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DETERMINATION OF BOVINE PROLACTIN BY CHARCOAL-DEXTRAN RADIOIMMUNOASSAY

RITVA MÄKELÄ and VAPPU KOSSILA

MÄKELÄ, R. & KOSSILA, V. 1976. **Determination of bovine prolactin by charcoal-dextran radioimmunoassay.** Ann. Agric. Fenn. 15: 145—162. (Agric. Res. Centre, Inst. Anim. Husb., 01300 Vantaa 30, Finland.)

Antibodies produced during this research were immunologically active in radioimmunoassay. Iodination of bovine prolactin was carried out by the chloramine-T technique. Labelled hormone possessed sufficient immunological activity and specific activity for radioimmunoassay. Determination of the percentage incorporation of iodine-125 into bovine prolactin was found satisfactory when based on the weights of the peaks of the iodination curve. Dextran coated charcoal was suitable for separation of the antibody-bound and free bovine prolactin. The assay conditions in this research resulted in a standard curve sensitive between the concentration levels of 0.05—0.8 ng bovine prolactin.

Index words: bovine, prolactin, radioimmunoassay.

INTRODUCTION

The purpose of this research was to apply radioimmunoassay of bovine blood prolactin in practice. The first reports on radioimmunoassay of bovine prolactin were published by SCHAMS and KARG (1969) and JOHKE (1969). The present paper gives the results of immunological activity in the antisera and labelled bovine prolactin produced during this research as well as the incubation conditions, separation of the antibody-bound and free hormone, and the standard curve. The work was part of NKJ project number 22, »Investigations on the interrelations between the hormone activity and productional capacity of domestic animals.»

METHODS AND MATERIALS

Hormone preparation

Bovine prolactin (NIH-P-B2) used for labelling, for production of antiserum and as a standard hormone was a gift from Dr. A.E. WILHELMI, National Institutes of

Health, Emory University, Atlanta, Georgia 30322, U.S.A. According to the information of the donor, its biological activity was approximately 19.9 IU/mg.

Antisera

Antisera were produced in rabbits and guinea pigs, mainly according to CLAUSEN's (1969) immunization schedule. Injections (0.5—5.0 mg NIH-P-B2/rabbit and 0.5—1.0 mg NIH-P-B2/guinea pig) were given subcutaneously in several sites near the lymphatic nodules of both species. Rabbit blood samples were taken from the central artery of the ear. Guinea pig blood samples were taken by heart puncture. Time tables for injections and bleedings for rabbits and guinea pigs are shown in Figs. 1 and 2.

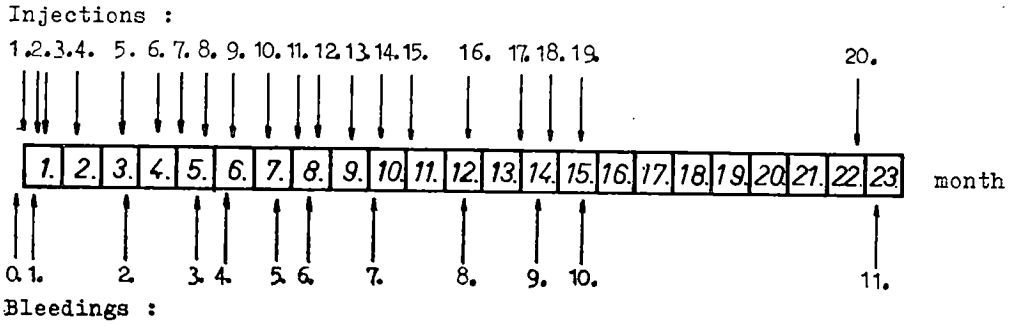

Fig. 1. Schedule of injections and bleedings for the rabbits.

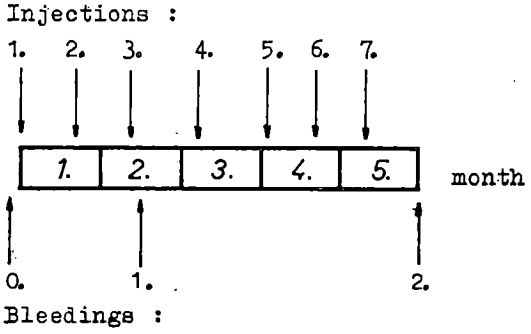

Fig. 2. Schedule of injections and bleedings for the guinea pigs.

One rabbit antiserum against bovine prolactin was a gift from Schams and Karg at the Institute of Physiology, Technical University of Munich, Weihenstephan. Antisera were stored at —20° C.

Purification of the rabbit antiserum (1 ml) was made by absorption, using lyophilized bovine brain (50 mg), liver (50 mg) and kidney tissue (50 mg), and serum (30 mg), either separately or combined.

Iodination

Iodination (labelling) of the bovine prolactin was carried out by the chloramine-T technique (GREENWOOD et al. 1963). 1—2 mCi of NaI-125 (Radiochemical Centre, Amersham) was used for each iodination. Reaction time was 20 or 30 sec. The labelled prolactin was purified by Sephadex G-100 gelfiltration (column 1 × 25 cm).

Repurification of the labelled prolactin was done by gelfiltration with Sephadex G-200 (column 1 × 25 cm). Both columns were presaturated with BSA. The elution buffer was 0.07 M veronal buffer, pH 8.6. Relative radioactivity of each fraction (8 drops) was measured with automatic gamma sample counters (Wallac, GTL$^{300-1000}$ or LKB-Wallac 1280 UltroGamma) to determine the elution pattern (iodination curve) of the radioactive subjects. Peaks of the elution pattern indicate in which fraction or fractions various radioactive materials are found.

To determine what amount (nCi) of the radioactivity is responsible for a given amount (ng) of the labelled hormone, the percentage incorporation of I-125 into the hormone had to be clarified immediately after purification of the labelled hormone. Two methods (a) and (b) were tested to determine the percentage incorporation of I-125 into the hormone.

Both methods were based on the weights of the peaks in the iodination curve. The hormone and iodine peaks of the iodination curve were cut off from the paper and weighed.

Method (a): In this method adsorption of free and bound I-125 into the reaction vial, pipette and column (Sephadex G-100) was assumed to take place to the same extent. The percentage incorporation (I %) of I-125 was estimated in the following way.

$$I\ \% = \frac{100 \cdot WHP}{(WHP + WIP)} \qquad a)$$

WHP = weight of the hormone peak (g)
WIP = weight of the iodine peak (g)

Method (b): In this method weights of the hormone and iodine peaks were corrected according to GREENWOOD et al. (1963). He found that when 2.0 mCi of I-131 was used in an iodination process the hormone peak contained 35.1 % of the hormone-bound iodine and the iodine peak contained 96 % of the free iodine. Thus the weights of the hormone and iodine peaks were corrected as follows:

$$CWHP = \frac{100 \cdot WHP}{35.1}$$

$$CWIP = \frac{100 \cdot WIP}{96}$$

CWHP = corrected weight of the hormone peak (g)
CWIP = corrected weight of the iodine peak (g)

The percentage incorporation of I-125 was then calculated in the following way:

$$I\ \% = \frac{100 \cdot CWHP}{(CWHP + CWIP)} \qquad b)$$

When the percentage incorporation of I-125 into the hormone and the amount of I-125 and hormone used in an iodination are known, the specific activity of the labelled prolactin can be calculated in the following manner:

$$SA = \frac{I\ \% \cdot AI}{100 \cdot AH}$$

SA = specific activity of the labelled hormone (μCi/μg)
AI = amount of I-125 (μCi)
AH = amount of hormone (μg)

Incubation

The incubation buffer used was 0.07 M veronal buffer, pH 8.6, containing 0.5 % BSA and 0.15 M NaCl. This buffer was used to dilute the standard and the labelled prolactin. Incubation buffer containing 1—3 % NRS was used to dilute the antiserum.

Incubation procedure for the antiserum dilution curve differed from that for the standard curve. In the former case 100 μl of diluted antiserum, 100 μl of the diluted labelled hormone and 500 μl of incubation buffer were incubated for 1—2 days at +4° C. In the latter case 100 μl of the standard hormone solution, 100 μl of diluted antiserum and 500 μl of incubation buffer were incubated overnight at +4° C. Then 100 μl of the diluted labelled prolactin was added. Incubation was allowed to continue for four days at +4° C. When guinea pig antiserum was used for the standard curve, only 100 μl of incubation buffer was added. For incubation, test tubes made of glass (12 × 100 mm), seldom of plastic (11 × 70 mm), were generally used in both cases. Buffers used in this research contained 0.1 % sodium azide (NaN_3).

Separation

In this research two methods were used to separate antibody-bound and free hormone. Separation was usually achieved by the

charcoal-dextran method ((JACOBS 1969, SCHAMS and KARG 1969). The double antibody method (SCHALCH and PARKER 1964) was also tested.

After incubation, 0.1 ml of horse serum diluted 1 : 4 with 0.07 M veronal buffer, pH 8.6, was added to each tube in the charcoal-dextran separation. After that, 0.8 ml of coated charcoal suspension (in the ice bath) was pipetted to the tubes for the antiserum dilution curve and 1.0 ml to the tubes for the standard curve. The coated charcoal suspension consisted of 0.07 M veronal buffer, pH 8.6, containing 5 % charcoal (Norit A) and 0.5 % dextran (T-110). Incubation was carried out in the ice bath for 15 min and centrifuging (2000 rpm) for 20 min at +4° C. Incubation and centrifuging in the tests on pages 18 and 19 were carried out at room temperature, as described by RATCLIFFE (1974). Radioactivity in the charcoal containing free hormone was measured.

Two kinds of antisera were needed for double antibody separation. The first antiserum, produced in the rabbit, contained specific antibodies against bovine prolactin. The second antiserum produced against rabbit IgG globulins in sheep contained precipitating antibodies. The second antiserum was a gift from the research team LEPPÄLUOTO—LYBECK—RANTA at the Institute of Physiology, University of Helsinki. The first immunological reaction occurred between hormone and specific antibodies. The second reaction occurred between hormone-antibody complexes and precipitating antibodies resulting in still larger complexes, which are found as a precipitate at the bottom of the test tube after centrifuging.

Phosphosaline (0.01 M phosphate buffer, pH 7.6, containing 0.15 M NaCl) was the stock buffer used in double antibody separation. Phosphosaline was used to dilute the second antiserum. Phosphosaline containing 0.1 % BSA was used to dilute the labelled hormone. Phosphosaline containing 1.0 % BSA was used as an incubation buffer. The first antiserum was diluted with phosphosaline containing 0.05 M EDTA and 3 % NRS.

After incubation, 200 μl of the second antiserum dilute (1 : 4) was added. The tubes were further incubated overnight at +4° C. They were then centrifuged (2500 rpm) for 30 min at +4° C. Supernatant solution was discarded. Radioactivity in the precipitates containing the antibody-bound hormone were measured.

Radioactivity measurements

During this research two automatic gamma sample counters were used to measure radioactivity. Counting efficiency of the Wallac GTL$^{300-1000}$ in the Isotope Laboratory, Agricultural Research Centre was about 45 % for iodine-125-isotope and that of the LKB-Wallac 1280 Ultro Gamma in the Isotope Laboratory, Faculty of Agriculture and Forestry, University of Helsinki was about 60 %. Radioactivity added to each tube was between 4 000—25 000 cpm when the former counter was used and about 30 000 cpm with the latter. These radioactivity figures were equivalent to 0.3—0.1 ng of labelled bovine prolactin. Each tube was measured for one or two minutes.

RESULTS AND DISCUSSION

Antisera

Immunological activity or titer of the antiserum is generally measured with the aid of the antiserum dilution curve. It is determined by incubating the constant

amount of the labelled hormone with the dilution series of the antiserum. The scale of antiserum dilution is usually logarithmic and the scale of the degree of immunochemical binding is arithmetic. The higher the degree of dilution of the antiserum responsible for 50 % binding of the constant amount of labelled hormone and the steeper the antiserum dilution curve, the more immunoreactive the antiserum.

Titers of different antisera can vary greatly, because production of antibodies depends on the quantity and quality of the material injected into the antiserum animal, preparation of the vaccine, adjuvant, species, site of injections, immunization schedule etc. (CLAUSEN 1969). There may also be marked differences between individul animals of the same species in ability to produce antibodies (PLAYFAIR et al. 1974).

If immunological activity of the antiserum or labelled hormone is reduced or the conditions for the immunological reaction change less optimal, the antiserum dilution curve reflects these changes by showing lower immunochemical binding percentages. Immunological activity of the antiserum and labelled hormone and the incubation conditions were tested in this research with the aid of the antiserum dilution curve.

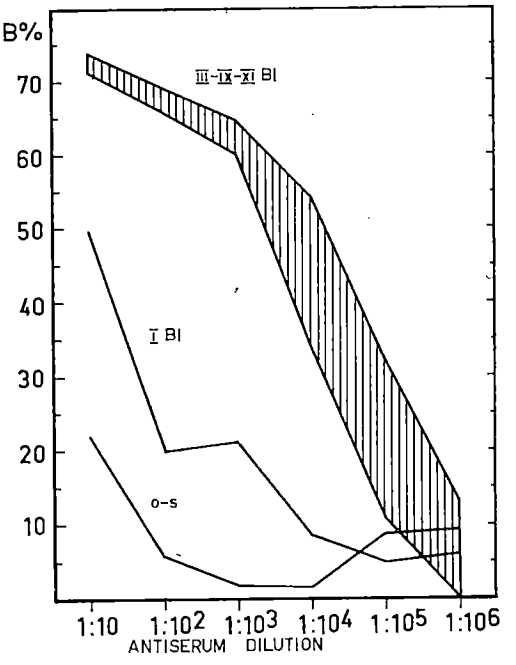

Fig. 4. Antibody titers of O-serum (O-S) and progressive antiserum bleedings (Bl) from rabbit 102 during immunization.

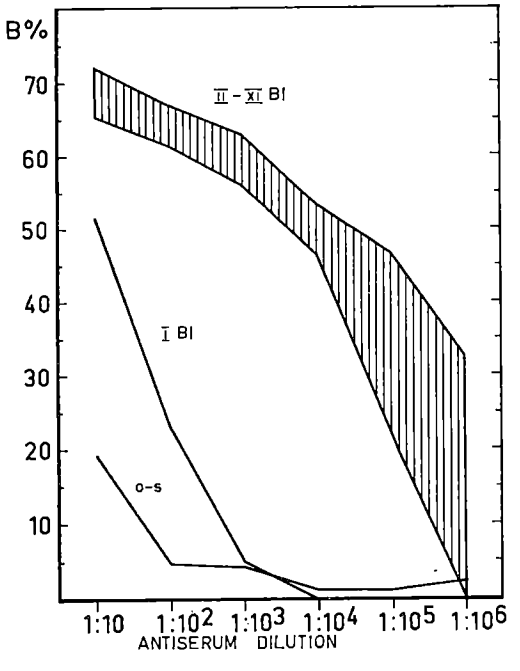

Fig. 3. Antibody titers of O-serum (O-S) and progressive antiserum bleedings (Bl) from rabbit 101 during immunization.

Antibody titers of the sera (0-sera) and progressive antiserum bleedings of the rabbit 101 and 102 during the course of immunization were determined as seen in Figs. 3 and 4, respectively. The titers of the first antiserum bleedings were rather low in both cases, but higher than the titers of 0-sera. Titers of the antiserum samples from the second or third bleedings up to the end of immunization did not vary significantly in either rabbit. These results indicated that maximal antibody content was already achieved after five injections. Observations in this research agree well with those made by HURN and LANDON (1971), who found that antiserum animals begin to produce

antibodies immediately after the first injection. The maximal antibody content in the antiserum is achieved after 2—6 injections. Further injections do not usually increase antibody content but may improve the quality of the antibodies. According to CLAUSEN (1969), at the beginning of immunization antibodies resemble IgM globulins, while long term immunization results in antibodies similar to IgG globulins. IgG globulins are particularly necessary for radioimmunoassay.

The dilution curve of the antiserum (rabbit 102, 9th bleeding) produced during this research and that of the antiserum donated by Schams and Karg were similar (Fig. 5). It may be expected that their immunochemical behaviour in radioimmunoassay will also be similar.

According to the dilution curves (Fig. 6), absorption of the antiserum (rabbit 102, 11th bleeding) by tissues and/or serum did not improve immunological activity in the antiserum. The immunochemical binding percentages were lower after absorption than before. On the basis of the antiserum dilution curve it could not be determined whether this was caused by a reduction in specific or unspecific antibodies. Specificity tests to reveal these phenomena have not yet been carried out in this research.

The effect of the dilution buffer on immunological activity of the antiserum was

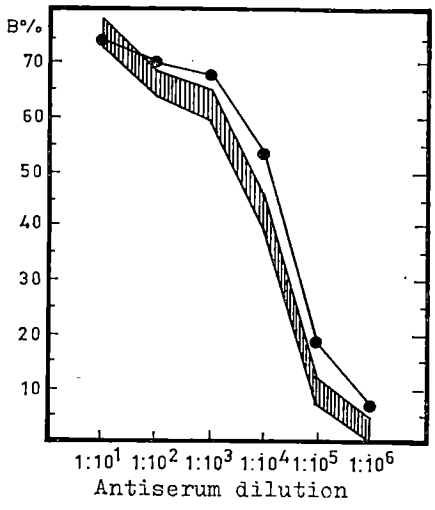

Fig. 6. Dilution curves of unabsorbed (●——●) antiserum (rabbit 102, 11th bl.) and that absorbed with bovine tissues and/or serum (closed area).

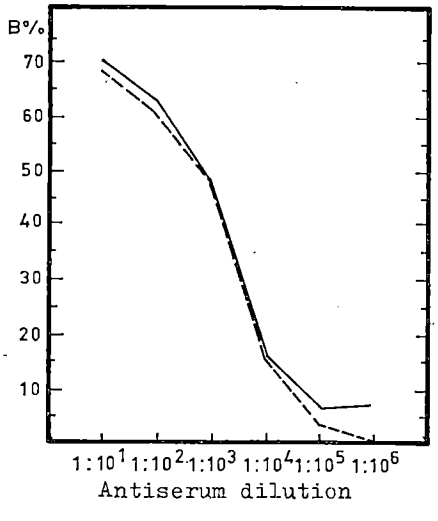

Fig. 5. Dilution curves of rabbit antiserum produced during this research (———— = rabbit 102, 9th bl.) and of that donated by Schams and Karg (— — —).

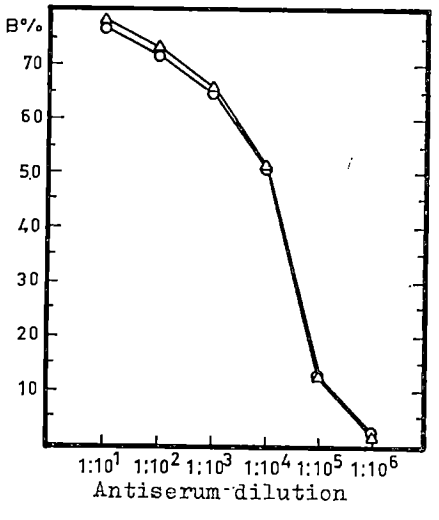

Fig. 7. Dilution curves of antiserum (rabbit 102, 9th bl.) diluted with veronal (O——O) and phosphate (△——▽) buffer.

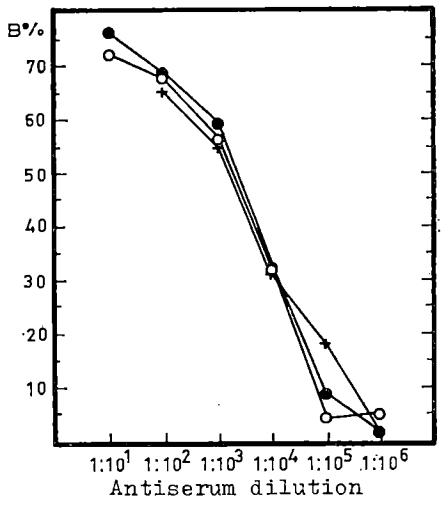

Fig. 8. Effect of length (●——● = 9 day, O——O = 24 days and ×——× = 30 days) of storage (at +4° C) of diluted antiserum (rabbit 102, 9th bl.) on the dilution curve.

tested using 0.07 M veronal buffer, pH 8.6, containing 0.5 % BSA and 0.01 M phosphate buffer, pH 7.5, containing 0.1 % BSA to dilute the antiserum (rabbit 102, 9th bleeding). Both buffers contained 0.9 % NaCl and 3 % NRS. Similarity in both antiserum dilution curves (Fig. 7) showed that the buffers were of equal value for diluting the antiserum, when it had been stored undiluted at −20° C and diluted after thawing. If antiserum is diluted with phosphate buffer before freezing, a phosphate buffer can have a markedly disadvantageous effect on the immunological activity of the antiserum (CHILSON et al. 1965).

Immunological activity of diluted antiserum stored at +4° C was also studied. Dilution series of the same antiserum sample (rabbit 102, 9th bleeding) were made 30, 24 and 9 days before determination of the antiserum dilution curves. All dilutions were stored at +4° C. Separation of antibody-bound and free prolactin was, as an exception, carried out by 1 % coated charcoal suspension (0.1 % Dextran T-110). According to the antiserum dilution curves (Fig. 8), immunological activity of the antiserum dilutions decreased very little with time during storage at +4° C. Diluted antiserum can thus be stored at +4° C for as long as one month without any marked loss of immunological activity. Also, according to HURN (1971), antisera can be stored in liquid form at +4° C for a long time and for even longer when diluted. The antisera are usually stored in lyophilized form or deep frozen at −20° C without preservatives. The most valuable antisera can also be preserved at −80° C (the temperature of solid CO_2) and even at −195° C (obtained with liquid nitrogen) (HURN 1971).

Iodination

The elution pattern or the iodination curve determined on the basis of relative radio-

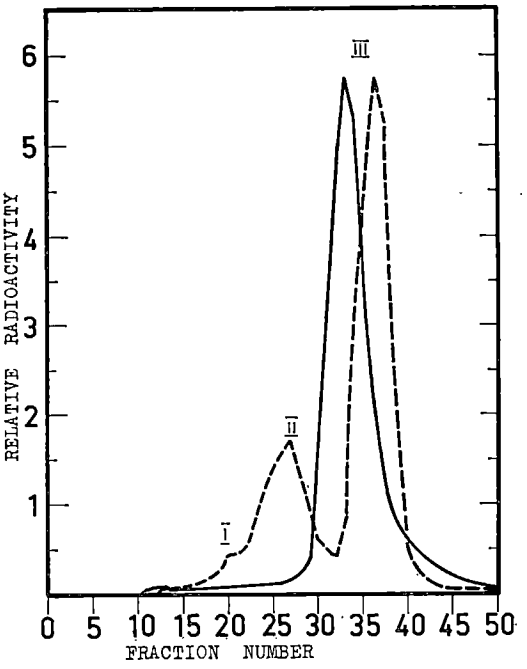

Fig. 9. Elution patterns of radioactive materials after Sephadex G-100 gelfiltration (purification of the labelled hormone) when bovine prolactin was present (— — — —) and absent (——).
I = aggregated BPR-I-125
II = monomeric BPR-I-125
III = free I-125

activity in the fractions of the first gelfiltration (Sephadex G-100) showed two distinct peaks and one less distinct peak (Fig. 9). Large molecules wander through the Sephadex column faster than small ones (GREENWOOD et al. 1963). The first peak, which was the least distinct and smallest one, presumably contained the aggregated bovine prolactin (see repurification of the hormone, page 146). The second peak in the iodination curve (Fig. 9) was a hormone peak containing iodine-125 bound to bovine prolactin (MW 24 000, ANDREWS 1966). The third peak was an iodine peak containing free iodine-125 (MW 126.9). The peaks were verified by iodination and purification procedures without hormone. When hormone was absent, the first two (obviously, aggregated and monomeric hormone, see page 153) peaks were also absent and only the third (free ionide) peak was present (Fig. 9). The iodination curve obtained for bovine prolactin in this research (Fig. 9) agrees well with that obtained by SCHAMS (1974).

The suitability of the two methods for estimating the percentage incorporation of I-125 into hormone was tested by applying them in iodination curves published by other researchers (Table 1). Percentages estimated according to methods (a) and (b) were compared with the values published by the other researchers. Percentages obtained by method (a) were lower than those obtained by method (b). Method (a) agreed more closely with previously published results than method (b). Method (a) was selected for routine determinations of the percentage incorporation of I-125 into bovine prolactin. If the iodination time was 30 seconds and the amount of I-125 beween 1.0—1.5 mCi, the percentage incorporation of I-125 into bovine prolactin varied between 32.9—91.1 % in this research (Table 2).

Table 1. Comparison between percentage incorporation of iodine into hormone estimated by methods (a) and (b) and the respective percentages in previous studies.

Reference	Percentages in previous studies	Percentages estimated by method (a)	Percentages estimated by method (b)
GREENWOOD et al. (1963)	60—75	65	84
SCHAMS (1974)	26.3	39	64
PEAKE (1974)	n. 60	65	84
HANDWERGER & SCHERWOOD (1974)	60—70	63	82
TYREY et al. (1974)[2]	43.8—62.5[1]	45	69

[1] Calculated according to specific activity
[2] Hormone labelled with I-131

Table 2. Percentage incorporation of I-125 into bovine prolactin and specific activity of the labelled bovine prolactin estimated on the basis of method (a) in five iodinations.

Number of iodination	Amount of I-125 mCi	% incorporation of I-125 into BPR	Specific activity of BPR-I-125, μCi/μg
1.	1.00	88.6	177.2
2.	1.00	70.9	141.8
3.*)	1.00	32.9	65.8
4.	1.22	91.1	222.3
5.	1.50	64.4	193.2

*) Iodination curve in Fig. 9.

Incorporation of I-125 into hormone depends on several factors: the amount of chloramine-T, I-125 and hormone as well as the reaction time, temperature, pH, ionic strength and volume of the reaction mixture (HUNTER 1971). The wide variation in percentage incorporation of iodine into bovine prolactin obtained in this research was not exceptional in comparison with results obtained by others (KIRKHAM and HUNTER 1971 and JAFFE and BEHRMAN 1974). The wide variation in the present research was most likely due to the variation in specific activity (μCi/μl) of iodine used in the iodinations. Because one iodine-125 batch was sufficient for more than one iodination, what was left of the batch was always stored for shorter or longer periods before the next iodination. During storage, specific activity diminished gradually. In order to keep the amount of radioactivity constant in each iodination, a larger volume

of radioactive iodine solution was used. This obviously lowered the percentage incorporation of I-125 into the bovine prolactin. Percentage incorporation of I-125 into hormone may vary markedly even under apparently similar conditions (JACOBS 1969, YALOW and BERSON 1969 and JACOBS 1974). This phenomenon is due to variations in the isotope in different batches (BERSON and YALOW 1966).

Specific activity of labelled bovine prolactin calculated in this research by method (a) varied between 65.8—222.3 μCi/μg (Table 2). The amount of labelled hormone for one assay has to be smaller than the amount of hormone in the sample to be studied. Consequently specific activity has to be sufficiently high, otherwise radioactivity measurements are not statistically reliable. In addition to the concentration of the hormone to be assayed, the specific activity derived also depends on the sensitivity of the counting system and the volume of incubation mixture to be counted (BERSON and YALOW 1973). Specific activity of approximately 100 μCi/μg has proved suitable for several proteohormone (HUNTER 1971). According to the findings of SCHAMS (1974), the specific activity of labelled bovine prolactin should not exceed 100 μCi/μg. However, labelled bovine prolactin with specific activity as high as 173 μCi/μg still displayed considerable immunological activity towards the antibodies in this research (pages 157 and 158). GREENWOOD et al. (1963) used human growth hormone with a specific activity of 250—590 μCi/μg and did not find any significant loss in immunological activity.

Iodination of the hormone can be carried out by several methods (see HUNTER 1971, 1974). The iodination method based on the chloramine-T technique (GREENWOOD et al. 1963) is convenient but damages the hormone more than other methods (MOUDGAL and MADHWA RAJ 1974). The reason is that chloramine-T is a strong oxidant. It oxidizes iodine to iodide, which substitutes hydrogen in the aromatic ring of tyrosine. Sodium metabisulphite, which is a reducing agent, stops the reaction. Damage to the hormone begins at labelling and continues from this stage on. The labelled hormone is not a stable compound. Damage to ruminant hormones is caused by aggregation of hormone molecules (CUNNINGHAM 1969). The smaller the amount of chloramine-T used in iodination, the less the damage to the hormone.

Alterations in labelled bovine prolactin after storage at +4° C were studied by repurification (Sephadex G-200 gelfiltration) of the labelled hormone 3, 9 and 23 days after iodination. Curves (Fig. 10) based on relative radioactivity of the fractions had three peaks. The first was obviously composed of aggregated BPR-I-125, the second of monomeric BPR-I-125 and the third of free I-125. According to the curves, damage to labelled bovine prolactin, after iodination continued

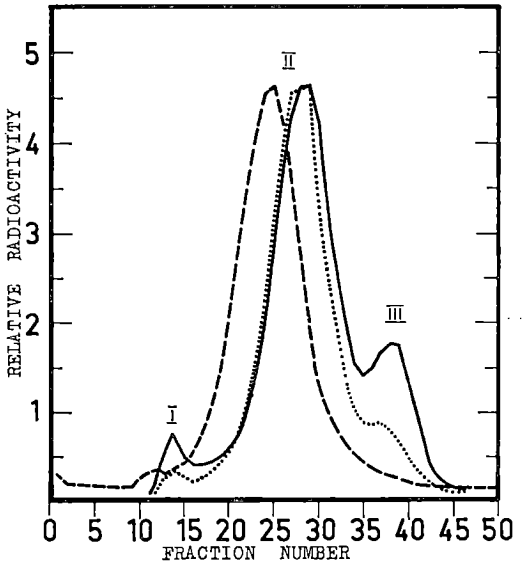

Fig. 10. Elution patterns of radioactive materials after Sephadex G-200 gelfiltration (repurification of labelled hormone) when labelled bovine prolactin was stored 3 (— — — —), 9 (. . . . . .) and 23 (———) days after iodination and purification.
I = aggregated BPR-I-125
II = monomeric BPR-I-125
III = free I-125

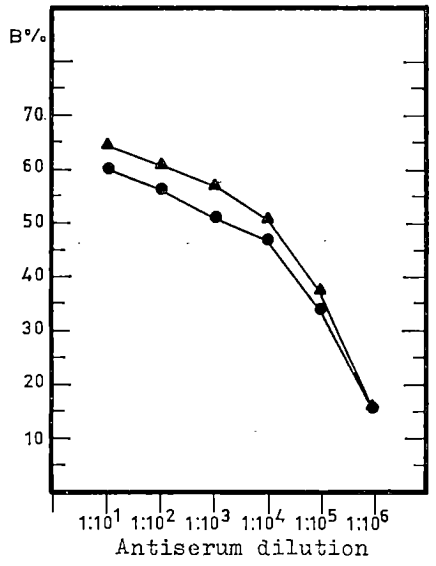

Fig. 11. Antiserum (rabbit 102, 9th bl.) dilution curves obtained by using purified (●——●) and repurified (▲——▲ labelled bovine prolactin.

during storage at +4° C, and the amounts of aggregated hormone and free iodine increased with time. JACOBS (1974) had similar results with labelled pig prolactin stored at −20° C. Gelfiltration with Sephadex G-200 was quite suitable for the separation of aggregated and monomeric bovine prolactin. This agrees well with the results of CUNNINGHAM (1969).

In order to find out the effect of repurification of labelled bovine prolactin on its immunological activity, antiserum (rabbit 102, 9th bleeding) dilution curves were prepared with purified and repurified labelled bovine prolactin. The interval between purification and repurification was three days (Fig. 10). The antiserum dilution curves obtained in this test (Fig. 11) indicated that immunological activity was higher in repurified hormone than in purified hormone. This agrees well with the results of BRYANT and GREENWOOD (1968) and PEAKE (1974). These workers observed that immunological activity of the aggregated hormone was significantly lower than that of the monomeric hormone. Lower immunological activity in purified labelled bovine prolactin in this research was obviously caused by the appearance of both aggregated hormone and free iodine.

Incubation

Immunological reaction between the antigen and antibodies takes place most completely under optimal conditions of temperature, time, pH, as well as salt and protein concentration (CLAUSEN 1969). Chemical conditions can be regulated by the incubation buffer.

The effect of the incubation buffer on immunological reaction was tested by using 0.07 M veronal buffer, pH 8.6, containing 0.5 % BSA and 0.01 M phosphate buffer, pH 7.5, containing 0.1 % BSA as an incubation buffer. Both buffers contained 0.9 % NaCl. Antibody-bound hormone was separated from free hormone by 0.5 % coated charcoal

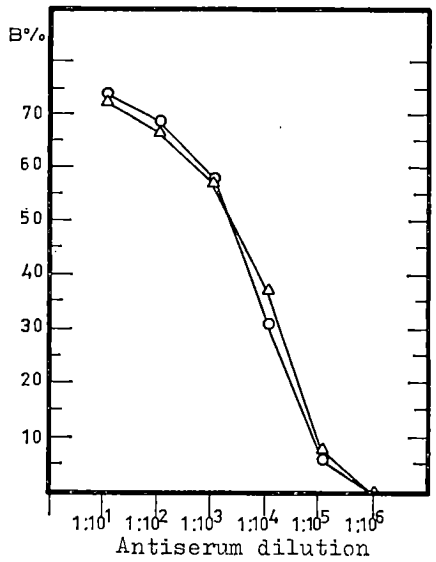

Fig. 12. Effect of incubation buffer (O——O = veronal buffer 0.07 M, pH 8.6 and △——△ = phosphate buffer 0.01 M, pH 7.5) on antiserum (rabbit 102, 9th bl.) dilution curve.

suspension (0.05 % Dextran T-110). Both antiserum (rabbit 102, 9th bleeding) dilution curves were similar (Fig. 12) indicating that both buffers were suitable for incubation.

Veronal, phosphate, tris or borate buffer are generally used as an incubation buffer in radioimmunoassays (ANON. 1974). pH varies between 7.4—8.6 and ionic strength between 0.01—0.04 M. The buffer systems are usually fortified with immunologically inactive protein (for example BSA). Specific protective agents such as sodium azide may also be added.

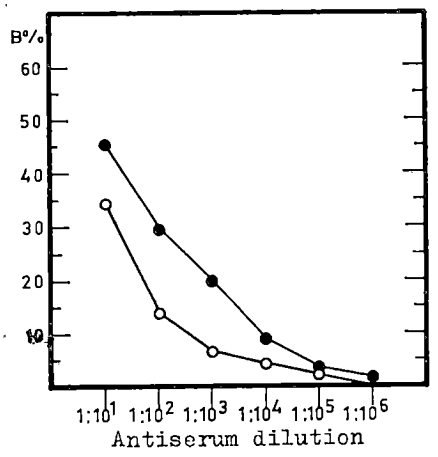

Fig. 13. Effect of molecular weight (●——● = MW 110 000 and O——O = MW 70 000) of dextran on antiserum (rabbit 102, 9th bl.) dilution curve in the charcoal-dextran separation.

Separation

Various methods for separation of the antibody-bound and free hormones have been developed to determine the extent of immunological reaction in the radioimmunoassay (RATCLIFFE 1974). Classification of the radioimmunoassays is also based on the separation technique. In this research the separation method mainly studied was the charcoal-dextran method. It was compared with the double antibody method.

The effect of the molecular weight of the dextran on the separation of the antibody-bound and free bovine prolactin was tested by coating charcoal with Dextran T-70 (MW 70 000) and Dextran T-110 (MW 110 000). In both cases the charcoal suspension contained 5 % charcoal and 0.5 % dextran. The antiserum (rabbit 102, 9th bleeding) dilution curve obtained by using Dextran T-110 coated charcoal in separation was markedly better than obtained by using Dextran T-70 (Fig. 13). According to these results the molecular weight of the coating material has an appreciable effect on the separation of the antibody-bound and free bovine prolactin.

Uncoated charcoal adsorbs all kinds of matter. Charcoal coated with material of a given molecular weight adsorbs only molecules smaller than the coating molecules (HERBERT 1969). Molecules to be separated must therefore have sufficiently different molecular weights. The molecular weight of bovine prolactin is 24 000 (ANDREWS 1966) and that of the antibodies (IgG globulin) 160 000 (CLAUSEN 1969). The molecular weight of hormone-antibody complex is thus 184 000. The influence of the coating material is most effective when 10 % of the weight of the charcoal to be coated is used (JACOBS 1969). Molecular weight of the coating agent should lie approximately halfway between that of the free hormone and that of the complex (HERBERT 1969). Dextran T-70 tested in this research failed to give satisfactory results in the separation process. SCHAMS (1969) used Dextran T-110 successfully as a coating agent. Similar experiences were also obtained in this research. Dextran T-110 is also one of the best coating agents for human GH (JACOBS 1969), the molecular weight of which, 21 500, (KARLSON 1974) is near that of bovine prolactin.

Significance of horse serum as a source of carrier proteins in charcoal-dextran separation was tested in this study. Separation was carried out both without adding horse

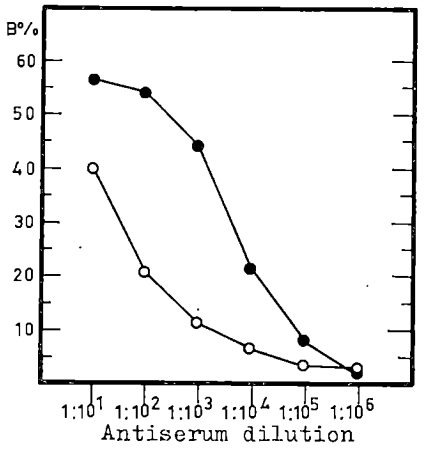

Fig. 14. Effect of horse serum on charcoal-dextran separation. Antiserum (rabbit 102, 9th bl.) dilution curves achieved with horse serum (●——●) and without horse serum (O——O).

serum and adding 0.1 ml horse serum diluted 1 : 4 (0.07 M veronal buffer, pH 8.6) immediately before the addition of the charcoal suspension. According to the antiserum (rabbit 102, 9th bleeding) dilution curves (Fig. 14), horse serum had a marked effect on the charcoal-dextran separation. The antiserum dilution curve with horse serum was significantly better than that without horse serum.

Separation conditions are optimal when adsorption of free hormone to the charcoal is maximal and adsorption of the complex is minimal. This cannot be achieved unless there is a sufficient amount of suitable carrier protein. Usually plasma or serum proteins reduce adsorption of the complex to the charcoal without inhibiting the adsorption of free hormone (YALOW and BERSON 1969). According to the observations of RATCLIFFE (1974) this selective influence is based on globulins. Serum or plasma can even substitute dextran as a coating agent. Charcoal suspension containing 10—30 % serum separates antibody-bound and free hormone at least as effectively as dextran coated charcoal suspension (RATCLIFFE 1974).

Charcoal-dextran and double antibody separation were compared in this research with the aid of antiserum (donated by Schams and Karg) dilution curves. In the former case the amount of labelled bovine prolactin was one fourth of that used in the latter case. Otherwise test conditions were similar. Charcoal separation was carried out without horse serum.

Immunological binding percentages of the antiserum dilution curve were higher in double antibody separation than in charcoal-dextran separation (Fig. 15). Because of the difference in the amount of labelled hormone in these two separations and the absence of horse serum in the charcoal-dextran separation, preference between the two separations could not be determined. This test, however, indicated that both methods were suitable for separation of antibody-bound and free bovine prolactin.

Dextran-charcoal separation is practical, quick and cheap. It may, however, be unspecific. ASHFORD et al. (1969) and RAPTIS (1971) compared various radioimmunoassays and observed that hormone

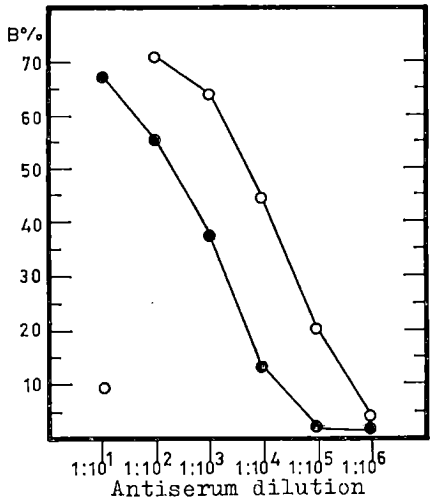

Fig. 15. Dilution curves of rabbit antiserum (donated by Schams and Karg) obtained by charcoal-dextran (●——●) and double antibody (O——O) separation.

concentrations obtained by the charcoal-dextran method were usually somewhat higher than hormone concentrations obtained by other methods. Charcoal may adsorb both immunologically active and inactive labelled hormone, free iodine and hormone-antibody complex. The charcoal-dextran method is dependent on the protein concentration of the samples. This can result in unspecific binding as high as 25 % (SCHAMS 1974).

Double antibody separation is specific because it is based on immunological reaction. It is expensive (production of the second antiserum) and tedious (separation demands overnight incubation). Furthermore, double antibody separation is also dependent on the protein concentration of the samples and on the anticoagulants (RATCLIFFE 1974).

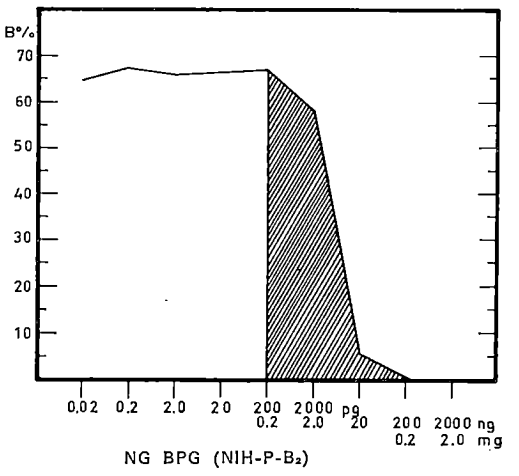

Fig. 16. The first trial to develop a standard curve for bovine prolactin using 0.3 ng labelled bovine prolactin (specific activity of 66 nCi/ng) and antiserum (rabbit 102, 9th bl.) diluted $1 : 10^3$.

Standard curve

The concentration level of the standard curve needed depends on the hormone concentrations of the samples to be investigated. Bovine blood may contain only a few ng prolactin per ml during the autumn and winter months (SCHAMS 1974). Furthermore, the sample volume to be assayed is 0.2 ml. Thus the standard curve for bovine blood prolactin must lie in a low concentration level.

The first trial to develop a standard curve for bovine prolactin was made by using 0.3 ng of labelled bovine prolactin (specific activity of 66 nCi/ng) and antiserum (rabbit 102, 9th bleeding) diluted $1 : 10^3$. Concentrations of the standard hormone solutions varied between 0.02 pg—2.0 mg/100 μl. The term maximal (immunological) binding means the percentage of labelled hormone bound to the antibodies in the absence of unlabelled hormone. According to the inhibition curve (Fig. 16), maximal binding was more than 65 %. The standard inhibition curve under these conditions lay between concentrations 0.2—200 ng of BPR. According to prolactin concentrations of bovine blood obtained by SCHAMS (1974), the concentration level for this standard curve was not low enough. This test, however, showed that all immunological reagents used were immunologically active.

Concentration levels of the standard curve are generally regulated by the amounts of labelled hormone (BERSON and YALOW 1968) and antiserum (HURN and LANDON 1971). By decreasing the amounts of these immunological reagents it is possible to achieve a more sensitive standard curve. The amounts of antiserum and labelled hormone have to be relative to each other. Standard curve conditions are ideal if the antibodies bind about 50 % of the labelled hormone in the absence of unlabelled hormone (MOUDGAL and MADHWA RAJ 1974).

Further trials to develop a standard curve for bovine prolactin were made using only 0.15 ng of labelled bovine prolactin (specific activity of 173 nCi/ng) and antiserum (guinea pig M2, 2nd bleeding) diluted as much as $1 : 10^3$, $1 : 10^4$, $1 : 10^5$ and $1 : 10^6$. Maximal bindings of labelled hormone to the

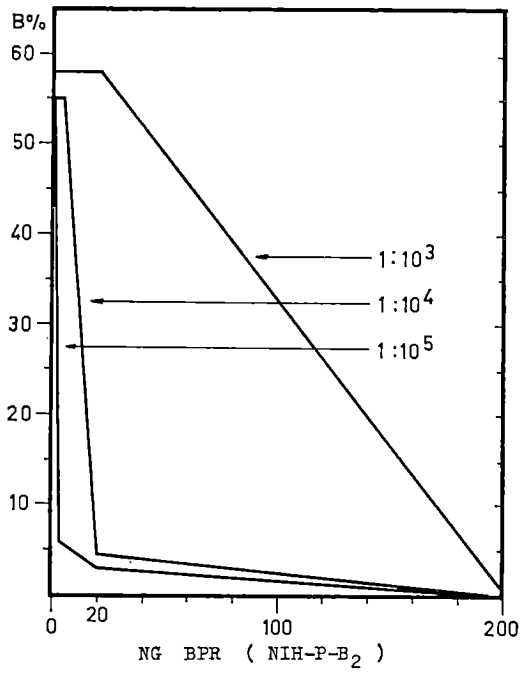

Fig. 17. Increase in sensitivity of the standard curve for bovine prolactin when concentration of the antiserum (guinea pig M2, 2nd bl.) decreased and when 0.15 ng labelled bovine prolactin (specific activity of 173 nCi/ng) was used.

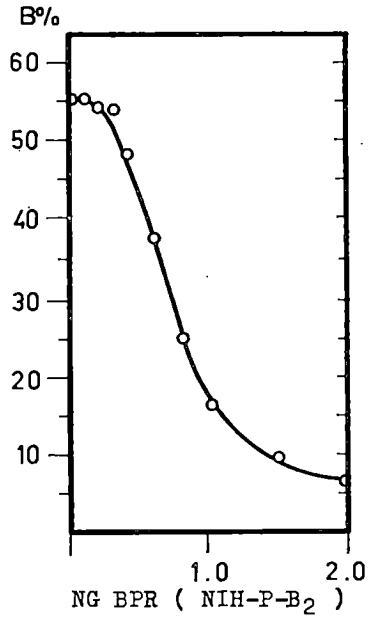

Fig. 18. Standard curve for bovine prolactin using 0.15 ng labelled bovine prolactin (specific activity of 173 nCi/ng) and antiserum (guinea pig M2, 2nd bl.) diluted $1 : 10^5$.

antibodies in various antiserum dilutions varied 57.9, 55.0, 54.8 and 16.6 % respectively (Fig. 17). When antiserum dilutions varied from $1 : 10^3$, $1 : 10^4$ to $1 : 10^5$, the concentration level of the standard curves varied from 20—200 ng, 4.0—20 ng to 0.05—2.0 ng of BPR respectively; an antiserum dilution of $1 : 10^6$ did not contain enough antibodies to give a satisfactory standard curve. The best standard curve in the tested conditions was obtained by an antiserum dilution of $1 : 10^5$ (Fig. 18). However, compared with the standard curve obtained by SCHAMS (1974), this curve was not sensitive either steep enough between the low concentrations of 0.05—0.3 ng BPR.

The guinea pig antiserum (M2, 2nd bleeding) dilution 1 : 50 000, 1 : 107 895, 1: 250 000 and 1 : 500 000 was tested under the similar conditions as the foregoing to achieve the most sensitive standard curve for bovine prolactin. Maximal bindings of labelled hormone to the antibodies were 51.3, 50.7, 44.5 and 35.2 (Fig. 19). Maximal binding percentages in this case were lower than in Fig. 17 because the labelled hormone was about two weeks older than in Fig. 17. Standard curves obtained with an antiserum dilution of 1 : 50 000 and 1 : 107 895 were not sensitive enough while the standard curve obtained with an antiserum dilution of 1 : 500 000 was too steep. Thus the most sensitive standard curve for bovine prolactin developed during this research was the one achieved by using antiserum (guinea pig M2, 2nd bleeding) diluted 1 : 250 000 and 0.15 ng labelled bovine prolactin (specific activity 173 nCi/ng) (Fig. 20). This standard curve was sensitive between concentrations of 0.05 ng—0.8 ng of BPR. It resembled closely the standard curve developed by SCHAMS (1974).

Effect of absorption of the antiserum (rabbit 102, 11th bleeding) using a brain-liver-

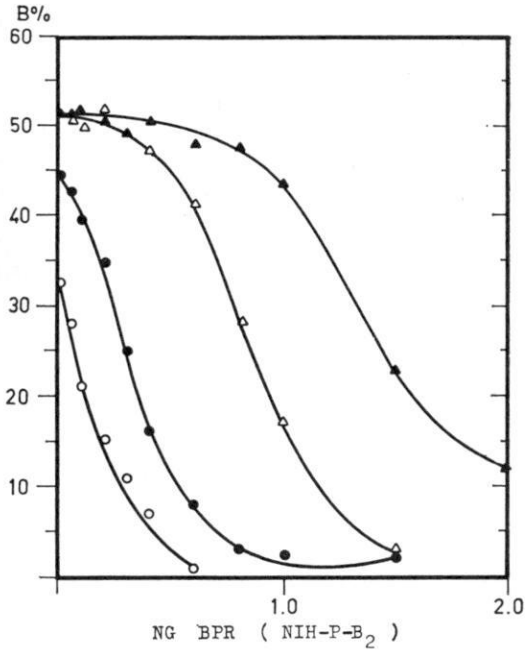

Fig. 19. Standard curves for bovine prolactin obtained by antiserum (guinea pig M2, 2nd bl.) diluted 1 : 500 000 = O——O, 1 : 250 000 = ●——●, 1 : 107 895 = △——△ and 1 : 50 000 = ▲——▲, when using 0.15 ng labelled bovine prolactin (specific activity of 173 nCi/ng).

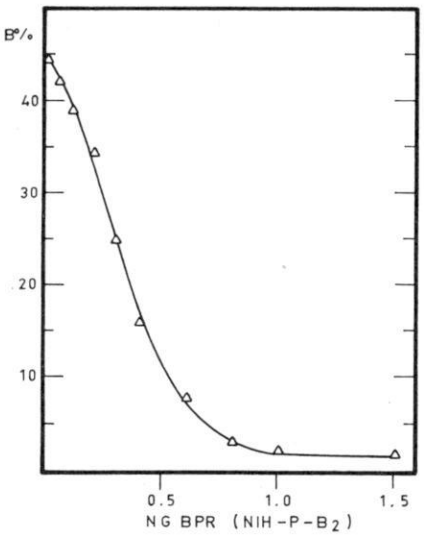

Fig. 20. The most sensitive standard curve for bovine prolactin developed during this research using antiserum (guinea pig M2, 2nd bl.) diluted 1 : 250 000 and 0.15 ng labelled bovine prolactin (specific activity of 173 nCi/ng).

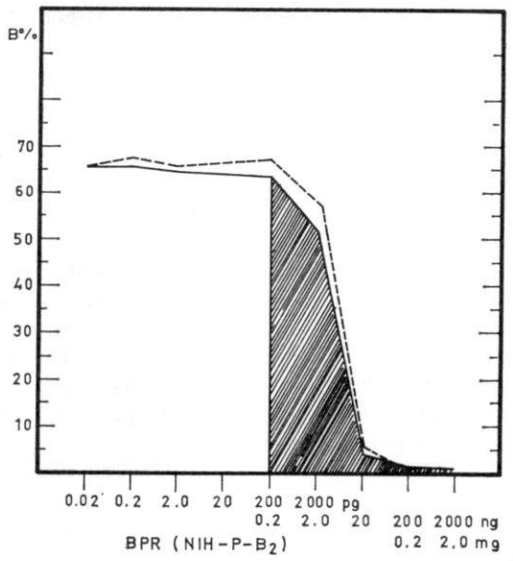

Fig. 21. Standard curves for bovine prolactin using unabsorbed (— — —) antiserum (rabbit 102, 9th bl.) and antiserum absorbed with bovine tissues and serum (———). In both cases antiserun was diluted 1 : 10^3 and 0.3 ng labelled bovine prolactin (specific activity 66 nCi/ng) was used.

kidney-serum mixture had no significant effect on the standard curve for bovine prolactin (Fig. 21).

LIST OF ABBREVIATIONS

NKJ	= Nordisk kontaktorganet för jordbruk
BPR	= bovine prolactin
I-125	= iodine-125
BPR-I-125	= bovine prolactin labelled with iodine-125
B %	= immunological binding percent of control
GH	= growth hormone
BSA	= bovine serum albumin
NRS	= normal rabbit serum
EDTA	= ethylenediaminetetra-acetic acid
cpm	= counts per minute

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Laboratory, Faculty of Agriculture and Forestry, University of Helsinki. Measurements of radioactivity have also been made at the Isotope Laboratory, Agricultural Research Centre. Sincere thanks are due to Prof. Esko Poutiainen at the Department of Animal Husbandry, University of Helsinki, for supporting this research in many useful ways, to Dr. Thomas Tallberg at the Second Department of Pathology, University of Helsinki, for his advices concerning immunization and iodination, to Mag. Phil. Antti Uusi-Rauva at the Isotope Laboratory, Faculty of Agriculture and Forestry, for much valuable advice on isotope techique, to Mag. Phil. Arja Paasikallio and Cand. Phil. Ulla Häkkinen for radioactive measurements made at the Isotope Laboratory, Agricultural Research Centre, to Dr. Dieter Schams and Prof. Heinrich Karg at the Institute of Physiology, Technical University of Munich, Weihenstephan, for the rabbit anti-BPR-serum, to Dr. Harry Lybeck, Dr. Juhani Leppäluoto and Tapio Ranta at the Department of Physiology, University of Helsinki, for the sheep anti-rabbit-IgG-serum and to Dr. A. E. Wilhelmi at the National Institutes of Health for the bovine prolactin preparation.

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SELOSTUS

Naudan prolaktiinin määrittämisestä radioimmunologisesti

RITVA MÄKELÄ ja VAPPU KOSSILA

Maatalouden tutkimuskeskus

Tämän tutkimuksen avulla luotiin pohjaa naudan prolaktiinin radioimmunologisen menetelmän käytäntöön soveltamiseksi verinäytteille. Sitä varten tutkittiin tuotettujen antiseerumeiden ja merkatun prolaktiinin immunologista aktiivisuutta, inkubointiolosuhteita, vasta-aineisiin sitoutuneen ja sitoutumattoman prolaktiinin erotusmenetelmiä, standardikäyrää ja sen herkistämistä sopivalle pitoisuusalueelle.

Tutkimuksen aikana tuotetut vasta-aineet naudan prolaktiinia vastaan osoittautuivat radioimmunologiseen menetelmään sopiviksi. Antiseerumit tuotettiin kaneissa. Vasta-aineita todettiin muodostuneen kanien vereen jo ensimmäisen ruiskutuksen jälkeen. Maksimaalinen vasta-ainepitoisuus saavutettiin viiden ruiskutuksen jälkeen. Tutkimuksen aikana tuotettu antiseerumi todettiin yhtä hyväksi kuin lah-

joituksena saatu antiseerumi. Absorboiminen erilaisilla kudos- ja seeruminäytteillä ei enää parantanut antiseerumin immunologista aktiivisuutta. Antiseerumeiden laimentamiseen sopivat sekä veronaali- että fosfaattipuskurit. Antiseerumilaimennukset säilyttivät suurimman osan immunologisesta aktiivisuudestaan säilytettäessä niitä jopa kuukauden ajan +4° C:ssa. Antiseerumeita naudan prolaktiinia vastaan tuotettiin myös marsuissa.

Kloramiini-T-tekniikkaan perustuvalla merkkausmenetelmällä saatiin merkattua naudan prolaktiinia, jolla oli sekä riittävä immunologinen aktiivisuus että spesifinen aktiivisuus radioimmunologista menetelmää varten. Suoritetuissa merkkauksissa 32.9—91.1 % jodi-125-isotoopista sitoutui prolaktiiniin. Jodauskäyrän piikkien painoihin perustuva jodin sitoutumisprosentin määritysmenetelmä osoittautui käyttökelpoiseksi. Merkatun prolaktiinin spesifiset aktiivisuudet vaihtelivat välillä 65.8—222.3 μCi/μg. Säilytettäessä merkattua prolaktiinia +4° C:ssa osa merkatusta prolaktiinista aggregoitui ja osasta merkattua prolaktiinia vapautui jodia. Näiden määrät lisääntyivät säilytyksen aikana. Sephadex G-100 ja Sephadex G-200-geelisuodatus soveltuivat aggregoituneen naudan prolaktiinin ja vapaan jodi-125-isotoopin erottamiseen monomeerisestä naudan prolaktiinista.

Tutkimuksessa käytetyt inkubointiolosuhteet osoittautuivat suotuisiksi. Sekä 0.07 M veronaalipuskuri, pH 8.6, että 0.01 M fosfaattipuskuri, pH 7.5, soveltuivat inkubointiin.

Vasta-aineisiin sitoutuneen ja sitoutumattoman prolaktiinin erottaminen toisistaan onnistui dekstraanilla päällystetyllä hiilellä. Erottuminen parani, kun erotusvaiheessa käytettiin hevosen seerumia ja kun päällysaineena käytettiin Dextran T-110:tä. Kaksoisvasta-ainemenetelmä oli todennäköisesti ainakin yhtä hyvä erotusmenetelmä kuin hiilimenetelmä.

Tutkimuksen koe-olosuhteissa saatiin naudan prolaktiinin standardikäyrä herkistymään hormonipitoisuusalueelle 0.05—0.8 ng. Tällöin käytettiin 0.15 ng merkattua naudan prolaktiinia ja marsun antiseerumia laimennettuna 1: 250 000.

ANALES AGRICULTURAE FENNIAE, VOL. 15: 163–167 (1976)

Seria AGROGEOLOGIA ET -CHIMICA N. 74 — Sarja MAA JA LANNOITUS n:o 74

UREA PHOSPHATE AS NITROGEN AND PHOSPHORUS FERTILIZER

JORMA KÄHÄRI

KÄHÄRI, J. 1976. **Urea phosphate as nitrogen and phosphorus fertilizer.** Ann. Agric. Fenn. 15: 163–167. (Agric. Res. Centre, Inst. Agric. Chem. and Phys., SF-01300 Vantaa 30, Finland.)

In a two-year pot experiment using 4 different types of soil urea ammonium phosphate (29–13–0) was compared with a combination of ammonium nitrate and monocalcium phosphate. Two levels of fertilization were applied in the experiment.

The two fertilizers gave equal dry matter yields of Italian rye grass in both years. The nitrogen and phosphorus contained in the urea ammonium phosphate were also as readily available to the plant on all the soils as were the respective amounts of nitrogen in the ammonium nitrate and phosphorus in the monocalcium phosphate. The plants receiving urea ammonium phosphate fertilization took up less calcium and magnesium than the plants receiving ammonium nitrate and monocalcium phosphate.

The results show that urea ammonium phosphate can be regarded as a serviceable fertilizer from which compound fertilizers containing potassium can also be produced.

Index words: Urea-ammonium phosphate, N uptake, P uptake.

INTRODUCTION

In recent years, urea has been used as a nitrogen fertilizer to an increasing extent in Finland. In 1962 50 per cent of the total nitrogen applied was in the of form single-nutrient nitrogen fertilizer, and the proportion of urea was less than one per cent. The corresponding figures for 1972 were 33 per cent and 14 per cent. There has also been a tendency to use urea as the nitrogen source in compound fertilizers. This is because urea has a high nitrogen content and is relatively low in price. Experiments performed in India, using a urea-superphosphate method, have rendered serviceable NP and NPK fertilizers (JEWELL 1962). In Great Britain and the United States a method has been developed based on urea and ammonium phosphate, and this method has yielded considerably higher nutrient contents in the final product (HEMSLEY et al. 1970).

In 1970 Institute of Agricultural Chemistry and Physics received for experimental purposes urea ammonium phosphate (29-13-0) from the Tennessee Valley Authority, in the United States. In 1970 and 1971,

comparisons were made between the new fertilizer and a control fertilizer ammonium nitrate and monocalcium phosphate, using pot experiments with 4 different types of soil.

MATERIAL AND METHODS

The urea ammonium phosphate contained 29.9 per cent total nitrogen, 10.3 per cent ammonium nitrogen, 12.3 per cent citrate-soluble phosphorus, and 11.6 per cent water-soluble phosphorus.

To compare the availability of the nitrogen and phosphorus, a pot experiment using 4 soil lots was established in the spring (Table 1). The experiment included two replicas. The experimental schedule was as follows:

cfl N 1000 mg/pot as ammonium nitrate (NH_4NO_3)
P 436 » as monocalcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$)
K 830 » as potassium chloride (KCl)

ufl N 1000 mg/pot as ammonium phosphate
P 413 » as ammonium phosphate
K 830 mg/pot as potassium chloride (KCl)

cf2 twice the quantities of cfl
uf2 twice the quantities of ufl

In both years the fertilizers were applied during the spring. All pots received annually: 2 g $MgSO_4 \cdot 7H_2O$, 10 mg H_3BO_3, 10 mg $NaMoO_4 \cdot 2H_2O$, 50 mg $MnSO_4 \cdot H_2O$, 50 mg $CuSO_4 \cdot 5H_2O$, 50 mg $ZnSO_4 \cdot 7H_2O$. An additional Fe 10 mg/pot was applied to the sphagnum peat. At the beginning of the experiment the loam, the gyttja clay and the sphagnum peat were limed with calcium carbonate at a rate of 12 grammes per pot.

The test crop was Italian rye grass (Lolium multiform L) co Barmultra. It was cut three times in both experiments.

Table 1. Soils: their physical and chemical properties. Nutrients leaching in acid ammonium acetate,pH4.65, soil: extractant v/v 1 : 10 and pH_{H_2O} 1 : 2.5 v/v.

	Loam	*Gyttja clay*	*Coarse sand*	*Sphagnum peat*
Particle size composition % $< 2\ \mu m$	26	59	6	—
$2-20\ \mu m$	42	19	17	—
$>20\ \mu m$	32	22	77	—
Org. matter %	7.7	4.3	6.6	72.7
Nutrients P mg/l	6.5	6.3	8.3	3.2
Ca »	200	1200	1580	290
Mg »	78	559	100	40
K »	100	370	120	40
pH_{H_2O}	4.5	5.1	6.3	3.5
Sample weight kg/pot	3.74	4.50	5.38	1.60
Moisture %	3.6	7.2	2.7	84.9

Table 2. Annual dry-matter yields of grass g/pot.

	Loam		*Gyttja clay*		*Coarse sand*		*Sphagnum peat*	
Treatment	*1970*	*1971*	*1970*	*1971*	*1970*	*1971*	*1970*	*1971*
cf 1	40.4	38.5	32.4	40.4	43.2	37.3	29.5	32.4
uf 1	40.8	37.6	32.8	38.4	45.1	35.9	27.5	32.5
cf 2	55.9	53.7	49.7	51.3	54.7	53.0	40.4	40.7
uf 2	58.0	51.2	52.8	45.6	53.1	39.0	48.5	48.1

L.S.D. 5 % 4.4 (1970)
5.8 (1971)

Table 3. Amounts of nutrients taken up by grass yearly, mg/pot.

Treatment	*Loam*		*Gyttja clay*		*Coarse sand*		*Sphagnum peat*	
	1970	*1971*	*1970*	*1971*	*1970*	*1971*	*1970*	*1971*
				N				
cf 1	1210	950	950	800	1250	810	860	730
uf 1	1190	950	930	790	1260	830	910	710
cf 2	2130	1720	1790	1580	2110	1670	1710	1510
uf 2	2080	1830	1830	1750	2000	1120	1630	1630
L.S.D. 5 %	120	(1970)						
	150	(1971)						
				P				
cf 1	140	160	100	130	110	130	220	260
uf 1	130	160	100	140	110	110	230	270
cf 2	210	240	180	190	200	210	400	490
uf 2	240	260	190	200	190	200	460	570
L.S.D. 5 %	70	(1970)						
	80	(1971)						
				K				
cf 1	1330	1090	1680	1610	1340	880	830	800
uf 1	1320	1090	1680	1560	1340	900	840	760
cf 2	2170	1930	2980	2410	2060	1740	1650	1540
uf 2	2180	1890	3060	2360	2120	1540	1620	1670
L.S.D. 5 %	100	(1970)						
	140	(1971)						
				Ca				
cf 1	420	310	210	230	430	340	290	330
uf 1	340	270	180	190	380	310	220	280
cf 2	600	500	360	360	540	590	440	510
uf 2	510	430	310	270	430	310	400	430
L.S.D. 5 %	40	(1970)						
	30	(1971)						
				Mg				
cf 1	120	90	90	90	150	100	100	100
uf 1	100	80	80	90	130	90	90	100
cf 2	180	140	150	150	200	150	150	160
uf 2	150	130	150	110	150	100	150	170
L.S.D. 5 %	15	(1970)						
	13	(1971)						

RESULTS AND DISCUSSION

Only slight differences could be established in the annual dry matter yields achieved with the different fertilizers (Table 2). On the sphagnum peat the urea ammonium phosphate produced higher yields than the ammonium nitrate and monocalcium phosphate at the higher level of ferlilization during both growing seasons. On coarse sand a less consistent but somewhat similar pattern emerges.

From the first to the second year the dry matter yield of grass receiving high urea ammonium phosphate fertilization seems to have declined more sharply than the yield of that getting the control fertilization. Compound fertilizers containing urea have usually proved useful on ley plants and cereals in short-term experiments (GASSER and PENNY 1967, SPRATT 1973).

In terms of the quantities of nutrients taken up by the grass, the urea ammonium phosphate was fully equal to the ammonium

Table 4. Nutrients available to plants in the soils after the experiment mg/l. The method is the same as that in Table 1.

Soil	*Treatment*	*P*	*Ca*	*Mg*	*K*
Loam	cf 1	8.3	1100	78	40
	uf 1	7.9	1000	80	40
	cf 2	15.0	1200	58	65
	uf 2	12.0	975	73	50
	cf 1	11.7	2500	563	200
	uf 1	12.6	2300	563	190
Gyttja clay	cf 2	16.4	2400	538	170
	uf 2	17 1	2300	558	190
	cf 1	10.3	1100	90	30
	uf 1	9 2	1050	97	30
Coarse sand	cf 2	15.0	1150	65	30
	uf 2	14 2	950	90	40
	cf 1	72.0	1350	109	50
	uf 1	54.5	1300	118	50
Sphagnum peat	cf 2	141.0	1350	70	40
	uf 2	567.0	1250	79	30
L.S D. 5 %		28.0	51	9	30

nitrate and the monocalcium phosphate as a source of plant nitrogen and plant phosphorus during both growing seasons (Table 3). Considerably more phosphorus was applied than was taken up by the plants, as can be seen from the phosphorus values of the soil analysis made at the end of the experiment (Tables 1 and 4). The plants took up a great deal more phosphorus from the sphagnum peat than they did from the mineral soils. Obviously this is because sphagnum peat does not retain phosphorus as effectively as mineral soils containing sesquioxides do (KAILA 1958, SALONEN et al. 1973).

The amounts of calcium and magnesium taken up by the crops reveal distinct differences between the types of fertilizer (Table 3). The uptake of calcium and magnesium in the grass receiving urea ammonium phosphate fell short of the amounts in the grass receiving ammonium nitrate and monocalcium phosphate. This was partly due to the water-soluble calcium contained in the monocalcium phosphate. In addition, where urea ammonium phosphate was applied, the grass took up the nitrogen in the form of ammonium and this caused a reduction in the uptake of divalent cations of calcium and magnesium (MULDER 1956). The difference was greatest in the yields of the first harvest, when the nitrification of the ammonium nitrogen had not yet got started. The decline in the pH values of the soil caused by the ammonium nitrogen was not very evident, except in the sphagnum

Table 5. pH-values of soils (0.1 N KCl v/v 1 : 2.5) Measured annually after the third harvest.

	Loam		*Gyttja clay*		*Coarse sand*		*Sphagnum peat*	
Treatment	*1970*	*1971*	*1970*	*1971*	*1970*	*1971*	*1970*	*1971*
cf 1	4.7	4.5	4.7	4.7	4.9	5 1	5.8	4.4
uf 1	4.6	4.4	4.9	4.6	4.7	5.0	4.5	4.3
cf 2	4.5	4.3	5.3	4.4	4.7	5.0	5.0	4 1
uf 2	4.5	4.3	4.6	4.5	4.8	5.3	4.0	3.9
L.S D. 5 %	1.0	(1970)						
	0.9	(1971)						

peat (Table 5). From the results it seems that urea ammonium phosphate is quite a useful fertilizer from all points of view. It is also possible to produce fertilizers containing potassium from it. Good examples of this include the NPK combinations 19-8-16 and 26-6-11, for which there would be a use in Finland (HEMSLEY et al. 1970).

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SELOSTUS

Ureafosfaatti typpi- ja fosforilannoitteena

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Maatalouden tutkimuskeskus

Urea-ammoniumfosfaattia (29-13-0) verrattiin kaksivuotisessa astiakokeessa ammoniumnitraattiin ja monokalsiumfosfaattiin neljällä maalajilla. Kokeessa käytettiin kahta lannoitustasoa.

Koekasvina ollut Italian raiheinä antoi urea-ammoniumfosfaatilla samansuuruiset sadot kuin ammoniumnitraatilla ja monokalsiumfosfaatilla kumpanakin koevuotena. Samoin urea-ammoniumfosfaatin typpi ja fosfori olivat kasville yhtä käyttökelpoisia kaikilla maalajeilla kuin vastaavat määrät ammoniumnitraatin typpeä ja monokalsiumfosfaatin fosforia. Urea-ammoniumfosfaattilannoituksen saaneet kasvit ottivat vähemmän kalsiumia ja magnesiumia kuin ammoniumnitraattia ja monokalsiumfosfaattia saaneet.

Tulosten perusteella urea-ammoniumfosfaattia voidaan pitää käyttökelpoisena lannoitteena, jonka pohjalta voidaan valmistaa myös kaliumia sisältäviä moniravinteisia lannoitteita.

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THE NECTAR RASPBERRY, RUBUS IDAEUS × RUBUS ARCTICUS — A NEW CULTIVATED PLANT

HEIMO HIIRSALMI and JAAKKO SÄKÖ

HIIRSALMI, H. & SÄKÖ, J. 1976. **The nectar raspberry, Rubus idaeus × Rubus arcticus — a new cultivated plant.** Ann. Agric. Fenn. 15: 168–174. (Agric. Res. Centre, Inst. of Hortic., SF-21500 Piikkiö, Finland.)

Since 1939, the Institute of Horticulture has been engaged in breeding a hybrid between the raspberry (*Rubus idaeus* L.) and the nectarberry (*Rubus arcticus* L.). The aim has been to develop a plant possessing the flavour of the nectarberry, but as easy to cultivate as the raspberry. The F_1 generation lacks practical importance, being almost sterile. F_2 and F_3 generations, obtained by free pollination, provided a number of plants whose yields, fruit size and flavour proved to be very promising. These were selected for further breeding and named nectar raspberries.

The nectarberry flavour was strongest in an individual of the F_3 generation, which received the name 'Merva'. Unfortunately, its yield was so poor that it could not be commercially introduced for general cultivation. However, a cross between the raspberry variety 'Malling Promise' and 'Merva' provided promising selections, one of which was put on sale in 1975, under the variety name of 'Heija'. Both vegetatively and from the point of view of cultivation, its properties are very similar to those of the raspberry, whereas the qualitative composition of its aroma greatly resembles that of the nectarberry.

Index words: *Rubus idaeus* L., *Rubus arcticus* L., nectar raspberry.

The nectarberry *(Rubus arcticus* L.), widely known for the delicate flavour of its fruit, occurs in subarctic Eurasia, mainly between latitudes 60 and 70. The possibility of cultivating this plant has been investigated in Finland since as early as 1930. Research has now progressed so far that experimental field plantings have been made and two selected natural strains, 'Mespi' and 'Mesma', were recommended for growers in 1972 (RYYNÄNEN 1972, 1973 a, 1973 b, 1974).

However, the productivity of the nectarberry is comparatively poor, and its fruit, which is rather small, is so tightly attached that it has to be picked together with the receptacle and calyx. Harvesting is further hampered by its low growing habit and the protracted fruiting season, which may last as long as two months. A desire to eliminate these negative characteristics as far as possible gave rise to the idea of breeding a plant that would be as easy to cultivate as the raspberry but would also possess the distinctive flavour of the nectarberry, which

is so suitable for use in the manufacture of liqueurs and soft drinks (VAARAMA 1951, 1956, MEURMAN 1956, ROUSI 1965 a, HIIRSALMI 1968 a, 1968 b, 1969).

Initial stage of breeding

It may be said that breeding and selection work began on the genus *Rubus* at the Institute of Horticulture in 1939—1940, when attempts were made to cross varieties of the raspberry *(R. idaeus* L.) with various strains of the nectarberry (*R. arcticus* L.). These attempts at cross-breeding resulted in four seeds from a raspberry variety of somewhat uncertain origin (VAARAMA 1951, ROUSI 1965 a), which had been pollinated by a nectarberry strain originally obtained from Maaninka in North Savo. Only one of these four seeds germinated. This minimal F_1 generation was then propagated many times. Unfortunately, in spite of repeated attempts, a primary cross between the raspberry and the nectarberry has never again been obtained.

Morphologically, the F_1 cross is intermediate between the raspberry and the nectarberry, but is clearly more similar to the latter. Its shoots, which reach a height of ca. 50 cm, are annual and spineless; its leaves are fairly small, thin, delicate and sparsely dentate, and its flowers are pink with the corolla longer than the calyx. It is also intermediate between the raspberry and the nectarberry in the taste and aroma of its fruit.

Unfortunately, however, this cross is almost sterile. Although it flowers abundantly from the middle of June till late in the autumn, only a few imperfectly formed fruits develop at the very end of the summer. In spite of this marked sterility, the meiosis of the F_1 generation was found to be fairly regular (VAARAMA 1948); the conjugation of the chromosomes was complete in 83.4 % of the divisions, seven bivalents being formed. Each of the parent species has the diploid chromosome number 14 (VAARAMA 1939). Disturbances in meiosis can thus account for only a small part of the sterility. The main cause is the poor tolerance of the anthers and pollen to low humidity during the summer, which makes fertilization impossible (VAARAMA 1948, 1951). As the atmospheric humidity increases in the autumn, a few fruits are formed. This feature, perhaps inherited from the nectarberry (HIIRSALMI 1975), is known as seasonal sterility and is most probably due to unfavourable combinations of genes.

The first F_2 seedlings were obtained in 1946 from the rare seeds resulting from natural pollination of the F_1 generation. Subsequent research showed that sterility had been largely overcome in the F_2 generation, the unfavourable gene combinations evidently having disappeared (VAARAMA 1951). Considerable variation existed between the individuals and some of them were not viable at all. They displayed many different combinations of the characters of the raspberry and the nectarberry. The most discouraging feature was that the flavour of the nectarberry occurred in only a few individuals of the F_2 and later generations, and even then was rather weak. On the other hand, besides that of the raspberry, these plants possessed many new and often unexpected flavours. It appears that inheritance of the flavour of the nectarberry depends on a complex gene system, which is easily lost in hybrids.

The number of hybrid individuals was increased considerably by growing new generations. The year 1952 was the first in which the F_2 and F_3 generations reached the fruiting stage on such a scale that it was possible to select a fairly large number of individuals whose productivity, fruit size and flavour were considered promising from the point of view of future breeding work. Their fruit was found to possess new types of flavours, most closely resembling different kinds of acid raspberries, which promised

to be very useful. The nectarberry flavour also occurred, although rather rarely and to varying degrees. The individuals possessing the nectarberry flavour were propagated vegetatively and are known as nectar raspberries.

Development of the nectar raspberry for cultivation

The nectarberry flavour was found to be strongest in an individual of the F_3 genaration, which was subsequently propagated vegetatively and given the name 'Merva' (Rousi 1965 a). Its fruit was favourably received by the foodstuffs industry, being considered suitable for such purposes as the preparation of liqueurs. 'Merva' resembles the raspberry in its growth habit, but is only half its height and much more slender. Its shoots are biennial and fairly spiny. Its leaves, which are noticeably smaller than those of the raspberry, have the reddish tinge found in the nectarberry. The flowers, with their white petals, are similar too but smaller than those of the raspberry. The fruit resembles that of the raspberry in shape and colour, and is easily detached from the calyx and receptacle. It is dark red when completely ripe and has something of the nectarberry in taste and aroma, although it is closer to the raspberry.

Unfortunately, 'Merva' is not particularly productive and its fruit yield varies widely from year to year. The fruit size is of almost the same order as that of the wild raspberry (Hiirsalmi 1971). Even in the best years, the yield on open ground is only ca. 10 kg/100 m². Another disadvantage is that pollination tests have shown 'Merva' to be self-sterile, though not as completely so as the nectarberry (Tammisola and Ryynänen 1970) and the F_1 cross. Thus, when 'Merva' is cultivated other hybrids must always be set beside it to pollinate it. Mainly for these reasons, 'Merva' has not been recommended for growers; nevertheless the plant may be considered to represent an important milestone in the present breeding programme, and has been used successfully in cross-breeding not only in the Finnish Institute of Horticulture but also in Sweden (Larsson 1969).

In recent years, work on improving the nectar raspberry has been continued at the Institute of Horticulture, Piikkiö, and a programme has been drawn up with the aim of obtaining increasingly suitable cultivars (Rousi 1965 a, 1965 b, Hiirsalmi 1968 a, 1973). Individuals are chosen each year for cross-breeding and self-pollination. Attention has primarily been concentrated on back-crossing different generations of the raspberry and nectarberry hybrids with raspberry varieties.

So far, the most promising results have been obtained with a cross performed in 1962 between 'Malling Promise' and 'Merva'. This cross was given the code number 62020, and the five most promising selections were chosen from it for further research, 003, 011, 018, 037 and 053. Morphologically, they are extremely similar to the raspberry, resembling it very closely in growth and height, and in the shape and size of the shoots, leaves and flowers.

Nos. 018, 037 and 053 proved to have the most suitable properties for cultivation (Tables 1 and 2). Their yield is in the same class as that of the raspberry variety 'Ottawa', which was used for comparison, and the size of the fruit is satisfactory, being even greater than that of 'Ottawa' in the selection 037. However, the berries are rather soft, and organoleptic tests showed that their flavour is somewhat weaker than in 'Ottawa', except in 053. It is notable that the nectarberry flavour is present in all three selections to a perceptible degree. They do not differ markedly from 'Ottawa' in the shape and colour of the fruit, or in their flowering time, ripening of shoots and winter hardiness. Selection 053 has the

Table 1. Properties of five selections from the cross 'Malling Promise' x 'Merva': average yield, size of berries and ripening of shoots in 1970—1974; average hardiness, and start and duration of flowering in 1971—1974; length and spininess of shoots in 1974. The raspberry variety 'Ottawa' was used for comparison.
Ratings: ripening of shoots: 0—100 = quite incomplete — quite complete; hardiness: 0—100 = all shoots dead — completely healthy; spininess: 0—10 = spineless — very spiny.

Selection / Variety	Total yield kg/100 m²	Saleable yield kg/100 m²	Wt of 100 berries g	Ripening of shoots 0—100	Hardiness 0—100
62020*003*	8	6	201	98	84
62020*011*	10	8	242	94	63
62020*018*	34	29	181	89	89
62020*037*	40	31	269	92	93
62020*053*	33	27	165	91	80
'Ottawa'	39	35	235	95	91

Selection / Variety	Start of flowering date	Duration of flowering days	Length of shoot cm	Spininess of shoot 0—10
62020*003*	20/6	15	117	—
62020*011*	21/6	18	123	—
62020*018*	19/6	15	189	4.0
62020*037*	19/6	17	174	5.3
62020*053*	19/6	15	212	3.3
'Ottawa'	18/6	17	158	5.8

Table 2. Properties of fruit of five selections from the cross 'Malling Promise' x 'Merva': average firmness, flavour, nectarberry aroma, shape and colour in the years 1970—1972.
Ratings: firmness: 0—10 = very soft — very firm; flavour: 0—10 = very poor — very good; nectarberry aroma: 0—10 = absent — very strong; shape: 1 = conical, 2 = oval, 3 = round, 4 = flatly rounded; colour: 0—10 = white — purplish red.

Selection / Variety	Firmness 0—10	Flavour 0—10	Nectarberry aroma 0—10	Shape 1, 2, 3 and 4	Colour 0—10
62020*003*	4.4	6.2	3.7	3	7.3
62020*011*	6.3	5.9	4.8	4	6.7
62020*018*	4.9	6.0	6.4	3	5.9
62020*037*	5.0	5.8	5.3	2	6.4
62020*053*	6.2	6.6	4.5	4	6.6
'Ottawa'	6.9	6.4	0.0	3	6.6

longest shoots, but since they bend to the side, they are not so high as the sturdier shoots of no. 018. In addition, it branches and forms new shoots more abundantly than 'Ottawa' and the other selections and is less spiny.

In the last few years, the composition of the substances responsible for the flavours of the nectarberry, the raspberry and their hybrids has been investigated in cooperation with the Technical Research Centre of Finland and the Department of Biochemistry of the University of Turku. The final results are not yet available but, for example, about 60 flavouring components have been isolated in the nectarberry. Instrumental investigation of the aroma of the three most promising nectar raspberry selections showed that their aroma distillate is remarkably similar in qualitative composition to that of the nectarberry. The chief constituents of the aroma of the nectarberry are also those of the nectar raspberry. However, in spite

of this qualitative similarity, the most noticeable constituent of the nectarberry aroma, a certain furane derivative, was found in only very small amounts in the nectar raspberry selections. Comparison with the raspberry showed that a number of its compounds are lacking in the nectar raspberry. It has inherited only very small amounts of the raspberry ionones and dihydroionones that have been observed to have an unfavourable effect on the aroma. For example, alpha- and beta-ionones, volatile compounds with a strong scent and taste that are characteristic of the raspberry, are present in the fruit of the nectar raspberry in concentrations of, at most, 1 % of that of the parent plant (HIIRSALMI et al. 1974). Selection 053 possesses particularly few ionones, whereas it contains comparatively large amounts of the constituents responsible for the aroma of the nectarberry. However, owing to the sourness of the fruit, these constituents are not so noticeable in organoleptic tests as they are, for example, in the sweeter fruit of selection 018.

The results of comparative investigations led to the decision to recommend selection 053 for general cultivation in spring 1975, under the variety name of 'Heija'. However, the suitability for cultivation of other promising nectar raspberry selections will be examined in further comparative experiments, and crossing and back-crossing will be continued, with the aim of developing still more valuable cultivars.

The nectar raspberry variety 'Heija'

The nectar raspberry variety 'Heija' was selected from the offspring of a cross between the raspberry variety 'Malling Promise' and the nectar raspberry selection 'Merva', performed in 1962 at the Institute of Horticulture. In cross-breeding performed at the Institute in 1940, a raspberry *(Rubus ideaeus* L.) variety of uncertain provenance had been pollinated by a nectarberry *(Rubus arcticus* L.) strain originating from Maaninka in North Savo. A single F_1 individual was obtained. A number of plants were selected from the offspring resulting from the free pollination of this individual and one of them yielded, by free pollination, 'Merva'.

'Heija' resembles the raspberry in its vegetative characteristics. Its growth habit and height, and the shape and size of its shoots, leaves and flowers are entirely typical of the raspberry. The shoots are pale brown and often over 2 m long, although their height is not so great, since they bend to the side. Slightly recurved 1—2 mm long spines occur throughout the shoot, but not very abundantly. A dense bush is formed, owing to branching and the abundant formation of new shoots. The leaves are clear green, turning greyish underneath with age and trifoliate. The middle leaflet is broadly cordate, ca. 5 cm long and 4 cm broad, and fairly evenly and sparsely dentate. The flowers are over 20 mm in diameter; the petals are white and narrow, ca. 7 mm long and 3 mm broad.

From the point of view of cultivation, the properties of 'Heija' are also comparable to those of the raspberry. Its yield is almost as high as that of the raspberry varieties commonly cultivated. The berries, which, like those of the raspberry, are easily detached from the receptacle, are flatly rounded and fairly small, the weight of 100 berries being ca. 165 g. They are dark red and slightly soft, and the nectarberry flavour is just perceptible in their fresh sour taste. A biochemical investigation has shown that the aroma distillate is very similar in its qualitative composition to that of the nectarberry. The fruit is good both fresh and frozen, and can be used to make pleasant jams and juices. In the foodstuffs industry, the chief use of the nectar raspberry will probably be as a base for liqueurs and soft drinks.

Exactly the same technique can be adopted

in cultivating 'Heija' as is used for the raspberry. Unlike the nectarberry, it is self-fertile, so that it can be planted out alone. Less favourable features are ist rather great susceptibility to the spur blight of raspberry, *Didymella applanata* (Niessl) Sacc., and its only moderate hardiness.

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SELOSTUS

Mesivadelma, Rubus idaeus × Rubus arcticus — uusi viljelykasvi

HEIMO HIIRSALMI ja JAAKKO SÄKÖ

Maatalouden tutkimuskeskus

Puutarhantutkimuslaitoksessa on vuodesta 1939 lähtien suoritettu vadelman (*Rubus idaeus* L.) ja mesimarjan (*Rubus arcticus* L.) välistä risteytysjalostusta. Pyrkimyksenä on ollut sellaisen marjakasvin kehittäminen, jossa mesimarjan aromi yhtyisi vadelman edullisiin viljelyominaisuuksiin. F_1-polven risteytymä on lähes steriili ja vailla käytännön merkitystä. Vapaan pölytyksen tuloksena syntyneistä F_2- ja F_3-polvista

on valittu joukko jalosteita, joiden satoisuus, marjakoko ja aromi ovat osoittautuneet hyvin lupaaviksi. Niistä on ryhdytty käyttämään nimitystä *mesivadelma.*

Selvimpänä mesimarjan aromi on todettu eräässä F_3-polven yksilössä, joka on saanut nimen 'Merva'. Valitettavasti sen satoisuus on kuitenkin ollut niin heikko, että sitä ei ole voitu laskea yleiseen viljelyyn. Risteyttämällä 'Malling Promise' -vadelma-lajike ja 'Merva' keskenään on saatu lupaavia jalosteita, joista yksi on vuonna 1975 laskettu viljelyyn lajikenimellä 'Heija'. Se muistuttaa sekä kasvullisilta että viljelyllisiltä ominaisuuksiltaan vadelmaa. Aromi muistuttaa kuitenkin kvalitatiiviselta koostumukseltaan suuressa määrin mesimarjaa.

ANNALES AGRICULTURAE FENNIAE, VOL. 15: 175–181 (1976)

Seria AGROGEOLOGIA ET -CHIMICA N. 75 — Sarja MAA JA LANNOITUS n:o 75

RADIOPHOSPHORUS USED TO FOLLOW ROOT GROWTH IN FIELD PLANTINGS OF SPRING CEREALS

ARJA PAASIKALLIO

PAASIKALLIO, A. 1976. **Radiophosphorus used to follow root growth in field plantings of spring cereals.** Ann. Agric. Fenn. 15: 175–181. (Agric. Res. Centre, Isotope Lab., SF-01300 Vantaa 30, Finland.)

Radiophosphorus solution was injected into the soil at different depths (15, 30, 50 and 80 cm), with an injection probe. The growth rate of cereal roots was examined in the field by counting the uptake of radioactivity on the shoots. The experiments were performed in three consecutive years in sandy and silty clay soil. Wheat was used in the first year, and barley in the two following years.

The root system of wheat reached a depth of 80 cm about 61 days after sowing, or on about July 3, in both soils. The root system of barley arrived at a depth of 80 cm about 44 days after sowing, or on about June 23, in the sandy clay soil.

In the first two experimental years, the growth rate of the cereal roots was nearly equal in normally and deeply ploughed soils. In the third year, with severe early summer drought, the early development of the barley roots seemed to be somewhat faster in soil with a deep plough layer. In the silty clay soil, the root system failed to extend beyond either the deep or normal plough layer.

The injection of radioactive tracer makes it possible to follow the growth rate of the root system in the field from an early stage of development. However, due to great variation between the individual counts, a high number of replicates is required for proper statistical evaluation of the results, this, in turn, makes the application of the method rather laborious.

Index words: root growth, radiophosphorus.

INTRODUCTION

A frequently used method in root research is to place radioactive tracer, by injection or otherwise, at different soil depths and to count the radioactivity taken up by the roots on the plant shoots (FOX and LIPPS 1960, NEWBOULD and TAYLOR 1964, HAAHR 1968, ECKERT and BLINCOE 1970, ROBERTSON et al. 1972 and ERIKSSON 1973). This method is particularly suitable for determining when the roots arrive at a given depth in the soil or for estimating maximum root length (PEARSON 1974).

Compared with the laboratory method reported earlier (PAASIKALLIO 1970) where

the radioactivity was spread in an even layer at a given depth in the soil, the injection method offers an easier way of placing the tracer.

The aim of this study was to test the suitability of the injection technique for evaluating cereal root growth in the field, without taking any plant cuttings.

MATERIAL AND METHODS

The experiments were conducted in three consecutive years in Tikkurila, in a field in which the Institute of Agricultural Chemistry and Physics was examining the effects of different methods of ploughing and fertilizer application on the yield of spring cereals. There were two experimental areas, one on sandy clay soil, the other on silty clay soil. In the first year (1968) the following treatments were given in both areas: 1) deep ploughing (30 cm) combined with placement of fertilizer in rows and 2) normal ploughing (20 cm) combined with broadcast application of fertilizer. In 1968, spring wheat (var. Svenno) was sown in the field on May 3. In 1969, one barley variety (Karri) was sown on the sandy clay soil and another variety (Pomo) on the silty clay soil on May 7, sowing being repeated two days later, in order to obtain denser growth. In 1970, barley (var. Pomo) was sown on the silty clay soil on May 10 and on the sandy clay soil two days later. Experimental quadrats measuring 80 × 150 cm were used and tracer was injected at depths of 15, 30, 50 and 80 cm in four adjacent quadrats. In 1968 tracer was not injected at a depth of 15 cm. The amount of injections per quadrat varied from 20 to 24. The injections were made in or between three sowing rows, at intervals of about 10—15 cm.

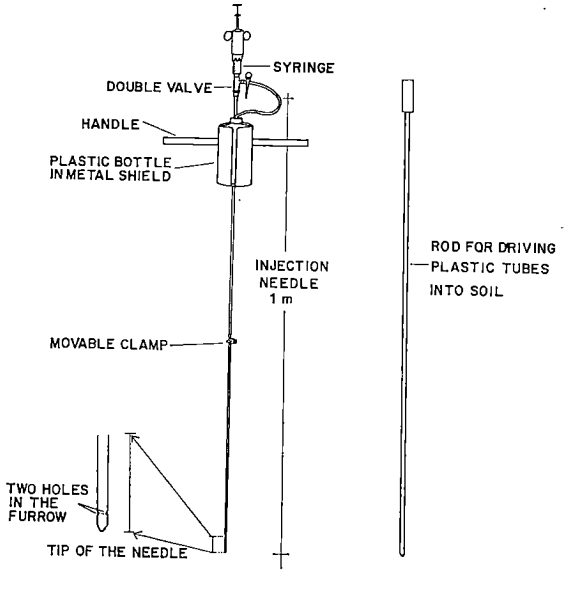

Fig. 1. The probe used to inject the tracer solution at different depths in the soil.

The injection probe (Fig. 1) was constructed according to LOEWENSTEIN (1965), with some modifications. Before the injection, plastic tubes were driven into the soil with a steel rod, the length of the tubes was determined by the injection depth. The tubes were not inserted into the soil until the seedlings were at least 2—3 cm long, or about two weeks after sowing. Two centimetres of the tube was left protruding above the soil surface and the tip of the metal rod penetrated 5 cm deeper than the lower end of the tube (Fig. 2). The tubes were left in the soil throughout the growing season. The injection probe was inserted into the plastic tubes and the tracer was injected about 2 cm beneath them, no tracer solution being left in the tube. The radioactive area in the soil was increased by the hole made by the rod below the tube. This appeared necessary, since the soil was very compact, especially in the deeper soil horizons. The sowing depth was about 5 cm and as the soil sank after sowing, the seeds lay about 2—3 cm beneath the soil surface. Each injection consisted of 3 to

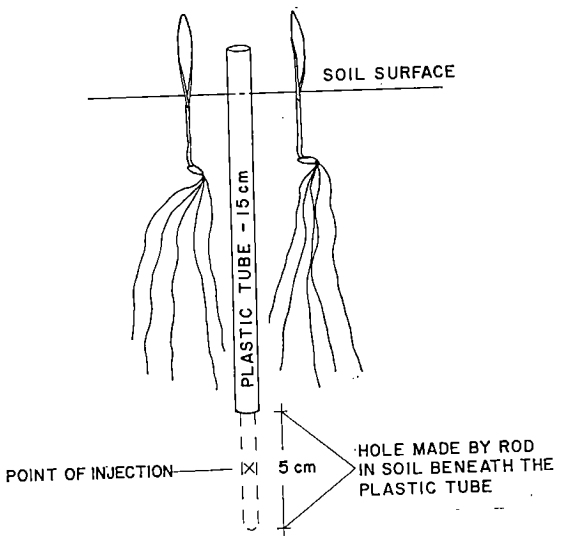

Fig. 2. The plastic tube inserted into the soil at seedling emergence. The point of injection is shown beneath the tube.

5 ml of neutral aqueous solution of sodium orthophosphate labelled with phosphorus-32, to which some carrier phosphorus was added. In 1968 125 μCi P 32 per tube was first injected at depths of 30 and 50 cm, and 200 μCi was injected at 80 cm about two weeks later. In the following years the radioactivity was decreased to 50 and 100—150 μCi, respectively, and the injections in the deepest soil horizons were carried out ealier, because when the shoots grew longer it was difficult to inject tracer without contaminating the aerial parts of the plants.

The radioactivity was counted on intact seedlings and shoots with a portable GM counter (RD-11, GM-tube GMP-533, Wallac, Turku). The readings were made separately at each injection site and were performed daily until the radioactivity was clearly detectable at all the sites in the quadrat, after which the measurements were made more seldom. During the measurements, the upper end of each plastic tube was covered with a lead cap. The mean radioactivity count was calculated for each experimental quadrat and the half-life corrections were made to the beginning of the measurements. The counts were found to vary widely between the different counting sites (20—24 sites per quadrat), so that the individual values could not be used as the basis of statistical treatment. The half-life of P 32 is 14.3 days. The shoot length was determined in each quadrat by taking the mean of four different measurements.

Weather conditions. In the experimental field, the average monthly temperature and precipitation (and the corresponding »normal» values) during the three growing seasons were as follows:

		May	June	July	Aug.
1968	°C	7.7	16.6	15.2	16.2
	mm	85	37	68	52
1969	°C	8.7	15.6	16.5	16.1
	mm	41	18	63	28
1970	°C	9.5	16.7	16.4	15.4
	mm	25	13	120	31
1931—1960	°C	9.3	14.3	17.0	15.4
	mm	40	48	73	75

In 1968, May was cooler and rainier than normal but June was dry, which was harmful to spring cereals especially those on clay soils. In 1969, May was a little colder than normal, but the precipitation was normal. The precipitation in June, July and August was below normal. In 1970, the average temperature in May was normal. The precipitation was 38 % lower than normal in May and 73 % below normal in June, but 64 % above normal in July (ANON. 1968—1970).

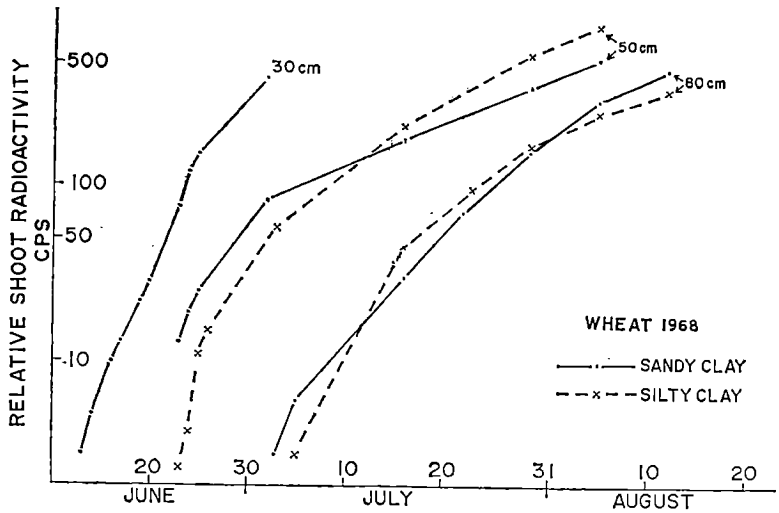

Fig. 3. The increase in the radioactivity of the wheat shoots after the roots had arrived at depths of 30, 50 and 80 cm in sandy and silty clay soil. The results from the areas with deep and normal plough layers are combined. Each point is the mean value of about 40 separate measurements.

RESULTS AND DISCUSSION

Experiments with wheat in 1968

Sandy clay soil. Fig. 3 shows the increase in the radioactivity of the wheat shoots after the roots had reached depths of 30, 50 and 80 cm in sandy and silty clay soils. The results of the areas with deep and normal plough layers are combined, because no clear differences were observed between them in the rate of growth of the roots. In sandy clay soil, the root system of wheat reached a depth of 30 cm on about June 13 and a depth of 50 cm about seven days later. It took about 13 days for the roots to grow from a depth of 50 to 80 cm. In the plot where the tracer was placed at 80 cm, radioactivity was detected in the wheat shoots on July 3, or 61 days after sowing. When the roots had arrived at a depth of 80 cm, the shoots had grown to a length of 50 cm. At the end of July, the shoots had reached their maximum length, which was about 68 cm.

Silty clay soil. Owing to the late placement of the tracer at a depth of 30 cm, no results were obtained from this quadrat. The roots arrived at depths of 50 and 80 cm at about the same times as in the sandy clay soil (Fig. 3). At the end of July, the length of the shoots was about 60 cm.

Experiments with barley in 1969 and 1970

Sandy clay soil. In both years, the growth of the root system of barley in this soil was rather good compared with the growth in the silty clay soil. In 1969, the injection at 15 cm was made too late (May 27); the roots had presumably passed this depth on about May 23—24. The roots arrived at depths of 30, 50 and 80 cm at about the same time in the deeply and normally ploughed soil, on May 29, June 11 and June 19 (Fig. 4). The root system of barley thus arrived at a depth of 80 cm

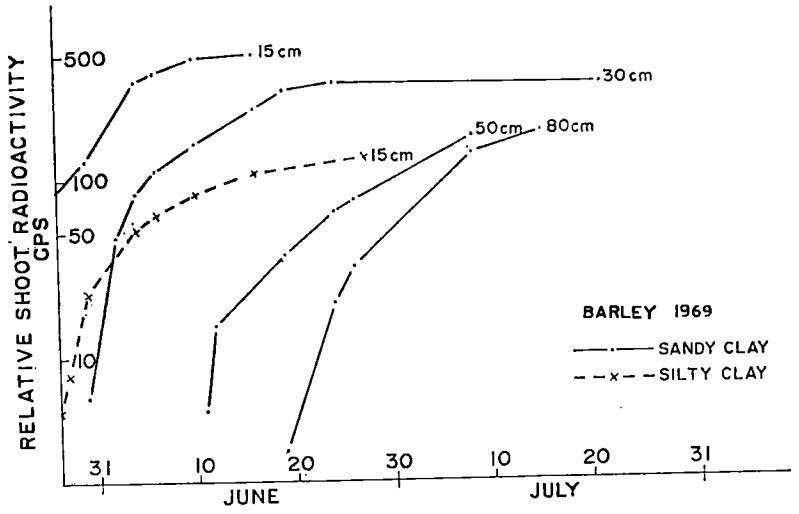

Fig. 4. The increase in the radioactivity of the barley shoots after the roots had arrived at depths of 15, 30, 50 and 80 cm in sandy clay soil, and a depth of 15 cm in silty clay soil in 1969. The results from the areas with deep and normal plough layers are combined. Each point is the mean value of about 40 separate measurements.

43 days after sowing. A difference in the rate of root development between barley and wheat was already detectable at a depth of 30 cm, but after this their growth rates were nearly equal (Fig. 5). The fact that the spring wheat »Svenno» is a late variety with respect to the growth of its aerial parts, is perhaps partly responsible for the great difference observed. However, the early development of wheat roots has been observed to be slower than that of other cereal species in a previous study, too (PAASIKALLIO 1970).

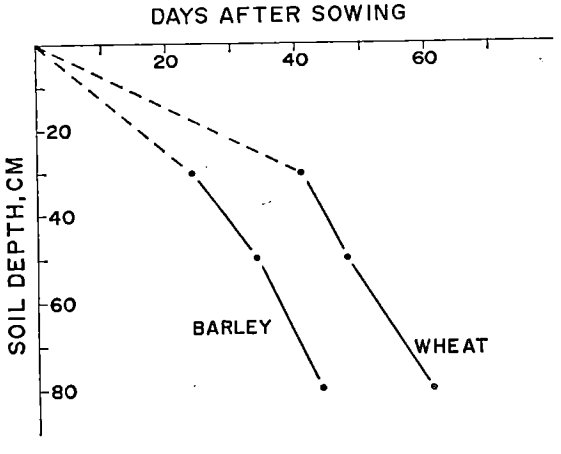

Fig. 5. The growth of wheat (1968) and barley (1969, 1970) roots from sowing to 30 cm, from 30 to 50 cm and from 50 to 80 cm as a function of time.

Fig. 6 shows the increase in the radioactivity of barley shoots in sandy clay soil with deep and normal plough layers in 1970. The injection depths were 15, 30, 50 and 80 cm. The root system arrived at a depth of 15 cm in the sandy clay soil at about the same time with both types of ploughing, on May 27. The roots reached a depth of 30 cm 7 days later in the deeply ploughed area and 12 days later in the normally ploughed area, or on June 3 and 8, respectively. The depth of 50 cm was reached about 10 days later in the deeply ploughed area and 7 days later in the normally ploughed area, or on June 13 and 15. With both types of ploughing a depth of 80 cm was reached about 11 days later, on June 26. The yields were 5 240 kg/ha in the deeply ploughed area and 4 560 in the normally ploughed area (LARPES, oral comm.). In 1970, the early summer was especially dry, which may

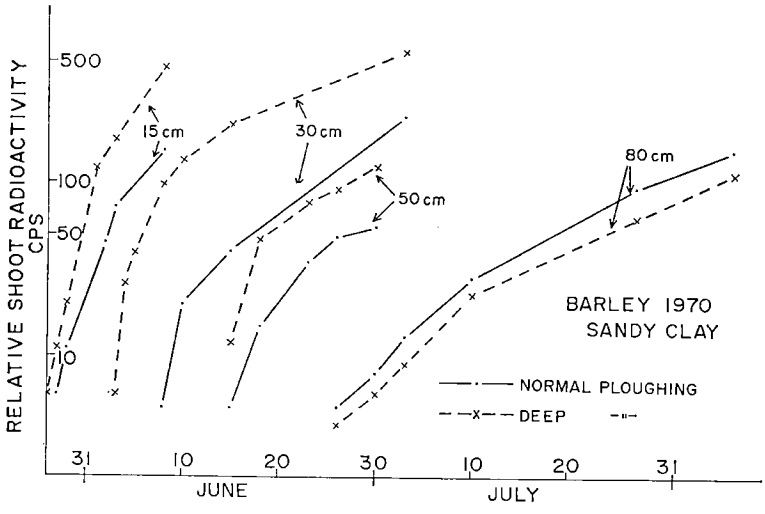

Fig. 6. The increase in the radioactivity of barley shoots in sandy clay soil with deep and normal plough layers after the roots had arrived at depths of 15, 30, 50 and 80 cm, in 1970. Each point is the mean value of about 20 separate measurements.

have been the reason for the difference in the growing rates of the roots in the soils with different plough layers. Deep ploughing has been reported to decrease yield losses caused by early summer drought in clay soils in southern Finland (LARPES 1973). Information on the soil moisture content might have been useful in interpreting the results, but moisture measurements were prevented by practical difficulties.

Silty clay soil. The vertical growth of the barley roots was evidently limited to the plough layer in both experimental years. In 1970 results were obtained only from the experimental quadrat where the injecting depth was 15 cm (Fig. 4). Drought in early summer may have limited root growth. Fig. 7 shows the growth of barley shoots and roots in 1970 in sandy and silty clay soils.

Compared with the »direct» method described here, the method in which cuttings are taken for analyses has the advantage that it gives information on changes occurring in the specific activity (P 32/P) of the plant, as a result of fluctuations in the intensity of its uptake of stable phosphorus during the growing season. However, the scantiness of the plant material that can be examined limits the application of the method, especially in investigations of early root growth (ERIKSSON 1973).

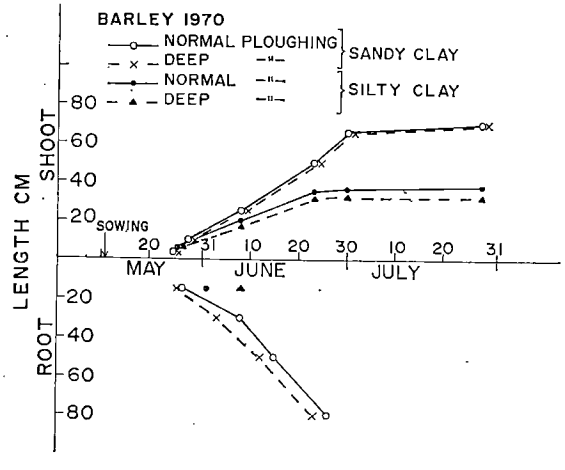

Fig. 7. The growth rate of barley shoots and roots in sandy and silty clay soils in 1970.

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SELOSTUS

Kevätviljojen juuriston kasvun seuraamisesta koekentällä radioaktiivisen fosforin avulla

ARJA PAASIKALLIO

Maatalouden tutkimuskeskus

Vuonna 1968 suoritettiin Tikkurilassa kevätvehnän ja vuosina 1969 ja 1970 ohran juuriston kehittymistä koskevia tutkimuksia radiofosforin avulla tarkoituksena lähinnä kokeilla injektiomenetelmän käyttökelpoisuutta juuristotutkimuksissa ilman leikkuunäytteiden ottamista.

Radioaktiivista fosforiliuosta injektoitiin maahan 15, 30, 50 ja 80 cm syvyyteen sitä varten valmistetulla ruiskulla (kuvat 1 ja 2) ja kasvin maasta ottama radiofosfori mitattiin kannettavalla geiger-mittarilla pellolla. Kun kasviin alkoi ilmaantua radioaktiivisuutta, osoitti se, että juuret olivat saavuttaneet sen maakerroksen, johon injektointi oli suoritettu. Tutkimuksia suoritettiin sekä hietasavi- että hiesusavimaalla, joilla oli suoritettu sekä syvä- (30 cm) että normaalikyntö (20 cm).

Vehnän juuristo saavutti 30 cm syvyyden kesäkuun 13. pnä, 50 cm syvyyden kesäkuun 20. pnä ja 80 cm syvyyden heinäkuun 3. pnä eli 61 päivän kuluttua kylvöstä. Eri kyntökäsittelyn saaneiden ja erilaisella maalajilla kasvaneiden vehnän juurten kasvunopeuden välillä ei havaittu eroja (kuva 3).

Hietasavimaalla ohran juuristo saavutti 80 cm syvyyden kumpanakin koevuonna keskimäärin 44 päivän kuluttua kylvöstä eli pari viikkoa aikaisemmin kuin vehnän juuristo (kuva 4). Ohran juuristo saavutti myös 30 cm syvyyden noin kaksi viikkoa aikaisemmin kuin vehnän juuristo (kuva 5). Vuonna 1970, jolloin kevät ja alkukesä olivat hyvin vähäsateisia, hietasavimaalla ohran juuriston varhaiskehitys näytti olleen syväkynnetyllä alueella jonkinverran nopeampaa kuin normaalikynnetyllä alueella (kuva 6). Hiesusavimaalla ohran juuriston pituuskasvu pysähtyi kumpanakin vuonna jo kyntösyvyyteen (kuvat 4 ja 7).

Menetelmän etuna (sellaiseen menetelmään verrattuna, jossa radioaktiivisuusmittaukset ja siihen liittyvät analyysit suoritetaan kasvinäytteistä laboratoriossa) on se, että sen avulla voidaan helposti tutkia juuriston kehitystä jo viljan orastamisvaiheesta lähtien, koska mittaukset voidaan suorittaa niukastakin kasviaineistosta ja kasvustoa tuhoamatta. Radioaktiivisuuden maahan sijoittaminen on selostetulla menetelmällä nopeaa ja vaivatonta. Toisaalta mittaustulosten suuren hajonnan takia tarvitaan monia kerranteita, mikä seikka tekee tällaisella tekniikalla suoritetusta tutkimuksesta melko suuritöisen.

ANNALES AGRICULTURAE FENNIAE, VOL. 15: 182—195 (1976)
Seria ANIMALIA DOMESTICA N. 42 — Sarja KOTIELÄIMET N:o 42

FACTORS AFFECTING THE COMPOSITION OF MILK

I. EFFECT OF ENERGY AND PROTEIN LEVELS IN GRASS SILAGE- AND PASTURE-BASED DIETS

ELSI ETTALA

ETTALA, E. 1976. **Factors affecting the composition of milk. I. Effect of energy and protein levels in grass silage- and pasture-based diets.** Ann. Agric. Fenn. 15: 182—195. (Agric. Res. Centre, Inst. Anim. Husb., SF-01300 Vantaa 30, Finland.)

The effect of the feed on the milk composition was investigated in nine indoor-feeding trials and eight pasture trials. In the indoor trials, 135 Ayrshire cows were used and milk analyses were made on 2606 samples from 188 lactations; in the pasture trials, 152 Ayrshire cows were used and 2054 samples were analysed from 243 lactations. In the indoor trials, the average composition of the dry matter consumed by the cows was 75 % silage, 13 % barley and 12 % hay; in the pasture trials it was ca. 99 % grass and ca. 1 % barley.

An increase in the energy supply, even an excess over estimated requirement, raised the content and yield of milk protein. The combined content of protein, fat and lactose was highest when the energy supply balanced the requirement. An increase in the protein supply raised the content and yield of milk protein, but an excess of protein over requirement increased only the protein content. In general, abundant supplies of energy and protein increased the proportion of protein in relation to the percentages of the other milk components.

An increase in the proportion of barley and N-free extract in the feed raised the contents of protein and fat in the milk, and also the yields of the milk and its different constituents, in both the indoor and pasture trials. The opposite effect was obtained by an increase in the average fibre content of the feed intake, and, accordingly, by an increase in the hay and fibrous silage fractions of the rations. An increase in the crude protein content of the feed raised the content and yield of milk protein. The effect of the feed on the lactose content was slight.

The total variation in the supplies of energy and protein and the composition of the rations explained 45.7 % of the combined variation of the protein, fat and lactose contents of the milk, when the milk yield and the lactation stage were kept constant.

Index words: silage, pasture, milk composition, energy intake, protein intake.

INTRODUCTION

One of the chief aims in recent research on cattle-feeding in Finland has been to achieve a satisfactory level of protein in diets unsupplemented with protein concentrates. Attention has mainly been concentrated on protein-rich pasture and silage; grass abundantly fertilized with nitrogen has proved to be a good source of protein both as pasturage (ETTALA et al. 1971 b) and as the raw material of silage (ETTALA and LAMPILA 1974.) In a series of experiments recently undertaken with varied concentrate-to-grass forage ratios, different applications of nitrogen to grass swards and different methods of preserving silage, milk analyses were also made to reveal the influence of the feeds on the milk composition.

The present paper examines the effect of dietary factors, primarily the supply of energy and protein, on the composition of milk. Attention is paid mainly to the milk protein content, but is also directed to its contents of fat and lactose, and to the yields of its different components. The second part of this study (ETTALA 1976 b) is devoted to the effect of other external factors on the composition of milk.

MATERIAL AND METHODS

Experimental procedures

The test animals were 287 Ayrshire cows. Some of them were used more than once in the trials, so that there were 431 lactations. The nine indoor-feeding trials (135 cows, 188 lactations) were performed in the years 1970/71—1972/73 at the Lintupaju farm of the Jokioinen estate and at the North Savo and Häme Experimental Stations. The eight pasture trials (152 cows, 243 lactations) took place on the Jokioinen estate (1970—1973), on the cattle Tikkurila farm of the Agricultural Research Centre (1969, 1971) and at the Häme Experimental Station (1970—1971). In the first three pasture trials, the lactose content was not determined; the lactose determinations in the summer trials were made for 132 cows and 188 lactations.

At the beginning of the trials, uniform feed was given during a preliminary period of 15—20 days. The indoor-feeding trials began in October — November, the pasture trials in May. The feed given in the preliminary period of the pasture trials was similar to that given in the indoor-feeding trials, consisting largely of silage. The change to the trial feeds was made gradually over a period of 5—10 days. The trials lasted 90—185 days (average 133 days).

The milk production of each cow was weighed separately at every milking. The protein, fat and lactose contents were determined at 10-day intervals on samples representing the milk produced by each cow during two days. Part of the milk sample was sent to a nearby dairy laboratory, where the fat content was determined; part was sent to a central laboratory farther away, for the determinations of protein and lactose — to the laboratory of the Central Cooperative Valio in Helsinki in 1969—1970 (pasture trials), to the Kainuu Cooperative Dairy at Sotkamo in 1970—1972 and to the laboratory of Kuivamaito Ltd. at Lapinlahti from spring 1972 onwards. Protein was measured with the Pro-Milk apparatus in Valio's laboratory and with the Infra Red Milk Analyser (IRMA) at the other laboratories. Lactose was also determined with the IRMA. The fat content was determined by the Gerber method or with Milko-Tester II. The milk samples were cooled

well and despatched in insulated boxes at the same time from all the trial localities. Potassium bichromate was used as preservative at a concentration of 0.1 %.

The IRMA was calibrated with control milk samples. Every twentieth sample was a control sample. Control samples were exchanged between the laboratories of the Kainuu Cooperative Dairy and Kuivamaito Ltd. and the results obtained were compared, control samples were also sent at monthly intervals to the Valio laboratory. The protein content of the control milk was determined by the Kjeldahl method and the lactose content by titrimetric analysis according to the IDF 28: 1964 standard (IDF Bulletin 1964).

The test animals and their feeding

The animals used in the indoor trials had calved 3.1 times, on average, and those used in the pasture trials had calved 3.9 times (Table 1). The time elapsed from calving to the middle of the trial periods averaged 143 and 129 days, respectively.

The silage given in the trials was prepared from grass fertilized with different amounts of nitrogen (ETTALA et al. 1974) and preserved with acid additives or with additives containing acid and formaldehyde (ETTALA et al. 1975 a and b). In the indoor trials, the cows were given silage *ad libitum*, but the individual intakes were measured. Each cow received 2 kg of hay a day and barley concentrate. The barley was given at the rate of 0, 1/3, 2/3 or 3/3 of the energy required for milk production exceeding 5 or 10 kg (4 %) (ETTALA and LAMPILA 1974). In the pasture trials, the cows fed on pasture fertilized with nitrogen at different rates, either without any supplement or with barley corresponding to 1/3 or 2/3 of the energy required for milk production exceeding 10 kg (4 %) (ETTALA et al. 1971 a and b). The production requirement was calculated as 0.4 feed unit (f.u. = 0.7 starch unit) and 60 g digestible crude protein (DCP) per kilogram 4 % milk, and the daily maintenance requirement as 3.8 f.u. and 320 g DCP per 500 kg liveweight (POIJÄRVI 1925, 1947). The cows were weighed at intervals of 30 days or less.

During the indoor-feeding period, the average dry matter (DM) intake of the cows was derived as follows: 75 % from silage, 13 % from barley and 12 % from hay (Table

Table 1. Mean weight, number of calving and time elapsed since last calving of test animals.

Variables	Indoor trials		Pasture trials	
	Mean	s.d.	Mean	s.d.
Liveweight, kg	476	50	485	48
No. of calvings	3.1	2.0	3.9	1.9
Time from calving to middle of trial, days	143	61	129	44

s.d. = standard deviation

Table 2. Feeds consumed, calculated nutrient supply and average composition of feed consumed in indoor trials.

Variables	Mean	s.d.
Feed consumed, kg/cow per day		
silage	41.2	7.8
DM of silage	9.4	1.9
barley	2.0	1.7
hay	1.8	0.8
total DM	12.6	2.3
Composition of DM of ration, %		
silage	75.0	10.3
barley	13.0	10.0
hay	12.0	4.6
crude protein	17.0	2.3
» fat	4.9	0.7
» fibre	25.4	3.7
N-free extract	44.6	4.5
Nutrient supply/cow per day		
f.u.	9.84	2.00
DCP, g	1479	384
supply-requirement, f.u.	−0.03	1.17
» » , DCP, g	244	376
protein/energy; DCP, kg/f.u.	0.15	0.03

Table 3. Least-squares analysis of effect of various factors on composition of rations eaten by cows, feed consumption and nutrient supply in indoor trials.

Properties	Regression variables				Factors					
	Days from calving		Milk yield		N. of calving		Years		Trial locality	
	b	F	b	F	% total var.	F	% total var.	F	% total var.	F
DM of ration, %										
silage	0.029	***	−1.451	***	3.6	***	3.4	***	1.6	***
barley	−0.006	*	1.883	***	4.7	***	19.1	***	12.4	***
hay	−0.021	***	−0.442	***	0.5	**	16.0	***	8.7	***
crude protein	0.002	*	0.014		0.2	*	4.2	***	41.6	***
» fat	0.002	***	−0.045	***	0.3	*	13.4	***	20.9	***
» fibre	−0.001		−0.539	***	1.0	***	38.6	***	8.6	***
N-free extract	0.001		0.726	***	2.6	***	17.0	***	7.3	***
Consumption and nutrient supply/cow per day										
total DM, kg	0.006	***	0.370	***	5.4	***	9.6	***	32.4	***
f.u.	0.007	***	0.432	***	6.0	***	4.7	***	12.8	***
DCP, g	0.001	***	0.046	***	1.9	***	1.9	***	11.9	***
supply-requirement, f.u.	0.005	***	0.020	**	4.8	***	2.5	***	16.8	***
supply-requirement, DCP, g	0.001	***	−0.015	***	1.9	***	1.7	***	9.0	***

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

2). On average, the DM of the ration contained: crude protein 17 %, crude fibre 25.4 % and N-free extract 44.6 %. Of the average f.u. received by the cows, 72 % was derived from silage and 18.5 % from barley. Of the DCP, ca. 81 % was obtained from silage, ca. 13 % from barley and the remaining 6 % from hay. The protein-to-energy ratio (DCP kg/f.u.) of the feed consumed averaged 0.15 (1: 6.7), but should have been 0.125 (1: 8), according to the norms. On average, the supply of energy balanced the requirement, but both surpluses and deficits occurred (ca. ± 2 f.u.) (Fig. 1). There was little underfeeding in respect of protein, but considerable overfeeding (Fig. 2). The variations occurring in the composition of the feed and the intake of the cows were mostly due to scheduled alterations in the level of concentrate, fluctuations in milk production, variations in the composition of the silage, and presumably also to individual variations in the intake of silage (Table 3).

The DM of the pasturage contained, on average: crude protein 19.6 %, crude fibre 25.3 % and N-free extract 41.3 % (Table 4). It was calculated that 1.36 kg of the DM of the grass made up one f.u. and that one f.u. contained 195 g of DCP. The feed value was calculated using a f.u. based on the starch value (NJF Fodermiddeltabel 1 1969: 5—7). In the feed of the cows receiving grass alone, the fibre content was as high

Table 4. Average chemical composition of grass, and barley ration given in pasture trials.

Properties	Average	s.d.
DM %	20.2	4.0
DM of grass, %:		
ash	11.1	1.5
crude protein	19.6	3.5
» fat	2.6	1.6
» fibre	25.3	2.5
N-free extract	41.4	3.8
DM kg/f.u.	1.36	0.10
DCP, g/f.u.	195	33.6
Barley, kg/cow per day	1.24	1.33

as in the rations of the indoor trials, and the crude protein content was higher, although the latter rations consisted of silage, barley and hay (Tables 2 and 4). When the pasture diet was supplemented with barley, its energy concentration rose and its protein content fell, as the level of the supplement was raised.

Statistical methods

Partial correlations and regressions were used to elucidate the influence of the feed. The influence of the milk yield and/or the time elapsed since calving was eliminated, i.e. they were kept constant. The partial regressions were calculated by stepwise multiple regression analysis. The canonical correlation was used to elucidate the combined effect of changes in the composition of the ration and the nutrient supply on the variation in the composition of the milk. Least-squares analysis was also employed in the study (HARVEY 1966).

RESULTS

Composition of the milk

The composition of the milk was analysed on 2606 samples from the indoor-feeding period and 2054 samples (1600 for lactose determination) from the pasture period. The average daily milk yield was 14.7 kg during the indoor trials and 18.6 kg during the pasture trials (Table 5). In the milk from the indoor trials, the average percentages of protein, fat and lactose were 3.33, 4.27 and 4.73; in the pasture trials, the corresponding percentages were 3.39, 4.17 and 4.56. The proportion of protein was higher in relation to the other two percentages in the pasture trials than in the indoor trials, but the ratio between milk fat and lactose was of the same size on the two types of feeding.

Table 5. Average milk yield of cows and composition of milk.

Properties	Indoor trials[1]		Pasture trials[2]	
	Average	s.d.	Average	s.d.
Milk yield, kg	14.7	4.1	18.6	4.6
Protein %	3.33	0.38	3.39	0.29
Fat %	4.27	0.47	4.17	0.47
Lactose %	4.73	0.30	4.56	0.29
P + F + L, %[3]	12.32	0.72	12.11	0.68
Protein, g	484	126	625	144
Fat, g	624	175	772	198
Lactose, g	703	218	862	240
Protein %/fat %	0.78	0.09	0.82	0.10
Protein %/lactose %	0.71	0.11	0.75	0.09
Fat %/lactose % .	0.91	0.12	0.91	0.12

[1] 2606 samples
[2] 2054 samples (lactose determined on 1600)
[3] protein % + fat % + lactose %

Effect of feed on protein content of milk and protein yield

In the indoor trials, the supplies of energy and protein were each positively and very significantly correlated with the protein content of the milk, when the effect of the lactation stage and the milk yield was eliminated (Table 6). The amount of barley concentrate and the contents of N-free extract and crude protein in the ration also had a very significant positive correlation with milk protein content, but the fibre content showed a negative correlation (Table 6). Fluctuations in the energy supply and the fibre content of the ration together explained 17 % of the variation in the milk protein content that was apparent when the milk yield and the stage of lactation had been eliminated (Table 7). Supplies of energy and protein

Table 6. Partial correlations of values describing composition of rations and nutrient supply with milk constituents in indoor trials.

Properties of rations and nutrient supply	Contents of milk constituents, %[1]				Ratios of constituents[1]			Yields, kg[2]			
	protein	fat	lactose	P+F+L[3]	protein/fat	protein/lactose	fat/lactose	milk	protein	fat	lactose
% of DM of rations											
silage	−0.12	−0.26	−0.04	−0.24	+0.13	−0.09	−0.22	−0.47	−0.46	−0.52	−0.48
barley	+0.25	+0.36	−0.01	+0.35	−0.10	+0.22	+0.33	+0.63	+0.65	+0.68	+0.61
hay	−0.17	−0.09	+0.09	−0.11	−0.08	−0.18	−0.11	−0.27	−0.32	−0.28	−0.23
crude protein	+0.28	+0.10	+0.01	+0.20	+0.16	+0.23	+0.09	+0.04	+0.18	+0.08	+0.04
» fat	+0.04	+0.01	−0.04	+0.01	+0.02	+0.06	+0.03	−0.19	−0.16	−0.17	−0.19
» fibre	−0.34	−0.17	−0.11	−0.31	−0.16	−0.24	−0.10	−0.50	−0.58	−0.50	−0.50
N-free extract	+0.22	+0.20	+0.13	+0.27	+0.02	+0.13	+0.12	+0.55	+0.56	+0.56	+0.55
Nutrient supply											
DM, kg	+0.16	−0.08	−0.10	−0.01	+0.22	+0.18	−0.03	+0.59	+0.58	+0.50	+0.55
f.u.	+0.30	+0.05	−0.08	+0.15	+0.24	+0.29	+0.08	+0.76	+0.75	+0.68	+0.72
DCP, g	+0.27	+0.06	−0.07	+0.15	+0.19	+0.26	+0.09	+0.41	+0.49	+0.39	+0.38
supply-requirement, f.u.	+0.17	−0.21	−0.05	−0.07	+0.36	+0.17	−0.17	+0.07	+0.15	−0.03	+0.05
» » , DCP, g	+0.21	−0.07	−0.07	+0.03	+0.26	+0.21	−0.04	−0.09	+0.03	−0.12	−0.11
protein/energy, DCP kg/f.u.	+0.13	+0.05	−0.06	+0.07	+0.07	+0.13	+0.07	−0.16	−0.07	−0.12	−0.17

[1]) Effect of lactation stage and milk yield eliminated
[2]) » » » » eliminated
[3]) protein % + fat % + lactose %
$P < 0.05$, $r > 0.13$; $P < 0.01$, $r > 0.17$; $P < 0.001$, $r > 0.21$ (observations 2606; d.f., number of feed samples −2; 247)

Table 7. Independent variables explaining significant percentage of variation in milk composition, and coefficients of determination when the effect of the lactation stage and milk yield is eliminated. The independent variables are those presented in Table 6.

Dependent	Independet	t-value	Total R^2 %	Regression equations
Protein % (y_1)	f.u. supply (x_1) fibre %/DM (x_2)	+ 7.33*** − 8.83***	17.0	$\Delta y_1 = 0.0696 \times \Delta x_1 - 0.0343 \times \Delta x_2$
Fat % (y_2)	barley %/DM (x_3) f.u. supply-requirement (x_4)	+13.30*** − 7.84***	17.8	$\Delta y_2 = 0.0259 \times \Delta x_3 - 0.0359 \times \Delta x_4$
Lactose % (y_3)	f.u. supply (x_1) N-free extract %/DM (x_5)	− 2.79** + 3.70***	2.8	$\Delta y_3 = -0.0229 \times \Delta x_1 + 0.0109 \times \Delta x_5$
P+F+L% (y_4)	fibre %/DM (x_2) barley %/DM (x_3) f.u. supply-requirement (x_4)	−11.07*** +12.10*** − 5.55***	18.6	$\Delta y_4 = -0.0635 \times \Delta x_2 + 0.0306 \times \Delta x_3 - 0.0775 \Delta x_4$
P %/F % (y_5)	f.u. supply-requirement (x_4)	+ 5.65***	12.7	$\Delta y_5 = 0.0278 \times \Delta x_4$
P %/L % (y_6)	f.u. supply (x_1)	+ 4.44***	8.4	$\Delta y_6 = 0.021 \times \Delta x_1$
F %/L % (y_7)	barley %/DM (x_3) f.u. supply-requirement (x_4)	+ 5.86*** − 3.05**	14.2	$\Delta y_7 = 0.0056 \times \Delta x_3 - 0.0163 \times \Delta x_4$
Milk, kg (Y)	f.u. supply (x_1)	+83.73***	57.1	$\Delta Y = 1.2851 \times \Delta x_1$
Protein, kg (Y_1)	f.u. supply (x_1)	+15.53***	57.0	$\Delta Y_1 = 0.0444 \times \Delta x_1$
Fat, kg (Y_2)	f.u. supply (x_1) barley %/DM (x_3)	+ 9.34*** + 9.35***	61.5	$\Delta Y_2 = 0.0354 \times \Delta x_1 + 0.0077 \times \Delta x_3$
Lactose, kg (Y_3)	f.u. supply (x_1)	+17.60***	51.9	$\Delta Y_3 = 0.0632 \times \Delta x_1$

[1]) Milk yield and lactation stage eliminated
[2]) Lactation stage eliminated
** $P < 0.01$; *** $P < 0.001$

exceeding the requirements also raised milk protein content (Figs. 1 and 2).

In the pasture trials, the effect of the dietary factors on milk protein content was very similar to that in the indoor trials (Tables 6 and 8). The amount of the barley ration and the energy and crude protein contents of the grass were very significantly positively correlated with milk protein content, when the stage of lactation and the milk yield were constant, but the fibre content showed a negative correlation (Table 5). A smaller amount of the variation in milk protein content was explained in the pasture trials (R^2 8.6 %, Table 9) than in the indoor trials (R^2 17 %, Table 8), which may be due to the fact that the feed intake could not be determined in the pasture trials.

The effect of the dietary factors on the protein yield was very similar to that exerted on the milk protein content (Tables 6 and 8), but the protein yield was not affected when the protein supply exceeded the norm. The partial correlation coefficients between the dietary factors and the protein yield were very similar to those between these factors and the milk yield (Tables 6 and 8), but the correlation between a surplus of

Table 8. Partial correlations of values describing composition of pasture and intake of barley with milk constituents in pasture trials.

Variables measured	Contents, %[1]				Ratios of constituents[1]			Yields, kg[2]			
	Protein	Fat	Lactose	P+F+L[3]	Protein/fat	Protein/lactose	Fat/lactose	Milk	Protein	Fat	Lactose
% of DM of grass:											
organic substance	+0.10	+0.15	+0.24	+0.26	−0.07	−0.03	+0.04	+0.06	+0.08	+0.13	+0.07
crude protein	+0.14	+0.02	+0.00	+0.09	+0.07	+0.06	+0.07	+0.12	+0.16	+0.10	+0.12
» fat	−0.03	−0.12	+0.06	−0.08	+0.09	−0.02	−0.18	−0.01	−0.02	−0.07	+0.04
» fibre	−0.15	−0.12	−0.05	−0.20	+0.01	−0.05	−0.17	−0.15	−0.18	−0.18	−0.15
N-free extract	+0.03	+0.17	+0.09	+0.19	−0.14	−0.02	+0.16	+0.02	+0.02	+0.11	+0.00
f.u./kg DM of grass	+0.17	+0.13	+0.12	+0.23	−0.01	+0.03	+0.14	+0.15	+0.19	+0.19	+0.15
Barley intake, kg	+0.22	+0.06	+0.09	+0.14	+0.09	+0.12	+0.01	[4]			

[1]) Effect of lactation stage and milk yield eliminated
[2]) » » » » eliminated
[3]) Protein % + fat % + lactose %
[4]) No correlations are given for the barley ration, since the barley was given to animals according to the milk yield

$P < 0.05$, $r > 0.08$; $P < 0.01$, $r > 0.11$; $P < 0.001$, $r > 0.13$ (protein and fat, observations 2054, d.f., number of grass samples −2; 639)

$P < 0.05$, $r > 0.09$; $P < 0.01$, $r > 0.12$; $P < 0.001$, $r > 0.16$ (lactose, observations 1600, d.f., number of grass samples −2; 477)

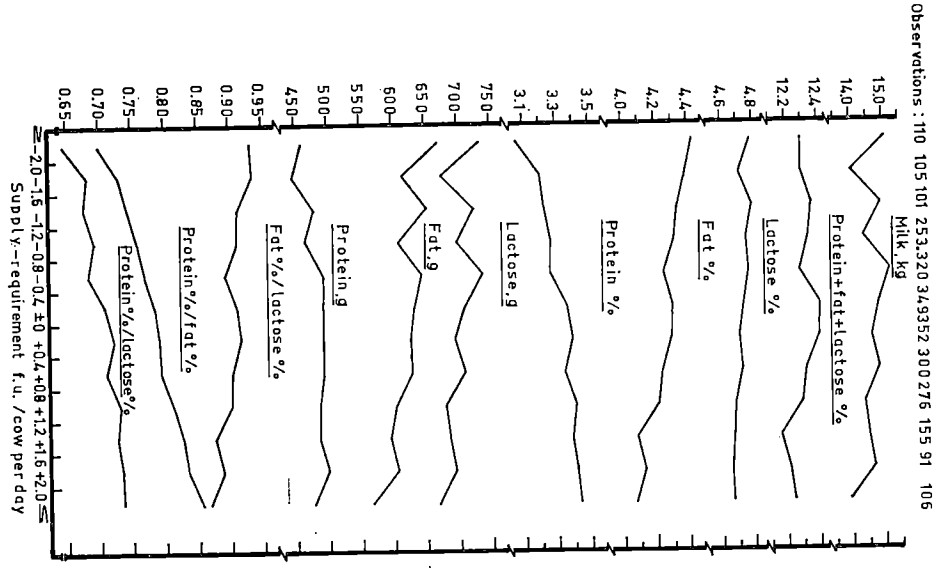

Fig. 1. Effect of relative net supply of energy on milk yield and milk constituents (effect of lactation stage and milk yield not eliminated).

Table 9. Independent variables explaining significant percentage of variation in protein and fat contents of milk, when the effect of the lactation stage and milk yield is eliminated

Dependent	Independent	t-value	R^2 %
Protein %	Barley, kg	+10.9***	5.3
	f.u./kg DM of grass	+ 7.2***	2.3
	DCP g/f.u. of grass	+ 3.5***	**0.6**
	Total		8.6
Fat %	N-free extract %/DM of grass	+12.3***	6.8
	Protein %/DM of grass	+ 6.5***	1.9
	Grass DM %	− 5.7***	**1.4**
	Total		7.2

*** $P < 0.001$

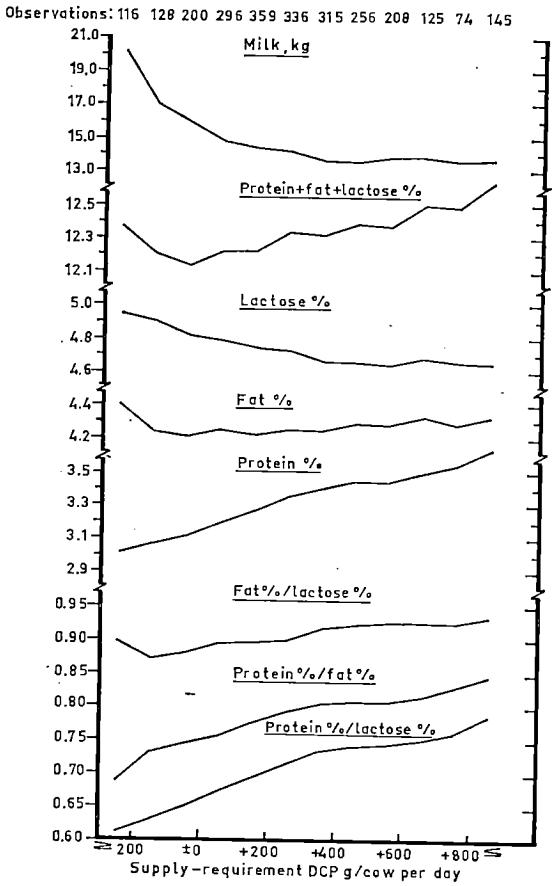

Fig. 2. Effect of relative supply of protein on milk yield and milk constituents (effect of lactation stage and milk yield not eliminated.

energy and the protein yield was stronger than that between a surplus of energy and the milk yield (Table 6). When the effect of the stage of lactation was eliminated, the fluctuation in the energy supply in the indoor feeds explained 57 % of the variation of both the milk and protein yields (Table 7).

An evident autocorrelation admittedly exists between the barley ration and the consumption of N-free extract and energy resulting from it, on the one hand, and the yields of milk and protein, on the other, since the amount of the barley supplement was determined according to the milk yield. In the pasture trials also, the energy value and the crude protein content of the grass were positively correlated with the protein yield, and the fibre content was negatively correlated (Table 8).

Effect of feed on fat content of milk and fat yield

The amount of N-free extract, and also the amount of barley given in the indoor rations are positively and significantly correlated with the fat content of the milk, when the effect of the milk production and the stage of lactation is eliminated (Tables 6 and 8). Milk fat showed a significant negative partial correlation with the fibre content of the ration and the amount of silage. A supply of energy in excess of the norm caused a very significant decrease in milk fat.

The influence of the dietary factors on the fat yield was very similar to their influence on the milk yield (Tables 6 and 8). In the indoor trials, the fluctuations in the energy supply and the barley ration explained 61.5 % of the variation in the fat yield, when the influence of the stage of lactation was eliminated (Table 7). This percentage may, however, be overestimated, owing to the method of deciding the amount of the barley ration. In the pasture trials, both the energy value and the crude protein content of the grass were positively correlated with the fat yield (Table 8).

Effect of feed on content and yield of lactose and protein + fat + lactose content of milk

The influence of the dietary factors on the lactose content was rather slight (Tables 6, 7 and 8). The chief positive correlation was that of readily digestible carbohydrates. The effect of the feed on the lactose yield was almost exactly the same as its effect on the milk yield (Tables 6 and 8).

The influence of the dietary factors on the combined protein, fat and lactose contents (P + F + L %) most closely resembled their influence on the protein and/or fat contents (Tables 6 and 8). In the material from which the influence of the milk yield and the stage of lactation had not been eliminated, protein in excess of the estimated requirement raised the combined content of these organic substances (Fig. 2), but the highest combined content was given by an energy supply agreeing with the norms (Fig. 1). According to the canonical correlation, the variation in the nutrient supply and the composition of the ration explained 45.7 % of the combined variation of the protein, fat and lactose contents of the milk, when the milk yield and the stage of lactation were constant.

Effect of feed on relationships between milk constituents

An increase in the supply of energy and protein, even above the standard requirement raised the protein content of the milk in relation to the contents of the other constituents (Tables 6 and 7, Figs. 1 and 2). The high protein content of the silage is presumably responsible for the fact that a positive correlation existed between the amount of silage and the protein-to-fat ratio of the milk. The amount of barley showed significant positive correlations with the protein-to-lactose ratio (Tables 6 and 8) and the fat-to-lactose ratio (Table 6). An increase in the amount of silage and a supply of energy in excess of the norm decreased the fat-to-lactose ratio (Tables 6 and 7, Fig. 1).

DISCUSSION

Since the milk yield and the stage of lactation influence the composition of the milk to an important degree (cf. part II, ETTALA 1976 b), their effects were eliminated when the effect of the feed was studied, i.e. they were kept constant, in order to obtain a clearer picture of its role. The effects of the supplies of energy and protein evident in the material before the elimination was performed may be seen in Figs. 1 and 2.

Grass herbage predominated in the experimental feeds (Table 2); its contribution to the f.u. consumption of the cows averaged 81.5 % in the indoor trials (as silage and hay) and 99 % in the pasture trials. The nutrition of the cows thus chiefly depended on their intakes of silage or pasturage. A balance of energy was generally achieved in the indoor trials, but there were also cases in which the energy obtained was considerably below or above the estimated requirement (ca. ± 2 f.u./cow a day) (Fig. 1); the protein supply mainly exceeded the norm (Fig. 2). The feed intake could not be measured in the pasture trials, but the excess of the protein supply over the norm was presumably even more pronounced than in the indoor trials.

In the present diets, the achievement of maximal percentages of milk protein and fat partly depended on an adequate supply of energy, especially the abundant readily digestible carbohydrates of barley or N-free extract in general (Tables 6 and 8). Energy in excess of the norms raised the protein content but depressed the fat content, thus increasing the proportion of protein in the milk in relation to the percentage of fat, and also to that of lactose. An increase in the fibre content of the ration (and thus also in the proportion of silage) lowered both the protein and fat contents. According to the regression equation obtained (Table 7), an increase in energy of, for example, 0.5 f.u. together with a decrease in fibre content of one percentage unit in the daily ration would cause a rise of 0.07 percentage unit in the protein content of the milk.

GÖNC (1971), HOLMES et al. (1957) and KIRCHGESSNER et al. (1965) also observed that milk protein decreased on silage diets, and they attributed this to an insufficient supply of energy. In many studies, an increase in readily digestible carbohydrates and/or a decrease in the fibre content have been observed to increase the formation of butyric acid and/or propionic acid in the rumen, thus raising the content of milk protein (BISHOP et al. 1963, HOOGENDOORN and GRIEVE 1970, HUBER and BOMAN 1966, HUBER et al. 1964, KIRCHGESSNER et al. 1965). YOUSEF et al. (1969) reported that high-grain feeding promoted the synthesis of protein. The high-grain rations increased the protein fractions synthetized within the mammary gland, especially α-casein and β-lactoglobulin, but serum albumins and other nitrogenous compounds decreased (YOUSEF et al. 1970). The increase in milk fat also caused by readily digestible carbohydrates in the present study is presumably due to the fact that the fibre content of the ration was at times remarkably high (Table 2).

KIRCHGESSNER et al. (1965) consider that the optimal dietary fibre content for the synthesis of fat is 18—22 % of DM; in the present study the average fibre content was over 25 % (Tables 2 and 4). In a number of investigations of low-fibre rations, increase in readily digestible carbohydrates depressed the content of milk fat, on account of the decrease in the formation of acetic acid (BISHOP et al. 1963, HOOGENDOORN and GRIEVE 1970, HUBER and BOMAN 1966, HUBER et al. 1964); in other investigations, it had no effect (CASTLE et al. 1958, 1959; CASTLE and WATSON 1961, HOLMES et al. 1957). Many workers report that an energy surplus increased and an energy deficit decreased milk protein (CASTLE et al. 1958, COMBERG and VOIGTLÄNDER 1959, HOLMES et al. 1956, 1957 HOLMES and ARNOLD 1960, HOOGENDOORN and GRIEVE 1970, ISAACHSEN et al. 1956), whereas the effect on milk fat was very slight (BURT 1957, CASTLE et al. 1958, 1959, CASTLE and WATSON 1961, HANSON et al. 1954, HOLMES et al. 1956, HOLMES and ARNOLD 1960, HOOGENDOORN and GRIEVE 1970, KAJANOJA 1944, LOGAN et al. 1959, POIJÄRVI 1952, ROOK and LINE 1962). In the investigation of ISAACHSEN et al. (1956) a large energy deficit also depressed milk fat.

An increase in the crude protein content of the feed and in the supply of protein, even when the supply was then greatly in excess of the requirement, also raised the protein content of the milk and at the same time increased its proportion in relation to those of fat and lactose (Tables 6 and 8). Unfortunately, in this study it was not possible to determine the different components of the milk protein. GÖNC (1971), and ORTH and KAUFMANN (1965) reported that grass silage increases only the non-protein nitrogen fraction in milk, and LEONHARD-KLUZ et al. (1973) found that large applications of nitrogen to pasture raised the contents of nitrate nitrogen and nitrite nitrogen in milk. POIJÄRVI (1952) found an increase in the content of non-protein nitrogen in the milk when the diet was supplemented with oil concentrate. In contrast, when ORTH and KAUFMANN (1964) and VIK-MO et al. (1975) used soya and casein supplements, respectively, the increases were mainly in the protein nitrogen of the milk and only partly in the non-protein nitrogen. A small surplus or deficit in protein supply generally did not affect milk protein content (HOLMES et al. 1956), but a marked surplus raised the content (LOGAN et al. 1959, ORTH and KAUFMANN 1964) and a marked deficit decreased it (ISAACHSEN et al. 1956, ROOK and LINE 1962). ORTH and KAUFMANN found that the protein content of milk increased when they raised the protein-to-energy ratio of the feed to twice that of normal rations. In the present study the average protein: energy ratio was above the standard requirement (cf. Tables 2, 6 and p. 4) and

was evidently very high at times in both the silage (Fig. 2) and pasture diets, so that the present results are consistent with those of ORTH and KAUFMANN.

The effect of the feed on the lactose content was observed to be small in this (Tables 6, 7 and 8) and many other studies (CASTLE et al. 1958, COMBERG and VOIGTLÄNDER 1959, HANSSON et al. 1954, HOLMES et al. 1956, 1957, HOLMES and ARNOLD 1960, HOOGENDOORN and GRIEVE 1970, ROOK and LINE 1962). Readily digestible carbohydrates increased milk lactose to some extent (Tables 6 and 8).

The combined content of protein, fat and lactose in the milk was highest when energy supply equalled the estimated requirement, and readily digestible carbohydrates played an important role in the achievement of an energy balance (Tables 6, 7 and 8).

In the indoor trials, the changes in the nutrient supply and the composition of the feed explained 45.7 % of the combined variation of the protein, fat and lactose contents of the milk, when the variations in the milk yield and the lactation stage were eliminated.

The effect of the feed on the yields of the different milk constituents was almost the same as its effect on the milk yield (Tables 6 and 8), but a surplus of energy had a significant positive correlation only with the protein yield. An increase in the already elevated protein-to-energy ratio decreased the yields, as did also an increase in the fibre content of the feed. The present results suggest that if optimal yields of milk protein and fat are to be obtained with diets containing abundant grass fodder, the fibre content of the feeds should be decreased and their energy concentration increased by giving more grain than the average ration used here, in both winter and pasture feeding.

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SELOSTUS

Maidon koostumukseen vaikuttavista tekijöistä

I. Energian ja valkuaisen saannin vaikutus maidon koostumukseen nurmikasvivoittoisessa ruokinnassa

ELSI ETTALA

Maatalouden tutkimuskeskus

Ruokinnan vaikutusta maidon koostumukseen on selvitetty 9 sisäruokinta- ja 8 laidunkokeen tulosten perusteella. Sisäruokintakauden kokeissa on ollut 135 ay-lehmää 188 lypsykaudella ja maidon koostumus on määritetty 2 606 näytteestä. Laidunkokeissa on ollut 152 ay-lehmää 243 lypsykaudella ja määritykset tehty 2 054 näytteestä. Lehmien keskimäärin syömästä kuiva-ainemäärästä on sisäruokintakaudella ollut 75 % säilörehua, 13 % ohraa ja 12 % heinää, laidunkaudella n. 99 % ruohoa ja n. 1 % ohraa.

Energia-annostuksen nousu, myös energian yliruokinta, on kohottanut maidon valkuaispitoisuutta ja -tuotosta. Energian yliruokinta on laskenut maidon rasvapitoisuutta. Valkuaisen, rasvan ja maitosokerin yhteispitoisuus on ollut korkein energiatasapainon vallitessa. Valkuaisannostuksen nousu on kohottanut maidon valkuaispitoisuutta ja -tuotosta, valkuaisyliruokinta kuitenkin vain valkuaispitoisuutta. Yleisesti ottaen on energian ja valkuaisen runsas saanti kohottanut valkuaisen osuutta maidossa muihin pitoisuuksiin nähden.

Ohran ja typettömien uuteaineiden osuuden kasvaminen ruokinnassa on kohottanut maidon valkuais- ja rasvapitoisuutta sekä maidon ja sen eri aineosien määriä niin sisäruokinta- kuin laidunkaudella. Syödyn rehun keskimääräisen kuitupitoisuuden sekä vastaavasti kuitupitoisten rehujen, säilörehun ja heinän, lisääntyminen ruokinnassa on vaikuttanut päinvastoin. Rehuannoksen raakavalkuaissisällön kohoaminen on nostanut maidon valkuaispitoisuutta ja -tuotosta. Ruokinnan vaikutus maitosokeripitoisuuteen on ollut vähäinen.

Energian ja valkuaisen saannin sekä rehuannoksen koostumuksen kokonaisvaihtelu on selittänyt sisäruokinnassa 45.7 % maidon valkuais-, rasva- ja maitosokeripitoisuuksien yhteismuuntelusta, kun maitotuotos ja tuotantovaihe ovat olleet vakioita.

ANNALES AGRICULTURAE FENNIAE, VOL. 15: 196–213 (1976)
Seria ANIMALIA DOMESTICA N. 43 — Sarja KOTIELÄIMET n:o 43

FACTORS AFFECTING THE COMPOSITION OF MILK

II. EFFECT OF STAGE AND NUMBER OF LACTATION AND SOME OTHER EXTERNAL FACTORS, AND SIGNIFICANCE OF CHOICE OF TEST DAYS IN ESTIMATION OF PROTEIN PRODUCTION ABILITY

ELSI ETTALA

ETTALA, E. 1976. **Factors affecting the composition of milk. II. Effect of stage and number of lactation and some other external factors, and significance of choice of test days in estimation of protein production ability.** Ann. Agric. Fenn. 15: 196–213. (Agric. Res. Centre, Inst. Anim. Husb., SF-01300 Vantaa 30, Finland.)

Nine winter and five summer trials were performed with Ayrshire cows. In the winter trials 135 animals were used and 188 lactations were studied; in the summer series, there were 132 cows and 188 lactations. On average, the cows in the winter trials had calved 3.1 times and those in the summer trials 4.0 times. The time elapsed from calving to the middle of the trials averaged 142 days in the winter trials and 116 in the summer trials. The composition of the milk was analysed at intervals of 10 days on samples representing the two-day production of each cow, and numbering 2606 in the winter trials and 1969 in the summer series.

The lactation stage had a strong effect on the milk yield, and, through it, on the yields of protein, fat and lactose. When the influence of the milk yield was eliminated, the lactation stage had only a slight effect on the yields of the various milk constituents. Percentage lactose was also very closely affected by the milk yield, decreasing in parallel with it. Percentage protein rose as the lactation proceeded and the milk yield decreased, the effect of the two factors being almost equally great. Percentage fat was not as strongly affected as the protein content by the lactation stage and the milk yield. Lactation number had only a small effect on the yields of the milk constituents and the protein content. As the number rose, there was a slight decrease in percentage fat and a clear decrease in percentage lactose, so that the proportion of protein in the milk increased.

When the protein producing ability of the cows was estimated from the records of several test days, the best result was obtained by using the means of determinations made 90, 140 and 220 days after calving. With this combination of test days, it was possible to explain 77.4 % of the variation in the protein percentage, and 62.4 % of the variation in the protein yield in the same year, and the correlations between the different years were also strongest (protein percentage: $r = 0.74^{***}$, protein yield: $r = 0.40^{***}$).

Index words: lactation, milk composition, protein in milk.

INTRODUCTION

The increase in the importance attached to milk protein, and the rise in the cost of animal testing are causing a reassessment of the use of milk analyses in selective cattle breeding. It has been suggested that protein determinations should be included among the criteria used in selection, and that the number of test days should be decreased. The use of protein determinations is supported by evidence that more rapid genetic progress is achieved when selection is based directly on such determinations rather than on various correlations (BERGMANN 1969, JENSEN 1971, MAIJALA 1974, PHILIPSSON 1973, SYRSTAD 1971, VARO 1960). On the other hand, it has been shown that selection made on the milk yield alone promotes the production of protein almost as well as when the choice is based directly on the protein yield itself (MAIJALA and VILVA 1974, ROOS 1971), which suggests that determinations of protein may not be of key importance, after all, in the selection of dairy cows for breeding.

In Finland, milk protein has receatly been adopted as a criterion in estimating the breeding value of bulls. The protein value used for this purpose is the percentage determined on a single test day in the first lactation of each of the cows sired by the bull. This method is fairly reliable if the number of cows is sufficiently large (MAIJALA and VILVA 1974, PHILIPSSON 1973, ROOS 1971, VARO 1964). If the protein content is adopted as the future basis of the price of milk, a network of central laboratories with automatic analysing equipment will have to be created, and this could also be used in estimating the breeding value of cattle. The cost of such investigations would then depend on the number of analyses, rather than on the number of components determined.

This study was undertaken with the present selection of dairy cows in mind. It examines the extent to which the reliability of information on the composition of milk, and especially on milk protein, is affected by certain external factors, such as the stage and number of lactation, and the choice and number of test days.

MATERIAL AND METHODS

The reader is referred to part I of this study the (ETTALA 1976 a: 183—185) for details of arrangement of the trials, trial localities, test animals and their feeding, and methods used to determine the percentages of milk protein, fat and lactose. The present investigation differs from part I in excluding those summer trials for which all three milk constituents were not determined, but all the winter trials are included.

Ayrshire cows were used — 135 in the winter trials (9) and 132 in the summer trials (5). Some of the cows were employed more than once, and the results in both the winter and summer trials are derived from 188 lactations. On average, the animals in the winter trials had calved 3.1 times and those in the summer trials 4.0 (Table 1). The time elapsed from calving to the middle of the trials averaged 142 days in the winter trials and 116 days in the summer trials. Milk analyses were performed at intervals of 10 days on samples representing the two-day production of each cow, and numbering 2606 in the winter trials and 1969 in the summer series (Table 1).

Table 1. Average percentages, percentage ratios and yields of milk constituents at the different trial localities in the winter and summer trials.

Trials	Cows	Calvings	Average days since calving	Samples	Percentages, %								Percentage ratios						Yields, kg or g							
					Protein		Fat		Lactose		P+F+L[1]		Protein/fat		Protein/lactose		Fat/lactose		Milk		Protein		Fat		Lactose	
					$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
WINTER TRIALS																										
Jokioinen	3	81	2.8	132	1432	3.19	0.34	4.13	0.42	4.78	0.38	12.10	0.64	0.78	0.09	0.67	0.09	0.87	0.10	15.1	4.0	476	114	622	171	727	213
Pohjois-Savo	3	27	3.4	158	816	3.54	0.36	4.43	0.48	4.70	0.28	12.67	0.65	0.80	0.09	0.76	0.11	0.95	0.13	14.1	4.2	494	138	618	172	669	221
Häme	3	27	3.7	150	358	3.40	0.36	4.41	0.49	4.64	0.34	12.44	0.86	0.77	0.08	0.74	0.10	0.95	0.11	14.6	4.1	493	141	645	200	683	220
Average	9	135	3.1	142	2606	3.33	0.38	4.37	0.47	4.73	0.30	12.32	0.72	0,78	0.09	0.71	0.11	0.91	0.12	14.7	4.1	484	126	624	175	703	218
SUMMER TRIALS																										
Jokioinen	3	96	4.2	111	1573	3.32	0.32	4.11	0.48	4.57	0.29	12.00	0.63	0.82	0.11	0.73	0.10	0.90	0.12	20.5	5.1	671	143	841	232	942	268
Tikkurila	1	18	2.7	167	234	3.46	0.35	4.56	0.52	4.66	0.27	12.68	0.61	0.77	0.10	0.75	0.10	0.98	0.14	16.0	4.6	546	132	725	210	753	240
Häme	1	18	3.9	99	162	3.24	0.45	4.14	0.53	4.78	0.33	12.16	0.85	0.79	0.11	0.68	0.10	0.87	0.14	19.2	4.1	612	109	788	167	921	209
Average	5	132	4.0	116	1969	3.33	0.34	4.17	0.51	4.59	0.29	12.09	0.69	0.81	0.11	0.75	0.10	0.91	0.13	19.8	5.2	652	153	823	226	918	267

[1]) protein % + fat % + lactose %

RESULTS

Mean values and differences between trials

The average daily milk production per cow was 14.7 kg in the winter trials and 19.8 kg in the summer trials (Table 1). In spite of the difference in production, the average protein content was the same in the two trial series (3.33 %), and the fat content was only slightly lower in the summer trials than in the winter series, 4.59 % *versus* 4.73 %. The ratio of the protein percentage to the fat percentage averaged 0.78 in the winter trials and 0.81 in the summer series.

Phenotypic correlations between the milk and its constituents

The milk yield had a significant negative correlation with the protein and fat percentages (−0.35***, −0.49*** and −0.19***, −0.16***) but a significant positive correlation with the lactose content (0.51***, 0.43***). A stronger correlation existed between the protein and fat percentages in the winter than in the summer trials, 0.45*** *versus* 0.17***. The corresponding correlations between the protein and lactose contents were −0.30*** and −0.23***. The correlation between the contents of fat and lactose was weak. The total percentage of the organic substances studied depended most closely on the fat content and least closely on the lactose percentage (Table 2).

The correlation between the milk yield and the yields of the various constituents were very strong (0.90*** — 0.99***) (Table 2). The protein percentage and the protein yield were positively correlated in the winter trials (0.06*) and negatively correlated in the summer series (−0.14***). The fat percentage and yield were positively correlated in both series (0.20*** and 0.27***), as were also the lactose percentage and yield (0.64*** and 0.59***). The total content of the or-

Table 2. Phenotypic correlations between the milk and its constituents.

Trials and variables	Protein %	Fat %	Lactose %	P+F+L %	P %/F %	P %/L %	F %/L %	Protein kg	Fat kg	Lactose kg
WINTER TRIALS										
Milk, kg	−0.35	−0.19	+0.51	−0.10	−0.17	−0.50	−0.41	+0.90	+0.92	+0.99
Protein %		+0.45	−0.30	+0.70	+0.56	+0.91	+0.51	+0.06	−0.18	−0.37
Fat %			−0.10	+0.85	−0.48	+0.39	+0.88	−0.01	+0.20	−0.19
Lactose %				+0.19	−0.20	−0.67	−0.56	+0.43	+0.47	+0.64
Protein+fat+lactose %					−0.10	+0.46	+0.61	+0.20	+0.22	−0.06
Protein %/fat %						+0.52	−0.30	+0.07	−0.36	−0.19
Protein %/lactose %							+0.65	−0.15	−0.35	−0.57
Fat %/lactose %								−0.22	−0.07	−0.46
Protein yield, kg									+0.90	+0.88
Fat yield, kg										+0.91
SUMMER TRIALS										
Milk, kg	−0.49	−0.16	+0.43	−0.18	−0.22	−0.59	−0.34	+0.92	+0.90	+0.98
Protein %		+0.17	−0.23	+0.52	+0.56	+0.88	+0.26	−0.14	−0.42	−0.48
Fat %			+0.01	+0.83	−0.70	+0.13	+0.88	−0.12	+0.27	−0.14
Lactose %				+0.31	−0.18	−0.67	−0.46	+0.40	+0.42	−0.59
Protein+fat+lactose %					−0.32	+0.25	+0.59	+0.01	+0.17	−0.09
Protein %/fat %						+0.51	−0.53	+0.00	−0.52	−0.24
Protein %/lactose %							+0.43	−0.31	−0.53	−0.66
Fat %/lactose %								−0.30	+0.04	−0.39
Protein yield, kg									+0.03	+0.90
Fat yield, kg										+0.89

$P < 0.05$, $r > 0.05$; $P < 0.01$, $r > 0.06$, $P < 0.001$, $r > 0.08$

ganic substances was positively correlated with the fat and protein yields, but negatively correlated with the lactose yield.

Effect of external factors on the composition of the milk

Least-squares analysis of variance (HARVEY 1966) was used to elucidate the effect on the milk composition exerted by each individual factor independently of the others. The linear regression variables were the milk yield and the stage and number of lactation, and the factors were the trial localities and years. The years were omitted from the analyses of the summer trials, since they had no effect.

The results show that the variables examined had a very significant effect ($P < 0.001$) on most of the values describing the composition of the milk in both the winter and summer trials (Table 3). The percentage of the variation explained was greatest for the yields of the various constituents (R^2 82 – 89 %), because the fluctuation in the milk yield explained ca. 50 % of their variation. As regards the composition of the milk, the percentages of the variation explained were greatest for the lactose content (52 % and 35.5 %), and smallest for the fat content (16.2 % and 9.7 %). In the case of the protein content, the variables explained 34.4 % of the variation in the winter trials and 32.8 % in the summer series. The contributions of the independent variables differed somewhat between the summer and the winter trials.

Milk yield and stage of lactation

The results shown in Table 3 indicate that the lactation stage and the milk yield each had its own separate effect on the composition of the milk, i.e. the level of the milk yield at the same stage of lactation had a

Table 3. Effect of various factors on milk composition and yield, results of least-squares analysis of variance.

Trials and variables	Regression variables									Factors				Total
	Milk yield			Lactation stage			Lactation number			Trial localities		Trial years		
	b	R^2 %	F	b	R^2 %	F	b	R^2 %	F	R^2 %	F	R^2 %	F	R^2 %
WINTER TRIALS														
Protein %	−0.0167	1.7	***	+0.0015	2.9	***	+0.018	0.8	***	10.8	***	4.0	***	34.4
Fat %	−0.0069	0.2	*	+0.0013	1.3	***	−0.023	0.9	***	8.1	***	2.5	***	16.2
Lactose %	+0.0259	6.9	***	−0.0011	2.8	***	−0.057	13.0	***	0.6	***	4.9	***	52.0
P + F + L %	+0.0023	0.0		+0.0016	0.9	***	−0.062	2.6	***	11.5	***	3.0	***	19.1
Protein %/fat %	−0.0027	0.8	***	+0.0001	0.3	**	+0.008	2.8	***	1.2	***	0.5	**	8.5
Protein %/lactose %	−0.0079	5.2	***	+0.0005	3.7	***	+0.013	5.6	***	7.3	***	4.2	***	51.2
Fat %/lactose %	−0.0067	2.8	***	+0.0005	3.0	***	+0.007	1.0	***	6.8	***	4.5	***	33.9
Protein yield, kg	+0.0299	51.3	***	+0.0002	0.4	***	+0.001	0.03	*	2.4	***	1.2	***	86.1
Fat yield, kg	+0.0411	49.8	***	+0.0001	0.1	***	−0.004	0.1	***	1.2	***	0.4	***	86.3
Lactose yield, kg	+0.0509	49.4	***	−0.0002	0.1	***	−0.007	0.4	***	0.01	**	0.2	***	98.1
Milk yield, kg				−0.0463	43.4	***	+0.307	2.0	***	0.0		0.6	***	45.7
SUMMER TRIALS														
Protein %	−0.0176	4.2	***	+0.0024	7.6	***	−0.009	0.3	**	0.8	***		[1]	32.8
Fat %	−0.0118	0.8	***	−0.0008	0.4	**	−0.022	0.7	***	5.5	***			9.7
Lactose %	+0.0257	12.4	***	−0.0008	1.0	***	−0.036	5.8	***	7.7	***			35.5
P + F + L %	−0.0037	0.0		+0.0009	0.2	*	−0.067	3.5	***	5.0	***			14.9
Protein %/fat %	−0.0024	0.7	***	+0.0007	5.2	***	+0.002	0.1		6.2	***			15.2
Protein %/lactose %	−0.0082	10.9	***	+0.0007	6.6	***	+0.004	0.5	***	4.1	***			46.1
Fat %/lactose %	−0.0078	5.9	***	−0.0000	0.0		−0.002	0.1		2.4	***			14.2
Protein yield, kg	+0.0296	58.5	***	+0.0004	1.2	***	−0.002	0.04	*	0.3	***			86.5
Fat yield, kg	+0.0388	46.0	***	−0.0003	0.2	***	−0.004	0.1	***	0.8	***			82.0
Lactose yield, kg	+0.0508	56.6	***	−0.0001	0.03	***	−0.007	0.3	***	0.3	***			97.0
Milk yield, kg				−0.0624	32.8	***	+0.264	1.0	***	1.0	***			42.2

[1]) The effect of the trial year was not significant in the summer trials and it has been omitted from the analysis.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

significant effect on many of the milk properties. The percentage of protein rose and that of lactose fell, both as lactation proceeded and as the milk yield decreased. Each of the factors had less effect on the percentage of fat than on that of protein; the stage of lactation even had opposite effects on the fat percentage in the summer and winter trials. The separate effects of the two factors on the total content of organic substances were only slight. The yields of the different constituents were very strongly affected by the milk yield, but only slightly affected by the lactation stage.

The effect of the time elapsed since calving decreased linearly in the least-squares analysis. The square of the time also had a statistically significant effect on many of the

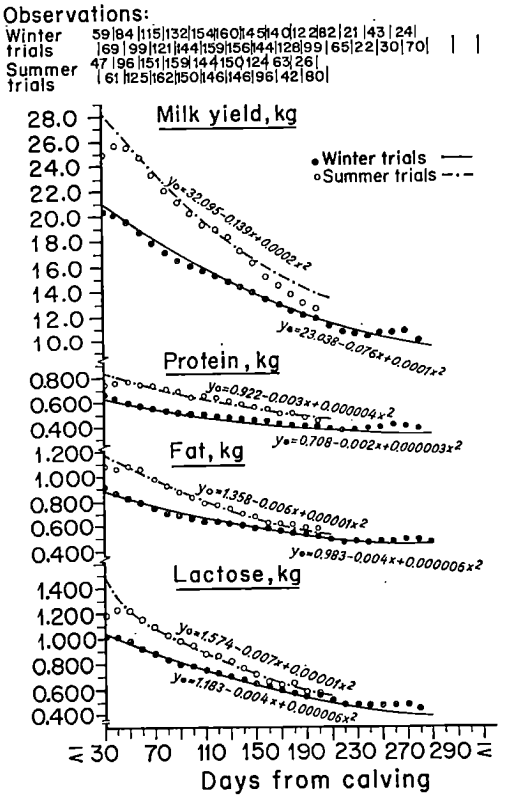

Fig. 1. Yields of milk and its constituents at different stages of lactation (x = time elapsed since calving in days; x^2 = square of time).

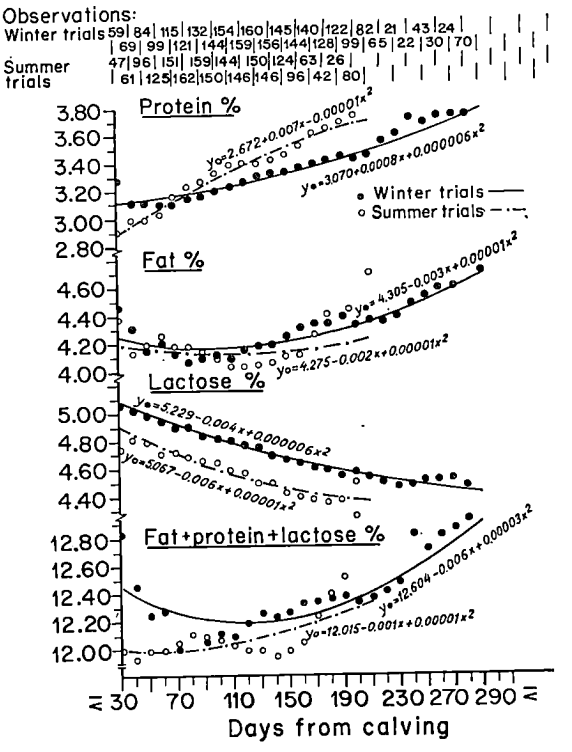

Fig. 2. Composition of milk at different stages of lactation (x = time elapsed since calving in days; x^2 = square of time).

properties. The regression equations and the curves are presented in Figs. 1—3. The effect of the milk yield has not been eliminated, so that the results are influenced by both the decrease in production and the progress of lactation.

Fig. 1 shows that in the winter trials the milk yield decreased evenly to ca. 10 kg a day, or to the 240th day of lactation, and then remained almost constant. In the summer trials, production was high at the beginning and decreased sharply throughout the trial period.

In the winter trials, the protein percentage rose fairly evenly, following the course of the regression curve, except in the initial and final stages of lactation (Fig. 2). In these trials, the percentage was lowest between the 50th and 70th days of lactation. In the summer trials, the protein content rose

rather sharply throughout the trial period, increasing in proportion to the rapid decrease in the milk yield. The percentage of milk fat did not follow the regression curve of the second degree equation as closely as the percentage of protein; it fluctuated irregularly, especially in the initial stage of lactation. It was lowest between the 80th and 110th days of lactation in the winter trials and between the 110th and 130th days in the summer series.

The lactose content fell as the lactation progressed, following the regression curve very closely (Fig. 2). The combined content of the organic substances varied in the same way as the percentages of fat and protein, its course being rather irregular, like that of the fat percentage. Its lowest values were recorded between the 70th and 90th day of lactation in the winter trials and between the 40th and 140th days in the summer series. The changes in the ratios of the milk constituents were chiefly linear (Fig. 3). As the lactation progressed, the protein content increased in relation to the other percentages, and the fat content rose in relation to the percentage of lactose. The relative increase in the protein content was particularly rapid in the summer trials.

The yields of the milk constituents fell, closely following the decrease in the milk yield and the regression curve (Fig. 1). The lactose yield dropped more rapidly than the others, since the lactose percentage decreased as well as the milk yield.

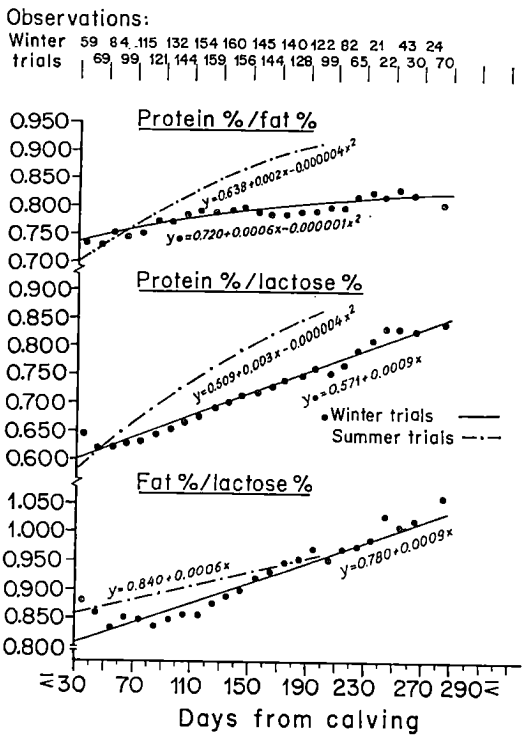

Fig. 3. Ratios between percentages of milk constituents at different stages of lactation (x = time elapsed since calving in days; x^2 = square of time).

Number of lactation

Table 3 shows the separate influence of the number of the lactation on the milk yield and composition, and Fig. 4 shows the effect it exerted together with the other variables. In each case the cows are divided according to whether they have calved 1, 2, 3, 4, 5—6, or 7 or more times. The number of observations in each group is shown in Fig. 4.

On its own, the lactation number had little influence on the percentage of milk protein, and even exerted opposite effects in the winter and summer trials (Table 3), the contradiction being evident both before (Fig. 4) and after (Table 3) the effect of the other variables has been eliminated. The milk of the cows in their first lactation had a lower protein content than that of the older cows in the winter trials but a higher content in the summer series. In the winter trials, the protein content of the first-lactation milk fell from about the 155th day to the end of lactation (Fig. 5). In the summer trials, this stage of lactation could not be examined, since the trials were shorter.

The percentage of milk fat fell as the number of the lactation rose (Table 3). When the effect of the other variables was

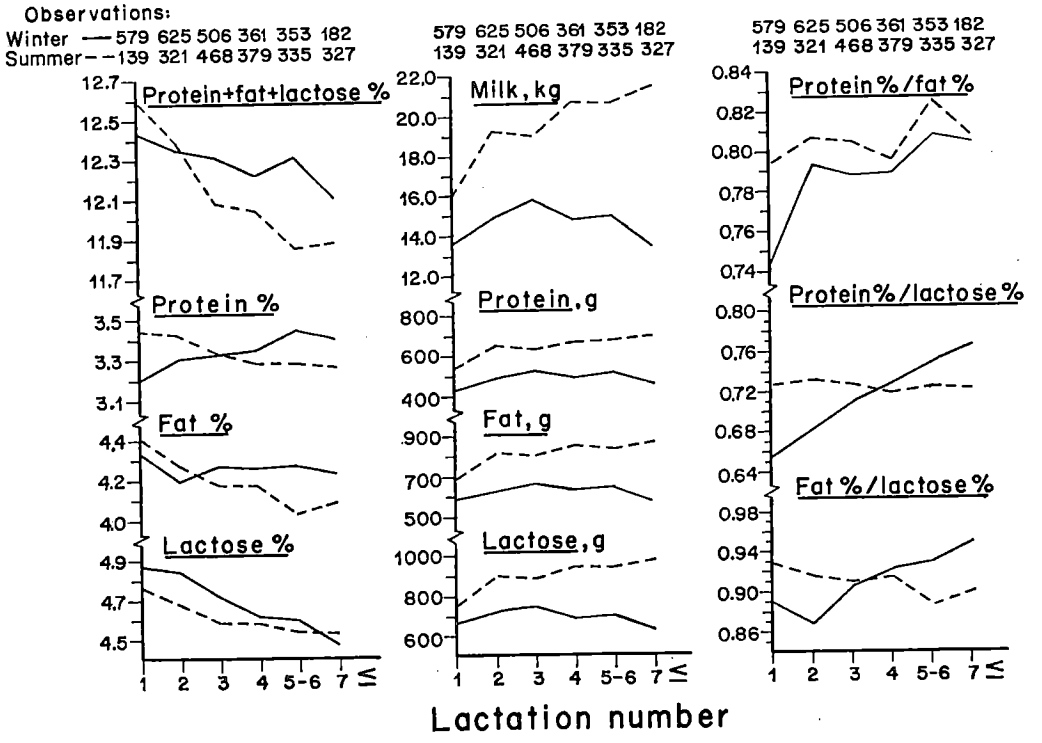

Fig. 4. Yields of milk and its constituents, percentages of constituents and ratios between the percentages plotted against lactation number.

eliminated, the negative correlation was of almost the same size in the winter and summer trials, but when no elimination was performed, the decrease in the fat content was smaller in the winter than in the summer trials, the lowest fat content in the winter series being recorded for the cows which had calved twice (Figs. 4 and 5).

The number of the lactation had the clearest influence on the lactose content (R^2 = 13.0 % in winter trials; 5.8 % in summer trials). The lactose content decreased as the number rose (Table 3, Figs. 4 and 5), as did also the combined content of organic substances (Table 3, Fig. 4). An increase in the number of preceding lactations generally raised the proportion of protein in relation to the other milk constituents.

In the summer trials, the milk yield rose fairly evenly with the lactation number, but in the winter trials the yield was highest in the third lactation (Fig. 4). On its own, the lactation number had little influence on the protein yield, even affecting it in opposite ways in the winter and summer trials (Table 3). The fat and lactose yields fell to some extent as the lactation number rose. When the influence of the other variables had not been eliminated, the yields of the different constituents followed the course of the milk yield fairly closely (Fig. 4).

Trial localities and trial years

The composition of the milk varied significantly with the locality (Tables 1 and 3). In the winter trials, the greatest differences occurred in the protein and fat contents and the combined percentage of organic substances (Table 3). The ratios between the percentages also differed notably. In contrast,

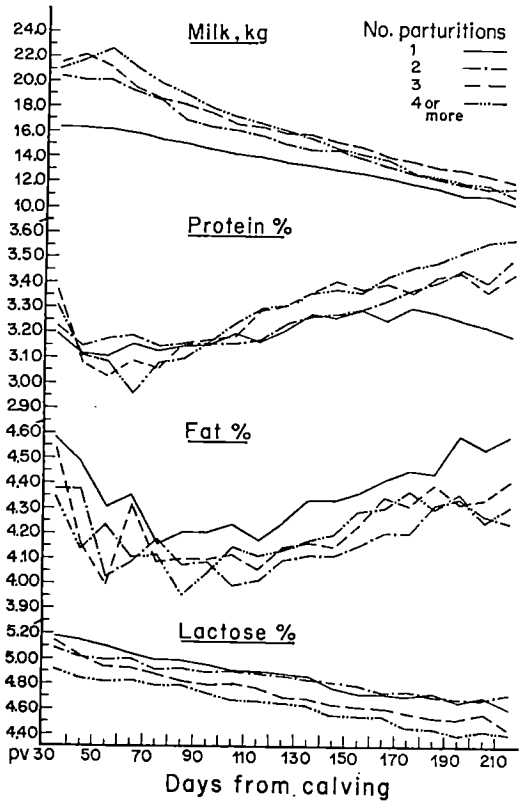

Fig. 5. Courses of milk yield and percentages of constituents during lactation in different calving groups (winter trials).

the milk yields differed very little between the trial localities (Tables 1 and 3), and in consequence there was also little difference in the yields of the constituents. The most pronounced difference was found in the protein yield in the winter trials (R^2 2.4 %). Differences between the trial years were also evident in the winter trials (Table 3), and here, too, the percentages of the constituents and the ratios between them differed more than their yields. The effect of the feed is treated in detail in the first part of this study (ETTALA 1976 a).

Weather in the grazing period

The average temperatures of the summer trials varied from 13.8 to 17.5° C at the

Table 4. Partial correlations between mean temperatures and precipitation in trials (5-day periods) and the values describing the yield and composition of the milk.

Variables	Temperature, C°	Precipitation, mm
Milk yield, kg	+0.07	+0.02
Protein %	−0.21*	−0.04
Fat %	−0.11	−0.15
Lactose %	+0.07	−0.17
P + F + L %	−0.13	−0.19
Protein %/fat %	−0.03	+0.12
Protein %/lactose % .	−0.21*	+0.07
Fat %/lactose %	−0.14	−0.06
Protein yield, kg	+0.01	+0.01
Fat yield, kg	+0.01	−0.05
Lactose yield, kg	+0.07	−0.03

The effect of the lactation stage was eliminated in the calculations
* $P < 0.05$ (d.f. 88)

different trial localities and in the different years. The temperatures also fluctuated considerably during the same summer (ANON. 1971—1973). When the influence of the lactation stage was eliminated, the temperature showed a significant negative correlation with the percentage of milk protein, and with the ratio of protein to lactose (Table 4). The temperature did not influence the yields of the milk and its various constituents. Precipitation did not show any significant correlation with the values describing the yield and composition of the milk; there was some evidence that rain has a decreasing influence on the contents of milk fat and lactose, and the combined content of organic substances, but no trends were apparent in respect of the yields of the milk and its constituents.

Most reliable test days

Phenotypic correlations between part and mean yields. The winter trials comprised a sufficiently large part of the lactation (30—290 days, Fig. 1) for the individual levels of production and the shape of the production curve to become apparent. The test days

Table 5. Phenotypic correlations between part records from different lactation stages and the means for the same cows (winter trials).

Days from calving	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230
No. of observations	84	99	115	121	132	144	154	159	160	156	145	144	140	128	122	99	82	65	53
Milk, kg	**0.88**	0.81	0.80	0.82	0.82	0.83	**0.87**	**0.88**	**0.85**	0.80	0.77	0.70	0.72	0.72	0.71	0.71	0.69	**0.79**	0.76
Protein, kg	**0.83**	0.75	0.76	0.79	0.79	0.80	**0.84**	**0.87**	**0.85**	0.81	0.72	0.69	0.64	0.68	0.70	0.72	0.74	**0.78**	0.77
Fat, kg	**0.84**	0.82	0.79	0.83	0.83	0.83	**0.85**	**0.88**	**0.87**	0.83	0.79	0.73	0.71	0.74	0.77	0.77	0.79	**0.83**	0.84
Lactose, kg	**0.82**	0.75	0.75	0.78	0.78	0.82	**0.86**	**0.87**	**0.83**	0.78	0.74	0.68	0.71	0.71	0.69	0.69	0.70	**0.79**	0.77
Protein %	0.74	0.85	0.86	0.86	**0.90**	**0.90**	0.87	0.85	0.83	**0.87**	**0.88**	0.83	0.84	0.87	**0.90**	**0.88**	0.85	**0.88**	0.85
Fat %	0.72	0.54	0.65	0.69	0.77	0.79	0.80	0.83	0.79	**0.88**	0.82	0.81	0.84	0.80	0.77	0.74	0.79	0.78	0.79
Lactose %	0.66	0.74	0.68	0.78	0.82	0.84	**0.89**	0.86	0.84	0.84	0.80	0.76	0.78	0.81	0.78	0.81	0.81	0.85	0.84
P + F + L % .	0.58	0.51	0.58	0.57	0.61	0.62	0.62	0.67	0.68	**0.73**	0.66	0.65	0.62	0.63	0.64	0.63	0.62	0.64	0.59

All the correlations are statistically significant $P < 0.001$.

best representing the mean production ability were sought by calculating simple phenotypic correlations between the results obtained in different stages of the same lactation and the same cows (Table 5). The correlations were calculated for the test days (10-day intervals; 50—230 days) for which the number of observations was at least 50.

The correlations of the yields of protein, and also those of milk, fat and lactose, with the mean values were greatest when 120 days had elapsed since calving ($r = 0.87^{***} - 0.88^{***}$) (Table 5). The yields were than at the same level as the average values. The correlations of the adjacent test days (110 and 130 days) were also very high. High correlations were also obtained at the beginning of lactation, 50 days from calving. In the latter part of the lactation the highest correlations were obtained on the 220th day.

Percentage protein was strongly correlated with the mean ($r = 0.88^{***} - 0.90^{***}$) at three different stages of lactation (90—100 days, 150 days and 190—220 days after calving) (Table 5). Percentage fat and the combined content of organic substances were most highly correlated with the mean values 140 days after calving ($r = 0.88^{***}$, 0.74^{***}), and the lactose content was most highly correlated on the 110th day ($r = 0.89^{***}$).

The results of these days were combined in different ways, according to the highest correlations of the protein yield, on the one hand, and those of the protein percentage, on the other (Table 6 a). A basis for comparison was obtained by taking combination A, with results from one test day in each of the middle months of the lactation (2—7), and, at the other extreme, combination H, comprising only the values from the two test days which were found to be best for the protein yield, the 50th and 120th day after calving. Most of the combinations contained the results from three test days, selected so as to reveal the shape of the lactation curve. The values of slightly different dates were also examined to discover the limits

Table 6. Correlations of combined results from different lactation stages with a) the means of the same lactation and b) the combined results of different years (winter trials).

a) Correlations between combined results and means

Combinations	A	B	C	D	E	F	G	H
Observations	745[2])	558[2])	388[2])	353[2])	308[2])	325[2])	375[2])	243[2])
Milk, kg	0.77[a]	0.78[a]	0.77[a]	0.80[ab]	0.85[c]	0.83[bc]	0.83[bc]	0.84[bc]
Protein, kg	0.75[a]	0.76[ab]	0.75[a]	0.79[abc]	0.83[c]	0.82[c]	0.81[bc]	0.82[c]
Fat, kg	0.79[a]	0.81[ab]	0.80[ab]	0.83[abc]	0.85[c]	0.84[bc]	0.83[abc]	0.83[abc]
Lactose, kg	0.75[a]	0.75[a]	0.76[a]	0.78[ab]	0.84[c]	0.82[bc]	0.80[abc]	0.81[abc]
Protein %	0.87[bcd]	0.88[cd]	0.89[d]	0.88[cd]	0.85[abc]	0.83[ab]	0.83[ab]	0.81[a]
Fat %	0.75[a]	0.79[ab]	0.79[ab]	0.82[b]	0.78[ab]	0.78[ab]	0.78[ab]	0.79[ab]
Lactose %	0.80[a]	0.80[a]	0.81[a]	0.84[a]	0.83[a]	0.81[a]	0.81[a]	0.80[a]
P + F + L %	0.62[a]	0.65[a]	0.64[a]	0.67[a]	0.64[a]	0.63[a]	0.63[a]	0.64[a]

b) Correlations between lactations

Observations	168[2])	133[2])	86[2])	84[2])	70[3])	70[3])	86[4])	55[2])
Milk, kg	0.53[b]	0.49[ab]	0.52[b]	0.54[b]	0.56[b]	0.51[ab]	0.26[a]	0.35[ab]
Protein, kg	0.39[a]	0.37[a]	0.35[a]	0.40[a]	0.36[a]	0.33[a]	0.31[a]	0.26[a]
Fat, kg	0.36[ab]	0.36[ab]	0.43[b]	0.40[b]	0.33[ab]	0.30[ab]	0.12[a]	0.17[ab]
Lactose, kg	0.56[b]	0.51[ab]	0.52[ab]	0.55[ab]	0.60[b]	0.56[ab]	0.31[a]	0.42[ab]
Protein %	0.68[ab]	0.69[ab]	0.73[b]	0.74[b]	0.62[ab]	0.61[ab]	0.59[ab]	0.49[a]
Fat %	0.38[a]	0.52[a]	0.53[a]	0.53[a]	0.34[a]	0.32[a]	0.30[a]	0.28[a]
Lactose %	0.48[a]	0.46[a]	0.39[a]	0.43[a]	0.58[a]	0.58[a]	0.54[a]	0.57[a]
P + F + L %	0.45[ab]	0.54[ab]	0.61[b]	0.55[ab]	0.35[a]	0.34[a]	0.42[ab]	0.34[a]
Protein %/fat %	0.37[a]	0.40[a]	0.36[a]	0.44[a]	0.41[a]	0.40[a]	0.34[a]	0.38[a]
Protein %/lactose %	0.74[abc]	0.76[bc]	0.78[c]	0.79[c]	0.72[abc]	0.73[abc]	0.63[ab]	0.59[a]
Fat %/lactose %	0.47[ab]	0.56[ab]	0.54[ab]	0.61[b]	0.47[ab]	0.45[ab]	0.34[a]	0.31[a]

The homogeneity between the correlations was tested by the χ^2-test. Values with different index letters in the same horizontal row differ significantly, a—d: $P < 0.05$.

[1]) Combinations
- A: 60 + 90 + 120 + 150 + 180 + 210 days from calving
- B: 90 + 120 + 150 + 190 »
- C: 100 + 150 + 200 »
- D: 90 + 140 + 220 »
- E: 50 + 120 + 220 »
- F: 50 + 120 + 210 »
- G: 50 + 90 + 120 »
- H: 50 + 120 »

[2]) All the correlations are statistically $P < 0.001$.
[3]) $P < 0.05$, $r > 0.22$; $P < 0.01$, $r > 0.29$; $P < 0.001$, $r > 0.38$
[4]) $P < 0.05$, $r > 0.21$; $P < 0.01$, $r > 0.28$; $P < 0.001$, $r > 0.35$
[5]) $P < 0.05$, $r > 0.26$; $P < 0.01$, $r > 0.33$; $P < 0.001$, $r > 0.42$

of the period within which the most favourable results are obtained.

The best estimate of the yield of protein and also of the other yields was given by combination E (50 + 120 + 220 days after calving) (Table 6 a). With it, it was possible to explain 68.9 % of the variation in the protein yield, 72.3 % of the variation in the milk and fat yields, and 70.6 % of the variation in the lactose yield. Other combinations which were equally valuable for estimating the yields were all those which included the test day at the peak of the initial period, the 50th day after calving (F —H). Combination A and combinations B — C, which were based on the highest correlations for

the protein content, were significantly weaker. Combination D, also belonging to the group based on the protein content, (90 + 140 + 220 days after calving), was comparable in value to combination E for estimating the protein and fat yields, explaining 62.4 % of the variation in the yield of protein.

The most reliable estimate of the average protein percentage was obtained from combination C (100 + 150 + 200 days) (Table 6 a). With it, it was possible to explain 79.2 % of the variation in the protein content. Combinations of comparable value were D (R^2 = 77.4 %), B, comprising four test days, and the comparative combination A. Another combination in the same class was E, which gave the best estimates of the yields. The combinations were all almost equally valuable for estimating the percentages of fat and lactose and the combined contents of the three organic substances, though D was slightly better than the others, explaining 67.2, 70.6 and 44.9 % of the variation in, respectively, the percentages of fat and lactose, and the combined percentage of the three constituents.

Correlations between lactations

The highest correlations between the results for protein in two production years were obtained with combination D (r = 0.40*** for protein yield, r = 0.74*** for protein percentage) (Table 6 b). However, the correlations between the values for the protein yield did not vary significantly from one combination to the other. The correlations between the lactations were much smaller for the protein and fat yields than for the corresponding percentages. The correlations for the ratios between the percentages of protein and fat were smaller than for the other ratios. The correlations between the milk yields in the different lactations were equally significant statistically in all the combinations (r = 0.35*** — 0.56***), except G.

Table 7. Correlations between results of two different years in initial, middle and final stages of lactation.

	Winter trials				Summer trials			Winter + summer trials	
Days from calving	50—100[2])	110—160[3])	170—230[2])	50—230[5])	50—100[3])	110—160[3])	40—180[5])	50—100[4])	110—160[5])
No. of observations	145	232	150	527	210	206	430	355	438
Milk, kg	0.16^{ac}	0.41^{b}	0.46^{bd}	0.58	0.46^{a}	0.63^{b}	0.69	0.37^{c}	0.54^{d}
Protein, kg	0.29^{a}	0.25^{a}	0.24^{a}	0.42	0.31^{e}	0.60^{f}	0.57	0.31^{c}	0.47^{d}
Fat, kg	0.02^{ce}	0.31^{d}	0.46^{df}	0.44	0.40^{c}	0.61^{d}	0.67	0.28^{c}	0.47^{d}
Lactose, kg	0.16^{c}	0.42^{d}	0.44^{d}	0.60	0.43^{a}	0.64^{b}	0.71	0.40^{c}	0.55^{d}
Protein %	0.61^{a}	0.71^{a}	0.68^{a}	0.73	0.19^{a}	0.37^{a}	0.35	0.40^{e}	0.62^{f}
Fat %	0.21^{e}	0.53^{f}	0.59^{f}	0.49	0.11^{c}	0.40^{d}	0.24	0.13^{e}	0.48^{f}
Lactose %	0.42^{a}	0.38^{ab}	0.19^{b}	0.45	0.55^{a}	0.59^{a}	0.63	0.50^{a}	0.49^{a}
P + F + L %	0.50^{a}	0.54^{a}	0.48^{a}	0.54	0.29^{a}	0.47^{b}	0.39	0.38^{a}	0.51^{b}
Protein %/fat %	0.19^{ce}	0.53^{df}	0.48^{d}	0.41	0.09^{a}	0.26^{a}	0.27	0.12^{e}	0.41^{f}
Protein %/ lactose %	0.56^{c}	0.74^{d}	0.74^{d}	0.78	0.32^{a}	0.46^{a}	0.52	0.43^{c}	0.65^{f}
Fat %/lactose %	0.18^{e}	0.52^{f}	0.59^{f}	0.55	0.14^{e}	0.47^{f}	0.25	0.15^{e}	0.50^{f}

[1]) The results of the winter and summer trials were pooled.
The homogeneity between the correlations was tested with the χ^2-test. Values with different index letters in the same horizontal row differ significantly. a—b: $P < 0.05$, c—d: $P < 0.01$, e—f: $P < 0.001$
[2]) $P < 0.05$, $r > 0.16$; $P < 0.01$, $r > 0.21$; $P < 0.001$, $r > 0.26$
[3]) $P < 0.05$, $r > 0.13$; $P < 0.01$, $r > 0.18$; $P < 0.001$, $r > 0.22$
[4]) $P < 0.05$, $r > 0.10$; $P < 0.01$, $r > 0.14$; $P < 0.001$, $r > 0.17$
[5]) All the correlations are statistically significant.

The correlations between the lactations were significantly lower in the initial stage (50—100 days after calving) than in the middle stage (110—160 days) (winter + summer trials) and in the final stage (170—230 days) (winter trials) (Table 7). The lactose content formed an exception. There was some difference between the results for the winter and summer trials; the correlations between the protein contents in the different lactations were notably higher in the winter than in the summer trials, and the reverse was the case with the protein yields.

DISCUSSION

One of the aims of this study was to discover the extent to which the influence of external factors interferes with selection of cows on protein yield; the other was to find the test days which give the most reliable results when production ability is assessed on a small number of samples. Records were not obtained from the whole lactation, but the winter trials comprised a sufficiently large part (ca. 30—290 days after calving, Fig. 1) to reveal the level of production and the normal variation in milk composition of the test animals. Irregular variation in the milk composition was already evident in the initial and final stages of this trial period (Fig. 2).

The fact that the study did not comprise the entire lactation explains why the average percentages of the milk constituents (protein 3.33 % and fat 4.27 %) were somewhat lower than those recently recorded by MAIJALA and VILVA (1974) for their large field material from the first lactations of cows sired by different bulls (3.53 % and 4.49 %). In their study, the mean time elapsed since calving was 184 days; in this study, it was 142 days. The milk protein percentage recorded here was of the same level as the average value obtained for dairy milk by PELTOLA et al. (1963) towards the beginning of the 1960s (3.34 %). The composition of Finnish dairy milk has been followed since the 1920s, during which time the fat and protein contents have risen continuously, while the lactose content has decreased (HIETARANTA and NIEMELÄ 1954, PELTOLA et al. 1963, VIRTANEN 1930). The fat content has increased more rapidly than the protein content, in accordance with the aim pursued in selective breeding, and the ratio of the protein content to the fat content has accordingly decreased (1922: 0.86, 1960: 0.78) (PELTOLA et al. 1963). In this study, the ratio was 0.78 in the winter trials. Thus, although the number of animals used in these experiments was not high, the composition of their milk seems to be rather representative of the average composition of Finnish dairy milk. However, although the material was sufficiently large for studying the external factors that interfere with selection, it was far too limited for an investigation of genetic differences.

The level of the phenotypic correlations between the milk yields and the contents of protein and fat (Table 2) were the same as in many other studies, according to the survey of, for example ROOS (1971). The correlation between the fat and protein percentages corresponded to that in other studies, too (JÄHNE and SCHWARK 1967, ROOS 1971), as did also the correlations of the fat and protein yields with the milk yield and with the percentages of the same constituents (ROOS 1971). On the other hand, the correlations of the lactose percentage with the contents of the other constituents and with the milk yield differed from those reported by ROBERTSON et al. (1956). HANSON et al. (1950) found the ratio of the lactose content to the protein content differed with the level of lactose.

The stage of lactation had a strong effect on the milk yield and, through

it, on the yields of all the constituents (Fig. 1). However, on its own, it exerted only a slight effect on the yields of the various constituents (Table 3). The protein percentage was affected to much the same extent by the lactation stage and the milk yield. The course of the protein content during the lactation was largely the same as in many other studies (BONNIER et al. 1946, DAVIS et al. 1947, HANSSON et al. 1950, KOSSILA 1968, KROSIGK et al. 1960, LANKAMP 1959. LONKA 1947, ROOS 1971, TREECE et al. 1961, VARO 1970, WAITE et al. 1956).

The fluctuations in the fat content during the lactation were also mainly in accord with the results of other investigations (BONNIER et al. 1946, DAVIS et al. 1947, JOHNSON et al. 1961, KOSSILA 1968, KROSIGK et al. 1960, LONKA 1947, ROOS 1971, WAITE et al. 1956). However, differences attributable to such factors as the breed of cattle are also apparent (DAVIS et al. 1947, ROOS 1971). GEISSLER (1974) reported that the rise in the fat content during lactation depended on the size of the content, low fat percentages rising sharply, while high percentages remained constant. The sudden rise in the fat content around the 150th—160th days of lactation (Table 2) has been attributed by LANKAMP (1959) to an increase in the rate of growth of the offspring.

The decrease in the lactose content during the course of lactation was nearly the same in this investigation as that observed by DAVIS et al. (1947), but larger than those reported by BONNIER et al. (1946) and WAITE et al. (1956). As the lactation progressed, the protein content increased in relation to the other constituents, and the fat content also increased in relation to that of lactose (Fig. 3).

As the number of the lactation rose, the milk yield increased — up to the third lactation in the winter trials and up to the oldest age classes in the summer trials (Fig. 4). The yields of the various milk constituents mainly depended on the milk yield (Fig. 4), and the individual influence of the lactation number was very slight (Table 3).

The variation in the protein content with the lactation number was mainly due to the changes in the milk yield; the influence of the lactation number itself was slight and even had contrary results in the winter and summer trials (Table 3). An exception was formed by the protein content in the first lactation; in spite of the smaller milk yield, it was lower than in the other age classes in the winter trials (Figs. 4 and 5). The difference was evident in the final stage of lactation and thus could not be examined in the summer trials.

KOSSILA (1968) also reported that cows in their first lactation had a lower percentage of milk protein in the middle and final stages than older animals. This was observed in a herd of Ayrshires over a period of 10 years. The fall in the protein percentage may have been due to a particularly high protein requirement in the growing animals. In the present trials the grain supplement given at that stage of lactation was very small, and it is also possible that the decrease in the protein content in the first lactation was due to the low intake of energy (cf. ETTALA 1976 a). In other investigations the protein content has generally been high in the first years of production and has then begun to fall gradually, though in some studies the decrease did not begin until the seventh to eighth year of production (GACULA et al. 1968, LANKAMP 1959, SARGENT et al. 1967, TREECE et al. 1961, WAITE et al. 1956). In some studies the percentage of milk protein was found to remain almost constant throughout the life of the cow (GACULA et al. 1965, VARO 1960). WAITE et al. (1956) observed a slight fall in casein nitrogen, accompanied by a slight rise in non-casein nitrogen.

The percentage of milk fat generally falls as the age of the cow increases (BAILEY 1953, GACULA et al. 1968, JOHNSON et al. 1961, LANKAMP 1959, SARGENT et al. 1967, SPIKE

and FREEMAN 1967, WAITE et al. 1956, VARO 1960), but deviations from the general pattern have occurred in the first years of production. In some studies the fat content has risen above the level of the first year (GACULA et al. 1968, JOHNSON et al. 1961, KOSSILA 1968, SPIKE and FREEMAN 1967, VARO 1960), in others it has decreased (JOHNSON et al. 1961, SPIKE and FREEMAN 1967, WAITE et al. 1956) or, as in the winter trials in this study, it has been exceptionally low in the second year of production (GACULA et al. 1968, LANKAMP 1959, SCHWARK and JÄHNE 1967). In this study the lactose content showed a very clear decrease as the lactation number rose (Table 3, Figs. 4 and 5). The same result was obtained by WAITE et al. (1956).

In this study it was not possible to examine the influence of the month of calving, because the trials were made only with animals that had calved in autumn (winter trials) or in early spring (summer trials). The examination of the influence of the season had to be limited to an investigation of the differences between the winter and the summer. The milk was relatively richer in protein in the summer than in the winter, since, although the average milk yield was ca. 5 kg higher in the summer, the protein percentage was as high as in the winter. In other studies also, the milk obtained from animals on pasture was richer in protein than that from animals on winter feeding, (LANKAMP 1959, MARCKMANN and WITT 1956 ROOK et al. 1960). ROOK et al. (1960) attributed this to the high energy value and protein content of the pasture.

The weather prevailing during the summer did not affect the yields of the milk and its constituents (Table 4), but high temperature was negatively correlated with the percentage of milk protein. The same observation was made by HIETARANTA and HOLOPAINEN (1960), when they compared the protein content of milk samples from a warm dry summer with those from a cool wet one. VOIGTLÄNDER et al. (1973) also found that high temperatures had a decreasing influence on the content of milk protein. The effect of temperature on the fat content was not equally clear (Table 4, HIETARANTA and HOLOPAINEN 1962, VOIGTLÄNDER et al. 1973).

It is evident from the above that, of all the external factors considered, the stage of lactation has the strongest effect on the protein yield. It should therefore be taken into account in estimating production ability, and the test days should be chosen in different stages of the lactation.

When only one test day is used, as when the breeding value of bulls is estimated from the first lactation of their daughters, the most reliable information on protein producing ability is obtained about 120 days after calving (110—130 days) (Table 5). This point in the lactation also provided the most reliable information on the yields of milk, fat and lactose. In many investigations, the months in the middle of the lactation have been found to be the most reliable for determinations of the milk and fat yields (4th—7th months) (KEOWN and VAN VLECK 1971, LAMB and MCGILLIARD 1967, MADDEN et al. 1959, SEARLE 1961, VAN VLECK and HENDERSON 1961).

If the protein yields is taken as a criterion in estimating the breeding value of dairy cows, it is advisable to use several test days, so as to reveal the shape of the milking curve. In one of the years in the present study, the best estimate of protein production ability was obtained with a combination of test days that included a day at the stage of peak production, 50 days after calving (combination E, 50 + 120 + 220 days after calving) (Table 6 a). However, external factors apparently affected the yields of the initial part of lactation more strongly than those of the middle and final stages, since the correlations between the different lactations were smaller in the earlier part (Table 7). For this reason, it is probably advisable to use later test days, which have also been

found to give a more reliable prediction of percentage protein. From the point of view of the whole material, the best result was obtained with combination D (90 + 140 + 220 days after calving). For practical purposes, these days are the same as those which have just been tentatively adopted in Finland for first-lactation estimates of protein production ability (90 + 150 + 210 days) (ANON. 1974). It is evidently unnecessary to determine the protein percentage in several different years, since the effect on the percentage of the lactation number is small (Table 3, Fig. 4), and the correlations between the protein percentages of different lactations are fairly high (Tables 6 and 7). However, this applies only if the feeding is well balanced and satisfies requirement (cf. part I, ETTALA 1976 a). It should also be noted that in this study the effect of irregular variations in the milk composition was decreased by using the combined samples of two days' milk production.

The milk yield should be determined in several different years, since it is rather strongly affected by the lactation number and is very sensitive to variations in the feeding (cf. part I, Tables 6 and 7). On the other hand, it would be possible to decrease the number of determinations made within the year; in this study, more accurate estimates were obtained when determinations were made at the three best times in the lactation than when they were made on four or six other test days (Table 6). VARO (1974) found that the mean of three daily yields, measured in the third, fifth and seventh month after calving, was as reliable an index of milk production as the mean of measurements made in six different months. The choice of test days indicated by the results of this study agrees with his.

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SELOSTUS

Maidon koostumukseen vaikuttavista tekijöistä

II. Tuotantovaiheen, poikimakerran ja eräiden muiden ulkoisten tekijöiden vaikutus maidon koostumukseen sekä määritysajankohtien merkitys lehmien valkuaisentuotantokyvyn arvioimisessa

ELSI ETTALA

Maatalouden tutkimuskeskus

Tutkimus sisälsi 9 talvi- ja 5 kesäkoetta. Talvikokeissa oli yhteensä 135 ay-lehmää 188 lypsykaudella, kesäkokeissa 132 ay-lehmää 188 lypsykaudella. Talvikoelehmät olivat poikineet keskimäärin 3.1 ja kesäkoelehmät 4.0 kertaa. Poikimisesta oli talvikokeiden puolivälissä kulunut keskimäärin aikaa 142 pv ja kesäkokeiden 116 pv. Maidon koostumus määritettiin 10 päivän välein kunkin lehmän kahden päivän maitoa edustavasta näytteestä, talvikokeissa 2606 ja kesäkokeissa 1969 kertaa.

Poikimisesta kulunut aika on vaikuttanut voimakkaasti maitotuotokseen ja sitä kautta valkuais-, rasva- ja maitosokerituotoksiin. Tuotantovaiheen itsenäinen, maitotuotoksesta riippumaton vaikutus on eri aineosien tuotoksiin ollut vähäinen. Myös maitosokeripitoisuus on laskenut lähinnä maitomäärän mukaan. Valkuaispitoisuus on kohonnut lähes yhtä paljon lypsykauden etenemisen ja maitotuotoksen alenemisen seurauksena. Kummankin vaikutus on rasvapitoisuuteen ollut pienempi kuin valkuaispitoisuuteen. Poikimakerran vaikutus on maidon aineosien tuotoksiin ja valkuaispitoisuuteen ollut pieni. Poikimakertojen lisääntyminen on jonkin verran laskenut rasvapitoisuutta ja selvästi maitosokeripitoisuutta ja siten kohottanut valkuaisen osuutta maidossa.

Kun lehmien valkuaisentuotantokykyä on arvioitu muutamien määrityskertojen perusteella, on paras tulos saavutettu käyttämällä 90, 140 ja 220 pv poikimisesta saatujen tulosten keskiarvoa. Tällöin on kyetty selittämään saman tuotantovuoden valkuaisprosentin muuntelusta 77.4 %, valkuaistuotoksen muuntelusta 62.4 % sekä saatu kiinteimmät korrelaatiot toiseen tuotantovuoteen (valkuaisprosentit: $r = 0.74^{***}$, valkuaistuotokset: $r = 0.40^{***}$).

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LUETTELO VUONNA 1975 JULKAISTUISTA MAATALOUSALAN TUTKIMUKSISTA JA KOESELOSTUKSISTA

List of agricultural research papers published in 1975

MAATALOUDEN TUTKIMUSKESKUS

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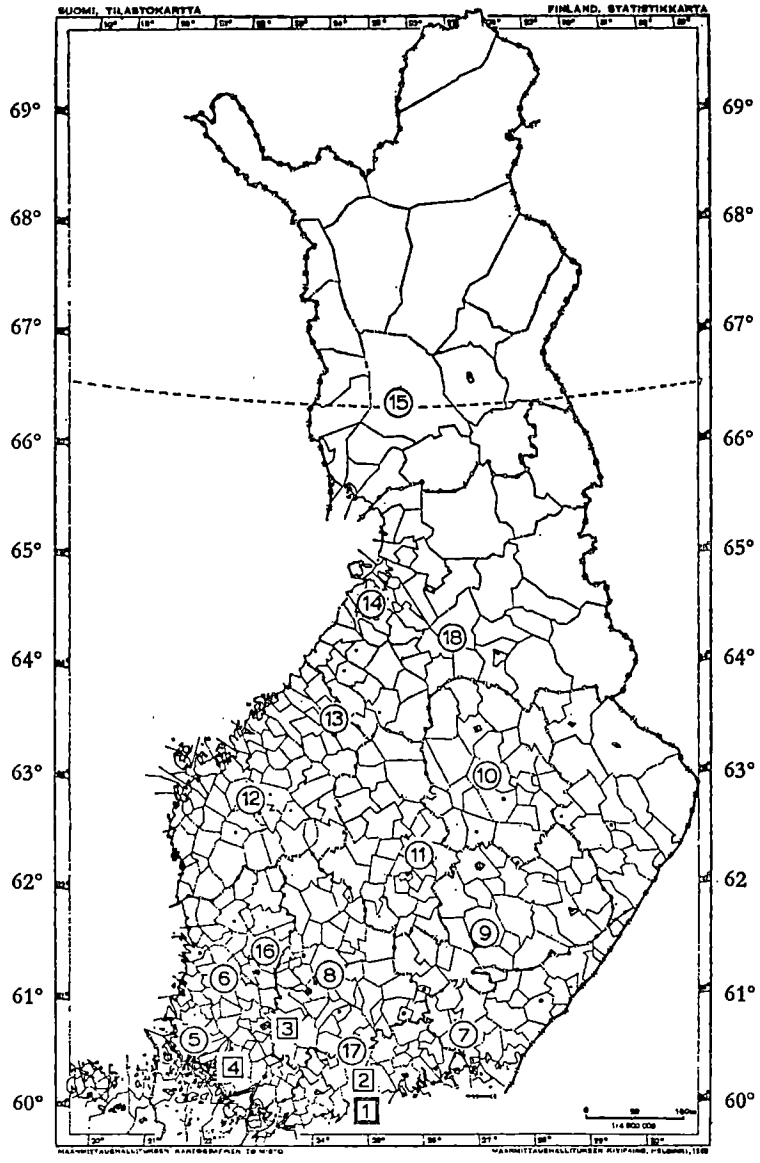

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