313	2.293	7.420	0.76
7:11:15	.77	.64	7.0	nd	8.164	1.440	6.925	.75
7:12:15	.78	.61	6.5	nd	6.701	5.872	4.897	.79
10:11:12	.78	.69	8.2	nd	3.827	3.871	8.556	.71

Jersey and Friesian 1958/59 (Sets 102 and 104) combined.

7:11:12	0.87	0.74	5.2	nd	1.960	22.400	-1.890	0.94
7:11:15	.86	.74	6.8	nd	3.232	11.751	4.904	.89
7:12:15	.85	.74	7.2	nd	4.200	9.060	5.735	.87
10:11:12	.90	.85	7.4	nd	6.307	9.445	5.190	.87

Appendix D (e)

Estimation of relationship between changes in correlation with total production and changes in intercorrelations and the effect on the standard error of the estimate.

Using manually calculated data

In any set or combination of sets, whenever various combinations of sampling times gave the same standard error of the estimate, the difference between the values for $\bar{r}$ (TOT) and $\bar{r}$ (INT) for each combination of sampling times was determined and the following relationship was established using thirty-one comparisons:-

$\bar{r}$ (INT) = 1.66 $\bar{r}$ (TOT) + 2.60 (Standard error of estimate 0.39 units)

The value of this relationship lies in the improvement it should introduce in the ability to select data most likely to give the best prediction equations. However its value when applied to an entirely fresh set of data has not been examined and furthermore the correlation coefficient relating the changes in $\bar{r}$ (TOT) with those in $\bar{r}$ (INT) was only 0.63 and so the changes are only moderately correlated. Nevertheless, the relative importance of the effect of changes in these values is useful, e.g. an increase of $\bar{r}$ (TOT) by 0.01 will generally mean an improved prediction provided $\bar{r}$ (INT) has not also increased by about 0.04.

Appendix E (a)

Regression coefficients and their standard errors of estimate for sampling times retained as contributing significantly to the prediction of production when prescribed data were processed by TRAP procedure.

Group 1 : Selected from sampling times considered likely to be significant on basis of manual calculation - sampling times 4,6,7,10,11,12,14,15,16 were used.

Group 2 : All odd periods.

Group 3 : All even periods.

Group 4 : Sampling times remaining in Groups 2 and 3 for each set. Examined set by set.

Group 5 : Sampling times selected in Group 4 for set 101 were tested for set 102 and vice versa and similarly for sets 103 and 104.

Group 1

Set No.	Sampling times	S.E. of Estimate %	byl:unp	bya:lnp	bya:lnp	byp:lnm	byq	byu
101	4:7:11:16	3.8	2.98	4.98	6.08	6.22		
102	4:6:7:12:14	2.9	2.59	2.06	2.90	5.87	6.29	
103	4:6:12:16	5.6	2.57	4.42	6.95	7.57		
104	4:7:10:14:16	5.4	3.14	2.96	6.98	3.70	3.87	
101/102	4:6:7:11:14:16	4.3	2.13	2.63	3.62	5.43	2.99	2.89
103/104	4:6:7:12:14:16	5.9	2.65	2.29	3.22	5.25	3.28	3.00

Group 2

101	5:7:11:15	4.7	5.34	3.57	6.77	3.70		
102	3:7:11:13:15	3.3	2.96	2.18	4.99	5.97	3.92	
103	3:7:15	5.3	5.19c	6.11	8.63			
104	1:5:11:15	6.2	2.73	3.19	7.93	6.37		

Group 3

101	4:8:10:12:16	4.7	2.64	5.78	2.64	3.30	6.27	
102	2:4:12:16	3.4	5.50	1.99	9.53	4.76		
103	4:6:12:16	5.6	2.57	4.42	6.95	7.57		
104	4:8:10:14:16	5.6	3.00	3.49	5.45	4.76	4.30	

Group 4

101	5:8:11:12	3.8	4.58	5.03	5.96	3.36		
102	3:7:12:13:15	2.9	3.70	1.86	7.06	3.67	3.64	
103	3:4:12:16	4.7	4.55	1.94	5.81	9.30		
104	5:10:16	5.8	5.35	9.39	5.54			

Group 5

101	2:7:11:12	4.8	3.95	4.24	7.03	3.56		
102	8:10:12:15	4.0	3.46	5.46	5.10	6.65		
103	1:4:11:16	6.0	4.57	2.10	5.61	8.39		
104	4:7:12:15	6.4	4.83	2.94	7.03	5.39		

Appendix E (b) (i)

Examination of "t" test rejection of variables using the TRAP procedure

The basis on which the "t" test rejects variables in this programme was examined, but it was not possible to determine unequivocally just why the rejected variable gave a low "t" value. If it had been possible to establish this basis, then it was proposed to pre-select variables for the programme and so reduce the number of computations necessary. It was considered that the major factor contributing to a rejection would be the inter-correlation between the variables and this was examined using the Group 1 data from sets 101 and 102 combined.

There was no consistent pattern in the rejection, e.g. variable 16 was discarded in favour of variable 15, despite the fact that this resulted in a higher intercorrelation between the remaining variables than would have been the case if variable 15 had been discarded in favour of variable 16. With variables 16 and 14 discard was in favour of a lower intercorrelation in four out of five cases, but in the fifth case this was reversed. The table below summarizes the observed changes.

Variables			Intercorrelation between retained variables	
			Higher	Lower
16	discarded in favour of	15	10	Nil
16	"	14	Nil	4
14	"	16	1	Nil
15	"	14	1	3
14	"	15	1	Nil
10	"	11	9	1
12	"	11	2	4

In almost every case discard of the variable resulted in a considerable increase in the coefficient of the favoured variable. Correlation between each of the two variables and the total production did not appear to be directly involved since discarding was not consistently in favour of either variable.

Appendix E (b) (2)

Relationship between "t" values of variables and the standard error of the estimate.

Using the combined data for sets 101 and 102 and sampling times 3,4,7,11,15, a comparison of the standard error of estimate and the "t" test of the sampling times retained shows that substitution of sampling times with low "t" values for those with higher "t" values generally resulted in increasing standard errors of estimate.

Sampling times	"t" value	Sampling times				
3	2.34					
4	2.48	4	4	7	7	7
7	4.15	7	7	11	11	11
11	4.68	11	11	14	14	15
14	2.57	14	15	15		
15	2.36					
Mean "t" value based on "t" values listed		3.47	3.42	3.44	3.88	3.73
S.E. of Estimate 4.45		4.7	4.9	5.3	5.4	5.7

The trend of increasing standard error of estimate with lower than "t" values is apparent but not consistent.

An examination on similar lines of the combined data for set 101/102. Group 1 shows the same trend.

Sampling times	"t" value	Sampling times											
4	2.94												
6	3.12	6	4	6	4	4	4	6	4	4	6	7	7
7	4.21	7	7	7	7	6	6	11	6	6	11	11	11
11	4.14	11	11	11	11	7	11	14	7	11	14	14	14
14	2.38	14	14	16	16	16	14	16	11	16			16
16	2.19												
Mean "t" value based on "t" value listed		3.46	3.42	3.42	3.37	3.12	3.15	2.96	3.60	3.10	3.21	3.58	3.23
S.E. of Estimate 4.3%		4.6	4.7	4.89	4.94	5.1	5.1	5.3	5.3	5.3	5.32	5.4	5.4

With this series discard was not strictly in order of increasing "t" values, nor was the least standard error associated with sampling times 4,6,7,11 as would have been expected. Interaction between individual sampling times is obviously affecting the selection.

Appendix F (b) (1)

Derivation of values for corrected sums of products and corrected sums of squares used to compute the regression coefficients for sampling times grouped as follows:- 6 and 7; 10,11 and 12; 15 and 16; 17 (total)

Corrected sums of squares and sums of products derived from correlation matrix

	Set 101/102	Set 103/104		Set 101/102	Set 103/104
6:10	1.5370	5.5649	10:15	1.0598	4.0087
6:11	1.2865	4.5367	10:16	1.0060	3.9990
6:12	1.3260	4.2342	11:15	1.1374	4.5739
7:10	1.7230	5.0966	11:16	1.0943	3.7896
7:11	1.4523	4.4191	12:15	1.1446	4.0661
7:12	1.5154	3.900	12:16	1.1173	3.7922
Mean value	1.4725	4.6253	Mean value	1.0932	4.0516
6:15	0.9593	4.8678	6:6	2.1572	9.6646
6:16	0.8142	4.1028	7:7	2.2457	9.1084
7:15	1.0336	4.9427	Mean value	2.2015	9.3865
7:16	0.9828	4.3318			
Mean value	0.9475	4.5613	10:10	2.5156	5.7771
			11:11	1.7506	5.0671
15:15	1.3502	6.4202	12:12	1.8719	4.3957
16:16	1.5019	5.5528	Mean value	2.0406	5.0800
Mean value	1.4261	5.9865			
			10:17	26.7470	86.8980
6:17	21.9693	112.6090	11:17	24.8696	81.6950
7:17	24.4143	108.4040	12:17	24.7012	72.8220
Mean value	23.1918	110.5065	Mean value	25.4393	82.8050
15:17	19.4801	93.2600	17:17	621.5916	2070.568
16:17	19.2782	85.4930			
Mean value	19.3792	89.3765			

Comparison of standard error of the estimate, the difference in lbs. of protein and the percentage difference in protein production predicted from the actual protein production when the partial regression coefficients based on mean-data for 6 and 7; 10,11 and 12;15 and 16, either for all data or for set 101/102 combined or for set 103/104 combined were applied to individual groupings of data in the individual sets as indicated.

	Set 101			Set 102		
TOTAL PRODUCTION :	8638 lbs.			TOTAL PRODUCTION :	6558 lbs.	
Sampling times and mean-data regression coefficients used.	Protein production predicted			Protein production predicted		
	S.E. of Estimate	lbs. diff. from Actual	% Diff.	S.E. of Estimate	lbs. diff. from Actual	% Diff.
	%			%		
6;10;15						
101/102	3.9	151	1.7	4.7	266	4.1
All-data	3.1	563	6.1	4.2	545	8.3
6;11;15						
101/102	4.4	48	0.6	5.2	228	3.5
All-data	3.8	473	5.5	4.9	512	7.8
6;12;15						
101/102	4.3	-202	2.3	6.4	99	1.5
All-data	3.1	395	4.6	5.1	412	6.3
6;10;16						
101/102	4.2	15	0.2	4.6	144	2.2
All-data	3.5	420	4.9	4.0	415	6.3
6;11;16						
101/102	4.7	-87	1.0	5.1	108	1.6
All-data	4.2	329	3.8	4.7	385	5.8
6;12;16						
101/102	4.5	-336	3.9	6.3	-7	0.1
All-data	3.4	251	2.9	4.9	283	4.3
7;10;15						
101/102	3.9	45	0.5	4.7	201	3.1
All-data	2.5	399	4.6	1.8	444	6.8
7;11;15						
101/102	4.4	-59	0.7	5.4	165	2.5
All-data	3.2	308	3.6	3.4	412	6.3
7;12;15						
101/102	4.3	-308	3.6	5.7	50	0.8
All-data	3.0	230	2.7	3.1	314	4.8
7;10;16						
101/102	4.1	-90	1.0	4.5	81	1.2
All-data	3.0	241	2.8	1.2	314	4.8
7;11;16						
101/102	4.6	-193	2.2	3.4	43	0.7
All-data	3.6	150	1.7	5.3	281	4.3
7;12;16						
101/102	4.5	-441	5.1	5.5	-71	1.1
All-data	3.4	73	0.8	3.2	181	2.8

Appendix F (b) (2) continued.....

	Set 103			Set 104		
TOTAL PRODUCTION :	13,847 lbs.			TOTAL PRODUCTION : 16,698 lbs.		
Sampling times and mean-data regression coefficients used.	Protein production predicted			Protein production predicted		
	S.E. of Estimate	lbs. diff. from Actual	% Diff.	S.E. of Estimate	lbs. diff. from Actual	% Diff.
	%			%		
6;10;15						
103/104	5.2	146	1.1	8.1	518	3.1
All-data	5.6	-32	0.2	8.5	153	0.9
6;11;15						
103/104	5.4	270	2.0	8.5	438	2.6
All-data	5.7	92	0.7	8.8	71	0.4
6;12;15						
103/104	5.8	-192	1.4	8.4	332	2.0
All-data	6.1	-371	2.7	8.8	-35	0.2
6;10;16						
103/104	5.3	-7	0.1	9.9	294	1.8
All-data	5.6	-186	1.4	9.3	-73	0.4
6;11;16						
103/104	5.4	118	0.8	9.6	213	1.3
All-data	5.9	-62	0.5	9.6	-154	0.9
6;12;16						
103/104	5.8	-346	2.5	9.9	108	0.6
All-data	6.2	-525	3.8	9.6	-259	1.6
7;10;15						
103/104	6.5	-70	0.5	7.7	-148	0.9
All-data	6.7	-234	1.7	8.1	-472	2.8
7;11;15						
103/104	6.6	93	0.4	8.1	-230	1.4
All-data	6.9	-109	0.8	8.7	-553	3.3
7;12;15						
103/104	6.9	-409	3.0	8.8	-335	2.0
All-data	7.1	-573	4.2	8.5	-659	3.9
7;10;16						
103/104	6.5	-223	1.6	8.5	-372	2.2
All-data	6.7	-388	2.8	9.1	-697	4.2
7;11;16						
103/104	6.6	-99	0.7	8.9	-454	2.7
All-data	6.9	-263	1.9	9.3	-777	4.7
7;12;16						
103/104	7.0	-560	4.1	8.9	-559	3.4
All-data	7.2	-727	5.3	9.4	-883	5.3

Appendix F (d)

Comparison of standard error of the estimate, the difference both as percentage and in pounds of protein from actual production using coefficients for 6 and 7; 10,11 and 12; 15 and 16 based on mean regression coefficients for sets 101/102 and 103/104, and for all-data and applied to groups of sampling times as indicated.

(NOTE : Standard error of estimate opposite group combination is "Actual". Standard error of estimate based on the specific regression equation for this data).

Production - Set 101: 8638 lbs. Set 102: 6552 lbs. Set 103: 13,874 lbs. Set 104: 16,698 lbs.

Sampling time combinations and mean regression coefficients used.	Set 101			Set 102			Set 103			Set 104		
	S.E.of Estimate	lbs. diff. from Actual	% Diff.	S.E.of Estimate	lbs. diff. from Actual	% Diff.	S.E.of Estimate	lbs. diff. from Actual	% Diff.	S.E.of Estimate	lbs. diff. from Actual	% Diff.
	%			%			%			%		
6:10:15	5.6			4.2			7.1			7.2		
101/102 or 103/104	3.4	66	0.8	5.2	204	3.1	5.0	350	2.5	8.3	492	2.9
All-data	6.6	421	4.9	4.2	456	7.0	6.2	-275	2.0	9.4	-178	1.1
6:11:15	4.2			4.3			7.1			7.3		
101/102 or 103/104	4.1	-48	0.6	5.9	163	2.5	5.2	484	3.5	8.8	398	2.4
All-data	9.5	312	3.6	5.0	417	6.4	6.4	-126	0.9	9.9	-276	1.7
6:12:15	5.2			4.6			6.5			7.5		
101/102 or 103/104	3.9	-320	3.7	6.2	38	0.6	5.8	-53	0.4	8.7	275	1.7
All-data	8.8	49	0.6	5.3	296	4.5	6.8	-684	4.9	9.8	-403	2.4
6:10:16	5.4			5.3			7.0			6.2		
101/102 or 103/104	3.7	-64	0.7	5.1	86	1.3	4.2	113	0.8	9.0	308	1.9
All-data	7.9	297	3.4	4.0	344	5.3	5.5	-407	2.9	10.1	-371	2.2
6:11:16	4.3			5.0			6.6			7.2		
101/102 or 103/104	4.3	-178	2.0	5.8	46	0.7	4.4	257	1.9	9.5	214	1.3
All-data	10.5	188	2.2	4.9	305	4.7	5.7	-258	1.9	10.5	-469	2.8
6:12:16	5.3			4.2			4.0			7.7		
101/102 or 103/104	4.2	-450	5.2	6.0	80	1.2	5.0	-280	2.0	9.4	120	0.7
All-data	9.9	-74	0.9	5.2	184	2.8	5.9	-816	5.9	10.5	-596	3.6
7:10:15	5.4			4.2			7.2			6.7		
101/102 or 103/104	3.3	-33	0.4	4.3	144	2.2	6.3	214	1.6	7.9	-208	1.3
All-data	6.4	279	3.2	2.4	369	5.6	7.1	-449	3.2	9.1	-714	4.3
7:11:15	3.5			4.3			7.4			7.0		
101/102 or 103/104	4.8	-146	1.7	5.2	103	1.6	6.5	358	2.6	8.4	-302	1.8
All-data	9.3	170	2.0	3.7	330	5.0	7.2	-300	2.2	9.6	-812	4.9
7:12:15	4.9			4.8			6.5			7.6		
101/102 or 103/104	3.9	-418	4.8	5.5	23	0.3	7.0	-179	1.3	8.3	-425	2.5
All-data	8.7	-93	1.1	4.1	209	3.2	7.6	-858	6.2	9.5	-939	5.6
7:10:16	4.9			5.3			8.1			6.2		
101/102 or 103/104	3.7	-163	1.9	4.9	26	0.4	5.7	-14	0.1	8.6	-392	2.4
All-data	8.7	159	1.8	2.0	257	3.9	6.4	-581	4.2	9.8	-907	5.4
7:11:16	4.1			5.8			9.1			6.7		
101/102 or 103/104	4.3	-276	3.2	5.1	15	0.2	5.9	132	1.0	9.1	-486	2.9
All-data	10.3	46	0.5	3.5	218	3.3	6.6	-452	3.1	10.3	-1088	6.0
7:12:16	4.8			4.9			6.6			7.5		
101/102 or 103/104	4.1	-548	6.3	5.4	140	2.1	6.3	-486	2.9	9.8	-580	3.5
All-data	9.7	-216	2.5	3.9	91	1.4	7.1	-990	7.1	10.2	-1132	6.8

Appendix E (f)

H.I.A., O.H.T., and C.O.R. results contrasted with each other and with actual yields - 50 lactations - After Campbell.

		Milk Yield	Fat %	Fat Yield
Average percentage error disregarding sign	H.I.A.	2.53	4.57	4.64
	O.H.T.	2.39	4.04	4.62
	C.O.R.	-	-	3.94
Average percentage error allowing for sign	H.I.A.	-1.69 ± 2.79*	+2.38 ± 5.49*	+0.72 ± 6.91*
	O.H.T.	+1.15 ± 2.73*	-1.15 ± 5.29*	+0.02 ± 6.39*
	C.O.R.	-	-	-1.26 ± 5.18*
Percentage cases within ±5% of actual	H.I.A.	84%	68%	64%
	O.H.T.	94%	66%	64%
	C.O.R.	-	-	68%
Range percentage error	H.I.A.	+6.65 to - 6.86	+17.17 to -16.84	+17.03 to -21.37
	O.H.T.	+5.86 to - 7.48	+ 9.59 to -14.96	+11.48 to -19.96
	C.O.R.	-	-	+ 9.65 to -14.97

* Standard deviation

H.I.A. : Herd Improvement Association testing procedure.

C.O.R. : Certificate of Record - milk yield determined daily and fat % monthly.

O.H.T. : Official herd test - milk yield and fat % determined at monthly intervals.

Appendix G (a)

Ranking of individual cows identified by herd test number using sampling times 4,7,11,16 and appropriate computer derived regression coefficients.

Set 101

Rank order of cows identified by herd test numbers

Production levels	Predicted	Actual	Predicted on common sampling date basis
lbs. protein ± 14 in 5% increments based on mean values.			
< 16	43	30	43,90
16-17	30,38	43	30
17-18	96,28,50	38,96,50	16,28,96
18-19	16,74,52	28	52,69,74
19-20	21,3	52,16	3,21,50
20-21	69,22	22,3,74,21,48	22,100,29
21-22	72,100,48,1	69,33,18,1	1,25,48
22-23	18,47,33	72,39,100,5,29,25	47,72
23-24	39,29,5,75	47	4,5,75
24-25	25	4	18,81
25-26	68,81,2,4	75,68,81,2	39,68
26-27	-	-	33
27-28	-	-	2

Set 103

20.7 - 22.1	128,160,142	160,128,142
22.1 - 23.5	137	129,137
23.5 - 24.9	129	138,161,148
24.9 - 26.3	143,138,156,158,127,169	127,158,143
26.3 - 27.7	148,187	169,130,156
27.7 - 29.1	147,161,139,130,131,180,171	147,187,141, 131,162,167
29.1 - 30.5	162,166,141,145,155,168	168,145,155,139,171
30.5 - 31.9	159,167,179	150,180,166
31.9 - 33.3	178,136	178
33.3 - 34.7	153,150	159,136,170,153

Absolute rank order is given by reading the cow numbers from left to right in each 5% incremental group.

Appendix 6 (c)

Comparison of ranking of cows which were common to both sets 101 and 102, ranking in terms of actual production.

	Absolute ranking		5% incremental group ranking	
Rank No.	Set 101	Set 102	Set 101	Set 102
1	43	5	43	5
2	96	74	96	74
3	32	22	32	22,43
4	22	43	22,74,21,48	96
5	74	96	33,18	48,18,33,75
6	21	48	100,5,25	21,32
7	48	18	47	100
8	33	33	4	4,47
9	18	75	75,68,81,2	68,2,25
10	100	21		81
11	5	32		
12	25	100		
13	47	4		
14	4	47		
15	75	68		
16	68	2		
17	81	25		
18	2	81		

Comparison of ranking of cows which were common to both sets 103 and 104, ranking in terms of actual production.

	Absolute ranking		5% incremental group ranking	
Rank No.	Set 103	Set 104	Set 103	Set 104
1	160	137	160	137
2	142	129	142	129
3	129	127	129,137	127,158
4	137	138	138,161,148,126	126
5	138	126	127,158,143	169,141
6	161	169	169,130,156	148,142,163,161
7	148	141	141,131,167	131,178,150
8	126	148	145,139	160,156,139,158,180
9	127	142	150,180,166	167
10	158	143	178	166,130
11	143	161	179,153	179
12	169	131	140	145,153
13	130	178		140
14	156	150		
15	141	160		
16	131	156		
17	167	139		
18	145	158		
19	139	180		
20	150	167		
21	180	166		
22	166	130		
23	178	179		
24	179	145		
25	153	153		
26	140	140		

Appendix G (a) (1)

Cows differing in rank by three or more using different sampling time combinations and mean-data regression coefficients for 6 or 7; 10,11 or 12; 15 or 16 on set 101 data for prediction.

	No. of ranks displaced in various combinations of sampling times	
Cow No.	Set 101/102 Regression Coefficients	All-data coefficients
1		6:11:16 3+
2		6:10:15 3+
18		6:10:15 3+ 7:10:15 3+
21	7:12:15 3-	
30	7:10:15 3+ 7:11:15 3+ 7:10:16 3+	7:10:15 3+ 7:11:15 3+ 7:10:16 3+
33		6:10:15 3+ 6:11:15 3+ 6:12:15 3+
39		6:10:15 3+ 6:11:15 3+ 6:12:15 3+
43	6:10:16 3- 7:10:15 3- 7:10:16 3-	
48		6:10:15 3+ 6:11:15 3+ 6:10:16 3+ 6:11:16 4+ 6:12:16 3+
69	6:12:15 4- 6:11:16 3- 6:12:16 5- 7:12:15 4- 7:12:16 4-	6:12:15 3- 6:12:16 4- 7:12:16 3-

Appendix G (e) (2)

Cases differing in rank by three or more using different sampling time combinations and mean-data regression coefficients for 6 and 7; 10,11 and 12; 15 and 16, on set 103 data for prediction.

Case No.	No. of ranks displaced in various combinations of sampling times: Set 103/104 regression coefficients	All-data regression coefficients
136	6:12:15 (3-) 7:12:15 (3-) 7:12:16 (3-)	6:11:15 (3-) 6:12:15 (3-) 6:12:16 (3-) 7:11:15 (3-) 7:12:15 (3-) 7:11:16 (3-) 7:12:16 (3-)
139	7:12:16	7:11:16 (3-) 7:12:16 (3-)
140		6:11:15 (3-) 6:12:15 (3-) 7:11:15 (3-) 7:12:15 (3-)
147	7:10:16 (3-) 7:12:16 (3-)	7:10:16 (3-) 7:11:16 (3-) 7:12:16 (3-)
148	6:10:15 (4+) 6:11:15 (4+) 6:12:15 (4+) 6:10:16 (3+) 6:11:16 (3+) 6:12:16 (3+) 7:10:15 (4+) 7:11:15 (4+) 7:12:15 (4+) 7:10:16 (3+) 7:11:16 (3+) 7:12:16 (3+)	6:10:15 (4+) 6:11:15 (3+) 6:12:15 (3+) 6:10:16 (3+) 7:10:15 (4+) 7:11:15 (3+) 7:12:15 (3+) 7:10:16 (3+)
150	6:10:15 (4+) 6:11:15 (5+) 6:12:15 (3+) 6:10:16 (4+) 6:11:16 (4+) 7:10:15 (4+) 7:11:15 (5+) 7:12:15 (3+) 7:10:16 (4+) 7:11:16 (4+)	6:10:15 (4+) 6:11:15 (4+) 6:10:16 (3+) 6:11:16 (4+) 7:10:15 (4+) 7:11:15 (4+) 7:10:16 (3+) 7:11:16 (3+)
159	All combinations 3- or 4-	All combinations 3, 4- or 5-
161	7:10:16 (3+) 7:11:16 (3+)	
168	6:12:15 (3-)	6:12:15 (3-) 6:12:16 (3-) 7:12:15 (3-)
169	6:11:15 (3+) 6:10:16 (3-) 7:11:15 (3+) 7:10:16 (4-)	6:11:15 (3+) 6:10:16 (4-) 7:11:15 (3+) 7:10:16 (4-)
178		7:10:16 (3-)
179	7:11:16 (3-) 7:12:16 (3-)	7:11:15 (3-) 7:10:16 (3-) 7:11:16 (4-) 7:12:16 (4-)
180	7:11:15 (3-) 7:12:15 (3-) 7:11:16 (3-) 7:12:16 (3-)	7:11:15 (4-) 7:12:15 (3-) 7:10:16 (3-) 7:11:16 (4-) 7:12:16 (4-)

Appendix G (f)

Cows differing in rank by three or more using mean regression coefficients for Set 101/102 for prediction

Cow No.	Set 101	Set 102
43	6:10:15 3- 6:10:16 3- 7:10:15 3- 7:10:16 4-	
69	6:12:15 4- 6:11:16 3- 6:12:16 5- 7:12:15 4- 7:11:16 3- 7:12:16 5-	
32		6:10:15 3+
5		7:10:15 3-
2		6:10:16 3-

Cows differing in rank by three or more using mean regression coefficients for Set 103/104 for prediction

Cow No.	Set 103	Set 104
136	7:12:15 3- 7:12:16 3-	
139	7:12:16 3-	
140	7:11:15 3-	
141	6:11:16 3-	
147	7:11:15 3+	
148	all times 3+ or 4+	
150	all times except 6:12:16, 7:12:15, 7:12:16, 3+, 4+ or 5+	
159	all times 3- or 4-	
161	7:10:15 3+ 7:10:16 3+ 7:11:16 3+	
166	6:10:15 3+	
169	6:11:15 3+ 6:10:16 4- 7:11:15 3+ 7:10:16 4-	
179	7:11:16 3- 7:12:16 3-	
180	7:11:15 3- 7:12:15 3- 7:10:16 3- 7:11:16 3- 7:12:16 3-	
126		6:11:16 3+ 6:12:16 3+
134		7:10:15 4- 7:11:15 4- 7:12:15 5- 7:10:16 4- 7:11:16 4- 7:12:16 4-
137		all times 3+ or 4+
138		6:10:15 3+ 6:11:15 3+ 6:12:15 3+
153		7:10:15 3- 7:10:16 3-
154		6:10:15 3+
157		6:11:15 3+ 6:12:15 3+ 6:10:16 3+ 6:11:16 3+ 6:12:16 3+
163		all times 4-, 5- or 6-
170		6:10:15 3+ 6:11:15 3+
172		6:10:15 3+
185		6:12:15 3- 6:10:16 4- 6:11:16 3- 6:12:16 5- 7:10:16 4- 7:12:16 4- 7:11:16 3-
190		7:11:15 3- 7:12:15 4- 7:10:16 3- 7:11:16 4- 7:12:16 4-