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SUITABILITY OF UNCASTRATED BOARS FOR MEAT PRODUCTION

KAIJA SUOMI and TIMO ALAVIUHKOLA

SUOMI, K. & ALAVIUHKOLA, T. 1986. **Suitability of uncastrated boars for meat production.** Ann. Agric. Fenn. 25: 81—90. (Agric. Res. Centre, Swine Res. Sta., SF-05840 Hyvinkää, Finland.)

In a meat production experiment, boars (= uncastrated boars) reached slaughter weight 8 % faster than castrated males and 5 % faster than females and consumed 11 % less feed per kilo live weight gain than castrated males and 6 % less than females. Production of lean meat was also greatest with boars. Calculations made from the trial results showed boar meat production to be more economically profitable than that of females and castrated males if only weight and fat content are taken into account in the pricing of carcasses.

For optimal gain, boars need more protein than castrated males. Boars could be fed fairly generously without increasing fat content excessively. It was not worth raising boars to over 100 kg, as the gain and feed conversion efficiency were then relatively worse than with boars under 100 kg. Members of the tasting panel also considered cutlets from the smaller boars (85—100 kg) slightly better in taste than those from larger boars (115 kg). 207 boars were raised for the trials. Cooking tests at the slaughter house found all the animals fit for human consumption. The tasting panel judged the quality of boar meat similar to that of castrated males and females.

Index words: uncastrated boars, gain, feed conversion efficiency, protein, energy, slaughter weight, boar taint.

INTRODUCTION

In most countries, including Finland, young boars are castrated if they are being kept for meat production. However, Ireland has completely given up the castration of young boars, and in Australia 80 % of boars are fed for meat uncastrated.

Several studies have shown that boars reach slaughter weight faster than castrated males and have a better food conversion efficiency (WALSTRA and KROESKE 1968, JONSSON et al. 1981 and RILEY 1982). Production of lean meat with boars is also greater than with castrated males (HANSSON 1970 and BEKAERT et al. 1973).

The aim in castrating young boars is to reduce their distinctive taint. This does not form in the fatty layer after removal of the testes as it does with uncastrated boars.

We know now that not all boars have this strong taint. The EAAP WORKING GROUP (1975) reported that 75—85 % of boar carcasses had no

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perceptible taint and that only 5—10 % of boars had a strong and distinct taint. According to MALMFORS and NILSSON (1978), 12—15 % of boars usually have taint.

The main cause of boar taint is 5-α androstene-3-one, shortened to androstenone, a steroid smelling of urine and sweat, which PATTERSON (1968) was the first to isolate. Boar taint has been compared (judged subjectively) with androstenone and various correlation coefficients have been obtained: FUCHS (1971) 0,75, NEWELL et al. (1973) 0,53 and MALMFORS and ANDRESEN (1975) 0,51. HANSSON et al, (1980) found in their research that androstenone alone explains 36 % of the variation in boar taint when skatole and indole are defined separately. Androstenone and skatole then together explain about 50 % of variation in boar taint. Skatole is produced during the decomposition of tryptophan and is thus a characteristic of both sexes. The source of boar taint is absorbed into the blood circulation and deposited in the fat from the age of 3 months. It is also stored in the salivary glands and is secreted from there into the saliva, with the aim of stimulating the sexual behaviour of animals of the opposite sex (ROACH 1982).

Boar taint is most noticable when the fat is heated. There are several methods of determining taint. In the view of WALSTRA and MATEMAN (1982) the soldering iron method is well suited to determining boar taint on the slaughtering line. After that, carcasses suspected of taint can be assessed separately using analytical methods. The ELISA method (enzymelinked immuno sorbent assay) is considered a quick way of measuring androstenone. According to WALSTRA and MATEMAN (1982), the ELISA method and sensory assessment are very comparable (correlation r = 0,71). RIA methods (radioimmunoassay) are also suitable for determining androstenone. A simple RIA method can be used to distinguish between animals with a low and high androstenone content (ANDRESEN 1979). FØRLAND et al. (1980) showed a positive correlation (r = 0,70) between the length of the Gl. bulbourethralis of boars and the androstenone content of their fat. The measurement is well suited to isolating tainted carcasses.

Analytical methods are more exact than sensory methods, because features other than smell may well influence the latter.

MATERIAL, METHODS AND RESULTS

Four trials were carried out to solve the research problem. Trial 1 compared the meat production capacity of boars, castrated males and females. Trial 2 investigated boars' protein need. Trial 3 compared two energy levels in boar raising and trial 4 studied the weight at which it is best to slaughter boars.

Trial 1. Meat production

One male, female and castrated male piglet was chosen from 44 landrace litters for meat production trial. Half of these received feed containing 13 % digestive crude protein and half 15 % digestive crude protein (Table 1). The animals were fed in pairs. At each protein level the animals between 25 and 100 kg in weight were

Table 1. Experiment 1. Composition of the diets.

	d. c. p. 13 %	d.c.p. 15 %
Barley %	84,3	79,5
Fish meal %	3,0	4,2
Soyabean meal %	9,0	12,6
Mineral + vitamin mixt. %	3,7	3,7
FU/kg [+)]	0,97	0,97
MJDE/kg	12,6	12,6
d. c. p. %	13,2	15,1
Ca g/kg	8,3	8,8
P g/kg	6,6	6,9
Lysine g/kg	7,6	9,0
Methionine + cystine g/kg	5,5	6,1

[+)] FU = 0,7 kg starch equivalent

given 1,2—3,0 feed units. The average amount was 2,2 feed units/animal/day.

In both groups the boars reached slaughter weight faster than the castrated males and females. The gain was greater at the higher protein level, the difference compared with castrated males then being 8 % (Table 2). The boars had a better food conversion efficiency than the castrated males. At the higher protein level, the difference was 11 %. There was no perceptible difference between boars and castrated males in loss at slaughter.

Table 2. Gain of boars, feed conversion efficiency and slaughter results compared with the results of castrated males and females.

	13 % d. c. p.			15 % d. c. p.		
	Boars	Gilts	Castrates	Boars	Gilts	Castrates
Daily gain, g/day	824 ^{ad}	811 ^{abde}	782 ^{be}	854 ^{ad}	813 ^{bde}	790 ^{be}
Loss at slaughter %	28,3 ^{ab}	27,9 ^{a}	28,5 ^{b}	28,2	28,0	27,7
Feed conversion efficiency, F.U./kg	2,60 ^{ad}	2,68 ^{bde}	2,78 ^{be}	2,50 ^{af}	2,67 ^{bfg}	2,79 ^{bg}
Side fat thickness, mm	18,4	19,1	19,7	15,9 ^{ad}	17,5 ^{ade}	20,4 ^{be}
Firmness of fat (scale 9—15)	12,2 ^{f}	13,1 ^{g}	13,2 ^{g}	11,9 ^{af}	12,3 ^{bf}	13,4 ^{g}
Colour of meat (scale EEL)	29,4 ^{adf}	36,1 ^{beg}	34,4 ^{be}	32,3	33,1	34,8
Meat in valuable cuts %	80,6 ^{a}	80,5 ^{ab}	79,2 ^{b}	82,5 ^{ad}	81,1 ^{bde}	78,8 ^{ce}

a — c = $p < 0,05$
d — e = $p < 0,01$
f — g = $p < 0,001$

The statistical handling done inside of protein levels.

There was a greater proportion of lean meat and a smaller proportion of fat in the boar carcasses than in the castrated males. Judged subjectively, the boars' fat was softer than the castrated males' and females'. The boars' lean meat was slightly darker than the castrated males' and females'.

A ten-person panel[1] made a sensory assessment of fried fat-free cutlets from both sexes. Only one member of the panel identified boar on the basis of a slight flavour difference. Consumers (41 persons)[2] were given an opportunity to taste boar meat. The cutlets, both of boar and normal pork cutlets, were fried and seasoned in the same way, ''restaurant style''. The consumers noticed no difference between the cutlets in taste or juiciness.

A financial calculation was made on the basis of the trial results. The slaughterhouse did not pay the normal price for pork for the boar meat, but a lower one corresponding to its use. All boar meat is used industrially. Thus the price of boar meat was estimate as the same as the meat of castrated males, while taking the better classification into account. Even at the lower protein level the boars yielded a better net profit per pig than castrated males, and at the 15 % protein level the difference was even greater.

Table 3. Experiment 2. Composition of the diets.

	d. c. p. 14 %	d. c. p. 15 %	d. c. p. 16 %
Barley %	82,2	79,5	77,1
Fish meal %	3,5	4,2	4,8
Soya bean meal %	10,6	12,6	14,4
Mineral + vitamin mixt. %	3,7	3,7	3,7
FU/kg $^{+)}$	0,96	0,96	0,96
MJDE/kg	12,5	12,5	12,5
d. c. p. %	13,9	14,8	15,6
Ca g/kg	8,5	8,8	9,0
P f/kg	6,8	6,9	7,0
Lysine g/kg	8,2	9,0	9,7
Methionine + cystine g/kg	6,0	6,1	6,5

$^{+)}$FU = 0,7 kg starch equivalent

Trial 2. Protein levels

This trial employed three protein levels: 14, 15 and 16 % digestive crude protein (Table 3). For each level, only boars from the same litter were

[1] Tasting panel made up of Swine Research Station staff.
[2] Tasting panels made up of Rotary Club members of Hyvinkää.

chosen, mostly Finnish landrace. Each protein level was divided half of the animals fed at that level received a single type of feed (S) throughout the trial, and half received a feed with higher protein content for the first six weeks and one with less protein for the rest of the time (though the protein content was the same as in feed S). The amount of feed at 25—100 kg live weight was 1,2—3,0 kg.

The boar gain increased significantly ($p < 0,05$) with a higher protein level (Table 4). In feed conversion efficiency there was an even greater difference between the protein levels ($p < 0,001$). There were no major differences in other characteristics. The systems being compared — one mixture from 25 to 100 kg weight or two feeds (25—40 and 40—100 kg live weight) — gave the same result.

Trial 3. Energy levels

There were two energy levels in the trial. Boars from the same litters were selected for each group. At gain intervals of 20—90 kg the animals in group I were given 0,75—2,65 kg of feed (L) and those in group II 0,90—3,15 kg (H). The average amount of feed was 1,8 and 2,1 kg/day respectively. The composition of the diet is shown in Table 5.

The boars given more energy (H) gained on average 20 % better and reached slaughter weight roughly 2 weeks earlier than those given less energy (L) (Table 6). The feed conversion efficiency was also 3,3 % better. The slaughter results showed no significant differences between energy levels.

Trial 4. Slaughter weights

In the slaughter weight trial the boars were raised to a final weight of 85, 100 and 115 kg. Twenty four animals from the same litters were selected for each group. The feed mixture was the same for all boars (Table 7) and the amount given was 1,2—3,0 kg/animal/day for feeding weeks 1—10 and 3,2 kg thereafter.

Table 4. Gain of boars, feed cenversion efficiency and slaughter results on different protein levels.

	14 % d. c. p.	15 % d. c. p.	16 % d. c. p.
Daily gain. g/day	814	823	856+
Loss at slaughter %	29,4	30,1	29,1
Feed conversion efficiency, F.U./kg	2,60	2,56	2,43+++
Side fat thickness, mm	16,6	14,7	15,0
Firmness of fat (scale 9—15)	12,0	12,0	11,9
Colour of meat (scale EEL)	39,0	35,9	38,8
Meat in valuable cuts %	82,5	82,9	83,2

Table 5. Experiment 3. Composition of the diet.

Barley %	41,5
Oats %	41,5
Fish meal %	6,5
Soyabean meal %	5,0
Dried skimmed milk powder %	2,0
Mineral — vitamin mixt. %	3,5
FU/kg +)	0,93
MJDE/kg	12,1
d. c. p. %	13,6
Ca g/kg	9,6
P g/kg	7,1
Lysine g/kg	9,2
Methionine + cystine g/kg	6,6

+) FU = 0,7 kg starch equivalent

Table 6. Gain of boars and feed conversion efficiency on a low or high feeding scale.

	L	H
Daily gain, g/day	650	781+++
Loss at slaughter %	29,3	28,9
Feed conversion efficiency, F.U./kg	2,73	2,64
Days in trial	101	86
Side fat thickness, mm	11,0	11,4
Firmness of fat (scale 9—15)	11,6	11,4
Colour of meat (scale GÖFO)	61	61
Meat in valuable cuts %	85,9	85,2

+++ = $p < 0,001$

Table 7. Experiment 4. Composition of the diet.

Barley %	64,5
Oats %	20,0
Fish meal %	5,0
Soyabean meal	7,0
Mineral + vitamin mixt. %	3,5
FU/kg +)	0,96
MJDE/kg	12,5
d. c. p. %	14,1
Ca g/kg	9,1
P g/kg	6,5
Lysine g/kg	8,2
Methionine + cystine g/kg	6,1

+) FU = 0,7 kg starch equivalent

Table 8. Gain of boars, feed conversion efficiency and slaughter results of different weights.

		I Final weight 85 kg	II Final weight 100 kg	III Final weight 115 kg
Slaughter weight, kg		61,1	73,0	84,3
Loss at slaughter %		29,7	28,0	27,1
Daily gain, g/day		811	865	881
Days in trial		73,5	88,0	104,5
Feed conversion efficiency, kg/kg		2,34	2,36	2,43
Side fat thickness, mm		13,6	15,5	16,9
Carcass length, cm		95,1	99,5	104,0
Meat in valuable cuts %		83,8	83,5	83,0
Carcass classification	E+	5	9	15
	E	11	9	5
	I	8	6	4
	IR	—	—	—

Table 8 shows that boar gain improved with rising final weight. The best gain stage, over 1 000 g/day, came between 60 and 90 kg, however (Fig. 1.). After this, gain slowed down. The boars' feed utilization deteriorated with higher final weight. Over the 100—115 weight interval proportionately more feed was consumed per kilo live weight than between 85—100 kg. The backfat thickness increased with higher final weight. More animals from group III (115 kg) than from the other classes would actually have been put in the top quality class if the carcasses had been graded. The meat percentage of the valuable parts of the carcass was roughly the same in all the groups.

Members of a professional tasting panel from the Finnish Meat Research Centre made a sensory assessment of cutlets from 19 animals in each group. The taste points given were 0—3 (0 = no boar-like taste and smell, 3 = strong boar-like taste and smell). On average, the members of the

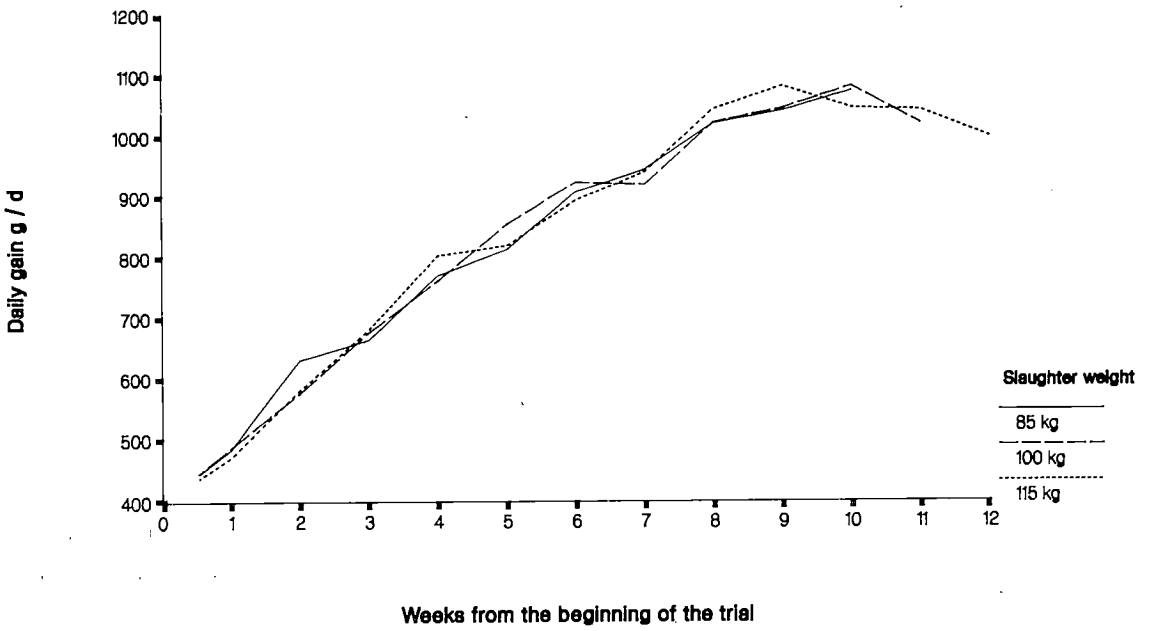

Fig. 1. Growth rates of the boars, slaughtered at different weights.

tasting panel gave the boars in group I 0,87 points, those in group II 0,89 points and those in group III 1,08 points. The Swine Research Station's own staff also sensorily assessed fried cutlets of boars from each group. The cutlets from boars in group III, i.e. the biggest animals, were found to have an unusual taste most frequently.

DISCUSSION

Meat production

With limited feeding boars have usually been found to gain faster than castrated males (WALSTRA and KROESKE 1968, JONSSON et al 1981, RILEY 1981). With *ad lib.* feeding, however, when castrated males can eat more than boars because they have a bigger appetite, this is not the case (KROESKE 1968). Limited feeding slows down castrated males' gain most, because their appetite is not satisfied (HANSSON 1970). BEKAERT et al. (1973), WOOD and MOTTRAM (1981) and ELLIS et al. (1983) found that loss at slaughter is greater with boars than with castrated males.

The softness of boar fat may be influenced by the fact that, according to SMITTHARD et al. (1980), it contains far more unsaturated fatty acids (61,6 % against 60,6 % in the fat of castrated males), in other words less palmitic acid and more linoleic and linolenic acid. Soft, floppy fat tends to loosen from the lean meat (FOSTER 1983). No such loosening of fat was noted in the boars in our meat production trials. WOOD et al. (1983) found in objective tests that the thin fat was firmer in boars than in castrated males, but that the opposite was true of thick fat. In general there are no very great differences in fat firmness between boar and castrated male.

The darker coloured meat of boars would be welcome here in Finland, where pork tends to be pale. British consumers consider that boar meat is darker, softer and more watery than other pork. They view these as negative qualities, because meat like this is difficult to cut and they think pork should look pale (ROACH 1982). MALMFORS and NILSSON (1978) found that boar meat is comparable to the meat of castrated males and females with a few exceptions. They were then comparing meat colour, frying loss, tenderness and juiciness. Boar had the lowest muscular fat content, the highest water content and the poorest commercial quality of back fat. The taste points given to the meat were only slightly lower than for castrated males and females. WOOD and MOTTRAM (1981) found that the higher water content in the muscular and fatty tissue of boar probably had little effect on the quality of the tissue.

Protein

Contrary to our findings, WALACH-JANIAK et al. (1980) did not find that protein had any significant effect on the gain of meat boars over the age span 91—182 days. The crude protein levels were 14,8, 16,3, 17,9 and 19,5 %. They found, in fact, that crude protein level does not need to be raised above 16,5 % if the energy content is sufficiently high. According to CAMPBELL and KING (1982), a rise in protein above 170 g of crude protein per kilo of feed over the weight interval 45—70 kg reduced the yield for castrated males, though with boars the yield did not fall until protein rose from 210 g to 231 g per kilo of feed. The fact that the pig material used for these protein tests differed probably explains the slightly deviant results.

In our trials, raising the protein level to over 16 % digestive crude protein would probably not have given a positive result. We calculated that cost of feed would be so high that the better gain would be no financial advantage, compared with the lower levels.

Energy

Raising the energy level did not make the boars' fat very much thicker, a conclusion also reached by TRAPNELL and COOKE (1978). They reported that raising the amount of feed thickened the fat more in castrated males and females than in boars, whose best feed conversion efficiency was attained with a daily amount of 2 kg. However, KALLWEIT and SCHRÖDER (1973) concluded that raising the amount of feed increased fat content and boar taint.

Slaughter weights

The Swedish researchers HANSSON and MALMFORS (1973) also came to the conclusion that boar gain improved with higher final weight. The final weights in their slaughter weight test were 70, 90, 110 and 130 kg. The best stage in pig gain was between 70—90 kg in their research. Thereafter gain deteriorated with boars, though more slowly than with castrated males and females. One reason suggested by these researchers was feeding intensity. The amount of feed remained the same after 85 kg live weight, i.e. 3,1 kg per day. The stabilization of feed quantity after c. 85 kg live weight at 3,2 kg per day obviously also contributed to the slower gain by our boars after 90 kg live weight. HANSSON and MALMFORS (1973) also noted that utilization of feed by boars deteriorated with higher final weight.

The slaughter weight test was aimed at discovering the weight at which boars should be slaughtered to attain maximum gain and feed conversion efficiency and the best slaughtering qualities, and that at which there would be no detrimental boar taint in the fat. Assessment of these qualities would indicate that it is not worth raising boars over 100 kg. Boars are on average 160 days old at this weight. The British veterinary association (ANON. 1981 a) has stressed that there is a low risk of taint if boars are slaughtered at under 160 days. In France and Sweden it has been found that androstenone content grows with a rise in slaughter weight. This effect is much clearer if the boats are fed *ad lib.* (ANON. 1981 b). The fat 42 % of fast-gaining boars (100 kg at the age of 150 days) contained more than 1 μg androstenone per gram of fat (BONNEAU and DESMOULIN 1979).

GENERAL DISCUSSION

KEMPSTER and CUTHBERTSON (1982) find that boar taint is not the only factor to be considered when boar meat is offered to consumers. In the retailer's view boar meat is floppy and the fat tends to detach itself from the muscular tissue. However, it is not known whether these drawbacks arise from the boars themselves of simply from the fact that boar has more meat on it. MALMFORS and LUNDSTRÖM (1982) report that consumer reactions to boar meat have been studied for 10 years in six European countries. The consumer view is that the smell of boar meat is less pleasant than that of normal pork, though the scatter is very considerable, from 5—35 %, compared with 3—10 % for castrate males and females. The results differ from country to country and for different products. According to the research done by JONSSON et al. (1981) one third of the boars slaughtered at 90 kg weight cannot be approved for human consumption. This study agrees with Norwegian and Swedish research. The results are based on biopsy samples taken from the live boars at 90 kg weight. The androstenone content of these samples was determined by an RIA method, and smell was assessed by members of a panel.

WILLEKE (1982) studied the heritability of androstenone in two lines, one with a low androstenone content and the other with a high one. Up to the fourth generation the androstenone content of the high line rose clearly and that of the low line fell slowly. The difference between the two lines still existed when androstenone content was measured at the age to 250 days. In WILLEKE's view the boars with a low level gave better production, i.e. more lean meat, than those with a high level.

JOHNSSON et al. (1981) found that boar taint is moderately heritable and that there is a high correlation between taint and the sexual hormone testosterone. In conclusion they say that it is possible to carry out selection with regard to boar taint. But before embarking on any extensive selection, it should be ensured that the boars' breeding ability and production capacity do not suffer. On the other hand, it is not essential to carry out selection with regard to boar taint as, according to ANON. 1981 a, the risk of boar taint is non-existent if they are slaughtered under the age of 160 days.

Vaccination (autoimmunization method) is considered one way of combating boar taint (WILLIAMSON and PATTERSON 1982). This reduces the formation of androstenone in the fatty tissue from 0,77 mg/kg of fat in the case of unvaccinated 93,9 kg boars to 0,06 mg/kg of fat with vaccinated boars weighing 92,2 kg. The vaccinated boars did not appear to suffer any side-effects, and did not deteriorate in male-type gain or in carcass features. The vaccine contains synthetic androstenone protein complex which appears "foreign" to the pig's immune system, triggers the production of antibodies. The vaccine is given at an early stage, i.e. during weaning, and a second time 7—9 weeks later if the boars are raised to 90 kg weight. The amount of androstenone then in the fat fell 93 %. This trial involved 200 boars (ROACH 1982).

Vaccination is a fast way of producing taint-free boars. They can then be raised as uncastrated boars to slaughter weight, the best selected for breeding purposes, and the rest sent to slaughter. For the time being vaccination is considered an expensive way of preventing boar taint, though it has given good results and the vaccine keeps well.

On the other hand, selection is slow and so far we are not sure whether or not it reduces the boars' breeding ability. It would be simplest to remove tainted boars from the slaughter line. Researchers believe that it would be useful to develop still faster and better methods of measuring taint.

The symposium on boar taint (MOERMAN 1981) recommended the following measures to get boar meat accepted:

1) No secret should be made of boar taint.
2) The members of the symposium recommended the following thresholds for androstenone content measured by an RIA method:
   fresh meat 0,5 µg/g of fat or less
   processed meat 1,0 µg/g of fat or less
3) Products should be developed which are made specifically of boar meat, and its good features should be exploited.
4) New methods should be developed for processing meat with a strong taint.
5) Negative publicity about boar meat should be combated.

Our meat production tests showed that gain, utilization of feed and production of lean meat were much better with boars than with castrated males. All the test boars at the slaughter house were also found to be free of boar taint in cooking tests, i.e. they would have been fit for human consumption, whereas they were now used for industrial purposes. Judged visually, the colour of the meat of castrated males in the test litters was on average paler than the boars', which would indicate that the boars were more resistant to stress than the castrated males. Taste tests seemed to show that the Finnish consumer would accept boar meat. The calculations made show that boars are more profitable in meat production than castrates.

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SELOSTUS

Leikkaamattomien karjujen soveltuvuus lihantuotantoon

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Maatalouden tutkimuskeskus

Nykyisin meillä leikataan kaikki lihantuotantoa varten kasvatettavat karjut. Suurimpana syynä leikkaamiseen ilmeisesti on se, että pelätään ennakkoluulojen karjun lihaa kohtaan johtavan sianlihan kokonaiskulutuksen laskuun.

Karjujen lihantuotanto-ominaisuuksia selvitettiin kokeellisesti vertaamalla niitä leikkojen ja imisien lihantuotanto-ominaisuuksiin. Samoin erilaisin maku- ja hajutestein arvioitiin karjujen lihan soveltuvuutta ravinnoksi.

Karjut kasvoivat leikkoja ja imisiä nopeammin teuraskypsiksi. Ero oli leikkoihin nähden 15 % sulavaa raakavalkuaista sisältävällä rehulla 8 %. Etuna oli myös se, että karjut käyttivät rehua vähiten kasvukiloa kohti. Ero leikkoihin oli samalla valkuaistasolla 11 %.

Karjujen liha oli tavallista sianlihaa tummempaa. Silava oli normaalia silavaa pehmeämpää, mutta toisaalta sitä voidaan pitää terveellisempänä, koska tyydyttymättömiä rasvahappoja oli runsaammin.

Tutkimuksen perusteella tehdyt taloudelliset laskelmat puoltavat karjun kasvatusta. Laskennassa karjun lihan hinta arvioitiin leikkojen lihan hintaa vastaavaksi ottamalla kuitenkin huomioon karjujen parempi laatuluokitus. Meillä karjun lihasta maksetaan nykyisin normaalia sianlihaa alhaisempi hinta (heikkoa hyväksikäyttöä vastaava hinta).

Kokein todettiin karjujen tarvitsevan optimaalista kasvuaan varten enemmän valkuaista kuin leikkojen. Karjuja voitiin myös ruokkia hiukan normaalia sikaa runsaammin niiden rasvoittumatta liikaa. Parhaimmat tulokset lihantuotannossa saavutettiin, kun karjut teurastettiin noin 100 kg:n painoisina.

Aistinvaraisten haju- ja makutestien perusteella voidaan arvioida, että suomalainenkin kuluttaja hyväksyy karjun lihan.

Kiitämme Lihateollisuuden tutkimuskeskusta ja Hyvinkään Rotaryklubin jäseniä karjun lihan aistinvaraisesta arvostelusta.

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TREATMENT OF STRAW WITH AMMONIA, UREA OR A UREA + UREAPHOSPHATE MIXTURE: EFFECT ON DIETARY INTAKE AND GROWTH OF YOUNG AYRSHIRE BULLS RAISED FOR BEEF

MARJA ALASPÄÄ

ALASPÄÄ, M. 1986. **Treatment of straw with ammonia, urea or a urea + ureaphosphate mixture: Effect on dietary intake and growth of young ayrshire bulls raised for beef.** Ann. Agric. Fenn. 25: 91—97. (Agric. Res Centre, Dept. Anim. Husb., SF-31600 Jokioinen, Finland.)

Oat straw was treated with ammonia (A), urea solution (US), urea granules (UG), urea + ureaphosphate mixture (3 + 1) solution (UUFS) or urea + ureaphospahte mixture (3 + 1) granules (UUFG). The aim of the dosage was to add of 25 kg nitrogen per ton of dry matter. Straw dried on the field was used as the control (C). The feeding-experiment was carried out with Ayrshire bulls between 139—345 days of age. *In vivo* digestibility was determined using wethers.

Losses of additives from straw were higher with granules than by spraying. The quality of all straws was good during winter and spring and mold growth did not became apparent until the summer.

The total nitrogen content of straw was increased by these ammonia-based treatments. However their influence on the digestibility of the organic matter of straw and energy value in fattening feed units was slight and insignificant. Further the treatments did not increase daily liveweight gain, carcass weight or the voluntary intake of straw significantly.

It is concluded that the protection of moist straw against molding is the most important function the ammonia-based treatments. Treatment with the liquid urea was found to be the most economical and practical.

Index words: straw as feed, ammonia treatment, urea treatment, urea + ureaphosphare mixture treatment.

INTRODUCTION

Alkali treatment has long been known in Finland as a means improving the nutritive value of straw. Sodium hydroxide has proved to be the most effective in this respect. However, as sodiumhydroxide has been found to be problematic both in the treatment and feeding of straw, it has sometimes been replaced with ammonia. In addition to slightly improving the nutritive value of straw, ammonia also functions as a preservative against molds in straw of high moisture content (SUNDSTOL et al. 1978, SCHMIDT et al. 1978). Methods for ammonia treatment are well established (SUNDSTOL et al. 1978). Since ammonia is easily released from

urea following its addition in to the moist straw, urea has replaced liquid ammonia for treatment purposes. Urea is also inexpensive and easily available, and pressure containers are not needed for its transport.

Ureaphosphate is a combination of two mole of urea and of one mole of ortophosphoric acid, and has been advocated for various plant materials treatments.

The aim of the present work was to examine the effect of treatment with ammonia, urea and a mixture of urea + ureaphosphate on molding, digestibility and the nutritive value of straw: on the voluntary intake and growth of young cattle raised for beef.

MATERIAL AND METHODS

Treatment of straw

Oats straw was used in the experiment. Fertilizer grade urea as well as a mixture of urea + ureaphosphate in both granular and liquid forms were applied.

The urea + ureaphosphate mixture consisted of 75 % urea and 25 % ureaphosphate. Solutions were prepared as follows: urea was dissolved in water (temperature + 60 °C) by 1 : 1,2 and the urea + ureaphosphate mixture by 1 : 1.

The aim of dosage was to add 25 kg pure nitrogen per ton of dry matter. On the basis of nitrogen content the amounts per ton of dry matter were: 30 kg ammonia (82,4 % N); 4 kg urea (46,7 %); and 63 kg urea + ureaphosphate mixture (39,4 % N) respectively. Anhydrous ammonia was injected using a perforated metal tube into a stack covered by a polyethylene sheet. Urea and the urea + ureaphosphate mixture were added during baling. Granules were strewn by an applicator placed above the compression chamber of the baler. Solutions were sprayed with an electric pump fitted with nozzles placed above of the pick up of the baler. After baling, the straw was stacked and sealed in a plastic sheet. Untreated straw was dried on the field and baled. All straws was stored in a barn. The straw was treated and baled during September 3.—8. 1981.

The mean daily temperature during the baling period range from + 3 °C to 17,5 °C. The dry matter content of the straw during treatment was on average 58,64 % (variation 57,4—77,7 %).

The chemical composition of the straw during baling is shown in Table 1.

Table 1. Chemical composition of straw during baling.

Field	Area ha	Number of samples	% in dry matter				
			Ash	CP	EF	CF	NFE
1	10,6	3	8,29	4,45	1,47	44,44	41,34
2	12,7	6	8,02	5,85	1,32	42,72	42,10

CP = crude protein, EF = ether extracts, CF = crude fiber, NFE = nitrogen free extracts

Feeding experiment

The feeding experiment was carried out during January 1.—August 8. 1982 with 46 Ayrshire bulls, whose average age was 138 days at the beginning of the experiment. Storage time of straw in the storage was 19 weeks. The animals were divided into six groups according to liveweight and age to form the six different diet groups. The animals were fed individually. Straw was offered *ad libitum*, but determing the intake. Standard barley meal was offered 50 g per kg metabolic liveweight. The control group was additionally supplemented with urea mixed into barley meal. A mixture of minerals and trace

elements and fat soluble vitamins were offered to all animals according to estimated requirements. Water was freely available.

Calcium carbonate was given to the animals fed straw treated with the urea + ureaphosphate mixture (10 g/kg DM straw consumed). Feed intake was determined by weighing the feeds and residues daily. Animals were weighed every second week and the feeding of concentrates adjusted accordingly. The experimental data was analysed by one way analysis of variance and differences among treatments were tested using Tukey's test (STEEL and TORRIE 1960).

Feed analyses

Standard laboratory routines were used for conventional feed analyses. The dry matter content was determined by oven drying as follows: 12 hours at + 60 °C and 8 hours at + 105 °C. The content of volatile fatty acid was determined by gas chromatography (HUIDA 1973). Total and water soluble nitrogen were determined from fresh samples using the Kjeldahl method and ammonium nitrogen was determined colorimetrically (MC CULLOUGH 1967). Wethers (6 × 6 latin square) were used for the determination of the *in vivo* digestibility.

RESULTS

Losses of additives from straw

In order to estimate the amount of solid and liquid additives retained in the straw, the phosphorus content of untreated straw and those of straw treated with the urea + ureaphosphate mixture were compared. On average 81,8 % of the additive was retained in the straw by spraying method and 43,8 % with granule method. It is assumed that about the same share of addititive was retained when urea alone was added in solution or granular form, respectively.

Chemical composition and quality of straw

The total nitrogen content was increased significantly by the treatments ($p < 0,05$); the highest values resulted from solution treatments (Table 2). These treatments did not result in any

Table 2. Average chemical composition of feeds during the feeding experiment. Treatments: C = untreated, control straw, A = ammonia, US = urea solution, UG = urea granules, UUFS = urea + ureaphosphate(3 + 1) mixture, solution UUFG = urea + urephosphate(3 + 1) mixture, granules.

		Treatments						
	barley	C	A	US	UG	UUFS	UUFG	
Dry matter, %	87,30	84,74	75,98	76,85	67,25	70,86	72,86	
In dry matter, %								
— ash	2,79	8,72^{a}	8,66^{a}	8,27^{a}	9,61^{b}	9,08^{a}	8,88^{a}	
— org. matter	97,03	91,28	91,34	91,73	90,39	90,92	91,12	
— crude protein (6,25 × N)	12,18	6,95^{a}	11,03^{b}	13,71^{cd}	10,43^{b}	13,99^{d}	11,78^{bc}	
— crude fat	2,20	1,30	1,47	1,47	1,28	1,45	1,37	N.S.
— crude fiber	5,86	43,11	43,15	43,22	43,23	42,31	42,84	N.S.
— nfe	76,79	39,93^{c}	35,69^{b}	33,34^{a}	35,44^{b}	33,18^{a}	35,14^{ab}	
pH		8,19	8,19	8,73	8,64	8,69	8,52	
Nitrogen								
— total		1,11^{a}	1,77^{b}	2,19^{cd}	1,67^{b}	2,24^{d}	1,88^{bc}	
— soluble		0,48^{a}	0,94^{b}	1,24^{c}	0,86^{b}	1,29^{c}	0,99^{bc}	
— ammonia		0,07^{a}	0,39^{b}	0,51^{bc}	0,34^{b}	0,70^{c}	0,47^{b}	
Digestibilities, %								
— org. matter		47,65	52,11	51,66	49,56	50,56	50,67	N.S.
— crude fiber		58,11^{a}	68,04^{b}	63,98^{ab}	64,70^{ab}	59,31^{a}	62,55^{ab}	
Feed value								
— kg DM per f.f.u.		3,81	3,56	3,55	3,72	3,89	3,61	

In calculation of feed value has used the correlation (42) to all treated straw. Means in the same line not having a superscript letter differ significantly ($p < 0,05$).

significant increase in the digestibility of organic matter ($p > 0,05$). In the case of fiber digestibility the difference between untreated and ammonia treated straw (10 percentage units) was significant ($p < 0,05$). The effect of urea treatment was 5—6 % and that of urea + ureaphosphate mixture 1—5 %, respectively.

The energy content of straw is expressed as fattening feed units (f.f.u.) which correspond to Kellner's starch equivalent in the ratio: 1 f.f.u. = 0,7 kg starch equivalent. The difference in the net energy values of straw among treatments or between untreated and treated straw were insignificant ($p > 0,05$).

The overall quality of straws was good and the treated straws smelled strongly of ammonia during the winter and early spring. The straws were dark yellow or brown in colour. Mold growth became apparent during the summer, apparently due to the warm weather, losses of ammonia from the stacks, and the penetration of air in to the stacks. In August, almost all treated straws were moldy to some extent, and therefore the feeding experiment had to be finished earlier than planned.

Table 3. Initial and final liveweights, carcass weights and daily liveweight gains of Ayrshire bulls. Treatments are shown in Table 2.

	Treatments						
	C	A	US	UG	UUFS	UUFG	
age at the beginning	137	139	139	139	139	142	
age, at the end	343	345	345	345	345	348	
live weight							
at the beginning	120	115	119	119	120	118	
at the end	299	303	314	311	315	305	
carcass weight, kg	134	138	144	142	145	136	N.S.
carcass percentage	44,7	45,7	45,9	45,7	46,1	44,7	
carcass quality class	I—	I—	I—	I—	I—	I	
daily gain, g	878	920	940	932	948	913	N.S.

N.S. = differences between means are insignificant ($p > 0,05$).

Table 4. Feed intake of bulls. Treatments are shown in Table 2.

	Treatments						
	C	A	US	UG	UUFS	UUFG	
Feed intake, kg DM per day							
— total	5,05	5,17	5,47	5,55	5,41	5,61	N.S.
— straw	2,48	2,66	2,90	2,99	2,82	3,06	N.S.
— barley	2,28	2,31	2,36	2,36	2,36	2,32	N.S.
— soy bean meal	0,07	0,07	0,07	0,06	0,06	0,06	
— urea	0,09						
— mineral mixture	0,14	0,14	0,14	0,14	0,16	0,16	
Crude protein, g per day							
— total	744	600	696	624	721	683	N.S.
— straw	172^{a}	284^{ab}	374^{b}	306^{ab}	402^{b}	367^{b}	
— barley	280	283	289	289	290	285	N.S.
Feed unit per day							
— total	3,25	3,38	3,50	3,48	3,41	3,49	N.S.
— straw	0,65	0,75	0,82	0,80	0,73	0,85	N.S.
— barley	2,54	2,58	2,63	2,63	2,63	2,59	N.S.
kg DM/kg daily gain	5,93	5,73	5,83	6,12	5,77	6,27	N.S.

Means in the same line not having a superscript letter differ significantly ($p < 0,05$).
N.S. = differences between means are insignificant ($p > 0,05$).

Feeding experiment

Data on the growth, liveweight gains and carcass weights of the animals are shown in Table 3, and those of feed intake in Table 4. Differences in the growth of the animals between groups were not significant ($p > 0,05$). Because the bulls were relatively young at the end of the experiment, carcass weights were low and no significant differences were found between the groups ($p > 0,05$) (Table 3). None of the treatments significantly increased voluntary intake of straw ($p > 0,05$). The daily dry matter consumption of untreated straw was 2,5 kg, while that of treated straw varied between 2,7—3,1 kg (Table 4). Energy intake as feed units mainly consisted of barley in all groups. Crude protein intake was the highest from UUFS and US — treated straw and lowest from ammonia treated straw (Table 4). However there were no significant differences in total protein intake between the groups because bulls in the control group received urea mixed into barley meal ($p > 0,05$). The animal's appetites were rather good throughout the feeding experiment and there were no ammonia poisonings, although the bulls were quite young at the beginning of the experiment. Adaptation time to urea begun 2 weeks prior to the onset of the feeding experiment.

Storage losses

Storage losses caused by mold were apparently due to escaped gaseous ammonia during the summer when the stacks were opened weekly for 4—5 month period. Amounts of moldy straw discarded during feeding are shown in Table 5. The average values of the total amount were lowest with the US and UUFS treatments (7 % of dry matter). The corresponding values in the case of UG and UUFG-treated straws were 21 and 22 % of dry matter, respectively.

Table 5. Amount of moldy straw during the feeding experiment calculated as % of dry matter of straw. Treatments are shown in Table 2.

	Treatments					
Date	C	A	US	UG	UUFS	UUFG
15.1—28.1	1,5	0	0	1,1	1,1	0,6
29.1—11.2	13,7	0	0	1,3	0	1,2
12.2— 1.3	4,5	0	0	2,1	0	0
2.3—12.3	0	0	0	0,6	0,7	0
13.3—26.3	0	0	0	3,1	3,0	1,5
27.3— 7.4	5,1	0	0	6,8	3,4	9,4
8.4—22.4	0	0,5	0,8	3,1	0	9,3
23.4— 5.5	6,2	1,1	0	7,6	7,2	1,2
6.5—20.5	9,5	28,8	2,7	33,0	5,4	7,0
21.5— 3.6	13,3	19,4	4,4	69,5	5,4	7,8
4.6—16.6	5,9	39,1	10,9	2,0	7,8	36,2
17.6—30.6	15,6	62,4	12,1	6,4	35,3	19,2
1.7—16.7	4,0	26,9	0,8	2,4	0,3	0,7
17.7—30.7	8,8	57,0	15,7	32,3	7,1	30,3
31.7— 9.8	15,0	11,9	24,1	48,5	0,6	73,4

DISCUSSION

The method of ammonia treatment and the dosage of all treatments (25 kg N per ton of dry matter) are based on the Norwegian stack method (SUNDSTOL and OWEN 1978). Methods for adding urea and the urea + ureaphosphate mixture in liquid or granule form are based on previous Finnish research (SULKA et al. 1982).

The treatments did not significantly improve the digestibility of organic matter, straw intake or the daily growth of the animals ($p > 0,05$). These results are in agreement with other results indicating that in addition to treatment, composition of the whole diet affects the improvement of the nutritive value of straw (HADJUPANAYITOU 1982, ORSKOV et al. 1983, CHESSION and ORSKOV 1984, WILLIAMS 1983/84, ORSKOV 1985). In addition such factors as the moisture content of straw during treatment, storage time, and in the urea treatment, the hydrolysis of urea to ammonia, influence the

effectiveness of the treatment (SCHMIDT et al. 1978, MBTAYA 1983, WILLIAMS et al. 1984, SUNDSTOL and COXWORTH 1984). One of the greatest disadvantages in using ammonia-based treatments is the loss of nitrogen in the form of gaseous ammonia during feeding (YASURICA and PEARCE 1983, HADJUPANAYITOU 1982, GORDON and CHESSION 1983, SUNDSTOL and COXWORTH 1984).

In the present work losses of nitrogen in the treatments during the feeding experiment were on average as follows: A 72 % from dry matter, US 56 %, UG 50 %, UUFS 56 % and UUFG 33 %. It was not possible to analyse the amount of nitrogen received by the animals from these treated straws, however.

It is concluded that the most important function of these ammonia-based treatments is that of the protection of moist straw against molding. In the present work liquid urea was found to be the most practical and economical means for achieving the best net yield (ALASPÄÄ 1984).

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SELOSTUS

Ammoniakki-, urea- tai urea + ureafosfaattiseoskäsittelyn vaikutus oljen maittavuuteen tai kasvuun ayrshire-sonneilla

MARJA ALASPÄÄ

Maatalouden tutkimuskeskus

Kauran olki käsiteltiin ammoniakilla (A), nestemäisellä urealla (US), rakeisella urealla (UG), nestemäisellä urea + ureafosfaatti (3 + 1) seoksella (UUFS) tai rakeisella urea + ureafosfaatti (3 + 1) seoksella (UUFG). Annostelun tavoitteena oli lisätä 2 kg typpeä kuiva-ainetonnille, ja typpipitoisuuksien perusteella säilöntäaineiden annostelutavoitteet kuiva-ainetonnia kohden laskettuna olivat seuraavat: ammoniakki 35 kg, urea 54 kg ja urea + urefosfaatti (3 + 1) seos 63 kg. Kontrollina oli pellolla kuivattu käsittelemätön olki (C). Kaikki käsitellyt oljet varastoitiin muovilla peitetyissä aumoissa. Ruokintakoe suoritettiin ayrshiresonneilla, jotka olivat 139—345 päivän ikäisiä. Sulavuus määritettiin pässeillä kokonaiskeruu-menetelmällä.

Säilönnän aikana tpahtuneet säilöntäaineen tappiot olivat suuremmat rakeisilla menetelmillä kuin nestemäisillä. Oljen laatu oli kaikilla säilönnöillä hyvä talven ja kevään aikana, mutta kesällä kaikki käsitellyt oljet alkoivat homehtua.

Mikään käsittelyistä ei lisännyt merkitsevästi keskimääräistä päivittäistä lisäkasvua, teuraspainoa eikä oljen maittavuutta. Orgaanisen aineen sulavuuden paraneminen ei myöskään ollut tilastollisesti merkitsevä.

Tämän tutkimuksen perusteella voidaan päätellä, että näillä ammoniakkipohjaisilla säilöntäaineilla on suurempi merkitys kostean oljen suojaamisessa homeita vastaan kuin rehuarvon parantajana. Nestemäinen urea osoittautui käytännöllisimmäksi ja taloudellisimmaksi.

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Seria ANIMALIA DOMESTICA N. 74 — Sarja KOTIELÄIMET n:o 74

EFFECT OF TREATMENT WITH UREA OR A UREA + UREAPHOSPHATE MIXTURE ON THE NUTRITIVE VALUE OF WHOLE CROP SILAGE

MARJA ALASPÄÄ

ALASPÄÄ, M. 1986. **Effect of treatment with urea or a urea + ureaphosphate mixture on the nutritive value of whole crop silage.** Ann. Agric. Fenn. 25: 99—103. (Agric. Res. Centre., Dept. Anim. Husb., SF-31600 Jokioinen, Finland.)

Whole crop silage was made from barley treated with urea solution (US), urea granules (UG), a urea + ureaphosphate(3 + 1) mixture solution (UUFS) or a urea + ureaphosphate mixture granules (UUFG). The barley was cut at the yellow or dough stage of maturity. The aim of application was to add 25 kg nitrogen per ton of dry matter of raw material. The prepared silages were tested in the experiment, which was carried out with Ayrshire bulls aged between 115 and 412 days. Silages were fed *ad libitum* and ration of barley meal was fixed, 3 kg per day.

Differences between urea or urea + ureaphosphate treatments in the average daily liveweight gains or carcass weights were insignificant. The dry matter intakes of silages were: US 3,45 kg, UG 3,14, UUFS 3,13, and UUFG 3,44 respectively. Intakes of US and UUFG treated silages were significantly higher than those of the other two treatments. Digestibility was determined with wethers. In the digestibility of organic matter there were no significant differences between treatments or maturity stages.

Index words: whole crop silage, urea treatment, urea + ureaphosphre treatment.

INTRODUCTION

Various additives have been tested earlier in the preparation of silage from whole cereal crop (KOMMERI and KONTTURI 1981). In the treatment of gaseous ammonia there have been problems caused by the spreading of ammonia in moist and dense material. The aim of the present work was to compare treatments of whole crop silage with urea or a urea + ureaphosphate mixture and to examine the effect of these treatments on the digestibility and nutritive value of whole crop silage.

MATERIAL AND METHODS

Preparation of silages

Barley was used in the experiment. The aim was to prepare the silage at the yellow ripening stage, but because of the late seeding of some fields, about 40 % of the silage was prepared at dough stage.

The additive compared was fertilizer grade urea and a urea + ureaphosphate mixture applied

both in granular and solution forms. The urea + ureaphosphate mixture consisted of 75 % urea and 25 % ureaphosphate. Solutions were prepared as follows: urea was dissolved in water (temperature + 60 °C) in a ratio of 1:1,2 and urea + ureaphosphate mixture in a ratio of 1:1.

The aim of the dosage was to add 25 kg nitrogen per ton of dry matter. On the basis of nitrogen content the amounts per ton of dry matter were 54 kg urea (46,7 % N) and 63 kg urea + ureaphosphate mixture (39,4 % N). Silages were made during August 17.—19. 1981. Barley was chopped with a precision-chop forage harvester and silages were stored in bunker silos. Preservatives were added manually when filling the silos. The chemical composition of the raw material is shown in Table 1.

Table 1. Chemical composition of whole crop silage during cutting.

	Stage of maturity	
	dough	yellow
Dry matter, %	31,22	33,43
In dry matter, %		
— ash	7,14	6,92
— crude protein	10,25	10,15
— ether extracts	2,28	1,85
— crude fiber	26,33	25,79

Feeding experiment

The feeding experiment was carried out from October 6, 1981 to June 20, 1981 with 45 Ayrshire bulls, whose average age was 155 days at the start of the experiment. The animals were divided into four groups according to liveweight and age, and the groups assigned to the four different silage diets. The animals were fed individually. Silages were fed *ad libitum* but the intake was measured. Three kilograms of barley meal was offered daily to all animals. A mineral and trace-elements mixture and fat soluble vitamins were given according to estimated requirements. Animals receiving urea + ureaphosphate treated silage were supplemented daily with 100 g calcium carbonate. Water was freely available.

The animals were weighed at the beginning of the experiment and thereafter every second week.

Analyses of feeds

Standard laboratory routins were used in performing the conventional feed analyses. Dry matter content was determined by oven drying: first by 12 hours at + 60 °C and then by 8 hours at + 105 °C. Volatile fatty acid content was determined by gaschromatography (HUIDA 1973). Total and water soluble nitrogen were determined from fresh samples using the Kjeldahl method, and ammonium nitrogen was colorimetrically determined (MCGULLOUGH 1967).

Digestibility was determined with wethers in a 4 × 4 latin square.

Statistical analyses

The experimental data, except those of the digestibility trial, were analysed by one way analyse of variance. The differences between treatments were tested using Tukey's test (STEEL and TORRIE 1960).

RESULTS AND DISCUSSION

Chemical composition and quality of silages

All treatments markedly increased the crude protein content of whole crop silage made from barley (Table 2). The differences between treatments were insignificant ($p > 0,05$).

Energy content is expressed as fattening feed units (f.f.u.), which correspond to Kellner's starch equivalent in the ratio of 1 f.f.u. = 0,7 kg starch equivalent. The f.f.u. values of these whole crop silages can be compared to that of average quality grass silage.

Though the fiber content was on average a bit lower in the dough stage of maturity of barley, there were no significant differences in the digestibilities of organic matter between treatments or maturity stages ($p < 0,05$).

With regard to mold growth the quality of all silages was good during the winter and spring. At the end of experiment in June, silages in all silos began to warm up and became moldy obviously due to losses of ammonia and the penetration of air into the silos during the feeding period.

Butyric acid content was high in all treated silages.

Feeding experiment

The daily average liveweight gain of the animals was about one kilogram and the carcass percentage from 52—53 % (Table 3). Differences between treatments were statistically insignificant ($p > 0,05$). These results are in agreement with earlier studies (KOMMERI and KONTTURI 1981).

Silages made up on average 53—56 % of the total DM intake (Table 4). Most palatable were the silages treated with US and UUFG and the difference between the two other additives was

Table 2. Average composition of feeds during the feeding experiment. Whole crop silage is described at dough and yellow ripening stage. Treatments: US = urea solution, UG = urea granules, UUFS = urea + ureaphosphate (3 + 1) mixture solution, UUFG = urea + ureaphosphate (3 + 1) mixture granules.

	Silages										
	Dough ripening stage					Yellow ripening stage					
	US	UG	UUFS	UUFG		US	UG	UUFS	UUFG		barley
Dry matter, %	28,65	27,55	28,80	29,56		32,00	31,61	30,87	32,62		87,01
In dry matter, %											
— ash	8,01	8,43	8,42	8,80	N.S.	7,53	7,80	7,95	8,13	N.S.	2,69
— org. matter	91,99	91,57	91,58	91,20	N.S.	92,47	92,20	92,05	91,87	N.S.	97,31
— crude protein (6,25 × N)	29,55	30,16	29,46	28,26	N.S.	30,22	30,86	30,87	28,17	N.S.	12,61
— crude fat	3,26	3,84	3,55	2,93	N.S.	2,53	2,49	2,52	2,31	N.S.	2,38
— crude fiber	27,41	27,46	26,76	27,49	N.S.	26,90	27,66	27,41	26,92	N.S.	5,23
— nfe	31,77	30,11	31,81	32,52	N.S.	32,82	31,20	31,25	34,47	N.S.	77,09
pH	7,63	7,09	7,55	7,90		7,63	7,52	7,48	7,69		
Vol. fatty acids, % in DM											
— asetic acid	$3,71^{ab}$	$4,44^{b}$	$3,68^{ab}$	$2,69^{a}$		4,38	4,58	4,10	3,43	N.S.	
— propionic acid	0,36	0,53	0,39	0,30	N.S.	0,44	0,42	0,38	0,29	N.S.	
— butyric acid	2,65	3,48	2,82	2,06	N.S.	$3,49^{b}$	$3,09^{ab}$	$2,77^{ab}$	$1,83^{a}$		
— valerianic acid	0,08	0,12	0,07	0,06	N.S.	0,18	0,13	0,14	0,10	N.S.	
— isovalerianic acid	0,12	0,11	0,07	0,05	N.S.	0,17	0,17	0,16	0,14	N.S.	
Nitrogen, % in DM											
— total	4,44	4,44	4,41	4,29	N.S.	4,46	4,56	4,60	4,27	N.S.	
— soluble	3,73	3,81	3,58	3,62	N.S.	3,75	3,83	3,79	3,50	N.S.	
— ammonium	2,74	2,85	2,69	2,51	N.S.	2,86	2,71	2,85	2,48	N.S.	
Digestibilities, %											
— org. matter	66,68	64,68	68,41	67,40		67,71	65,37	66,40	67,92	N.S.	
— crude fiber	56,46	58,16	59,79	58,84		48,74	49,34	55,27	54,23	N.S.	
Feed value											
— kg DM per f.f.u.	1,45	1,49	1,41	1,45		1,47	1,49	1,47	1,46		

N.S. = differences between means are insignificant ($p > 0,05$).
Means in the same line not having the same superscript letter differ significantly ($p < 0,05$).

Table 3. Initial and final liveweights and carcass weights of Ayr bulls. Treatments are shown in Table 2.

	Treatments 1	2	3	4	
Age, at the beginning	155	155	155	157	
— at the end	412	412	412	414	
Liveweight					
— at the beginning	151,0	147,2	145,5	150,0	
— at the end	427,5	405,8	412,0	419,7	
Carcass weight, kg	222,6	214,3	215,8	218,3	N.S.
Carcass percentage	52,1	52,7	52,3	52,0	
Carcass quality class	I	1+	I	I	
Daily gain, kg	1,03	0,95	0,94	0,99	N.S.

N.S. = differences between means are insignificant ($p > 0,05$).

Table 4. Average feed intake of Ayr bulls. Treatments are shown in Table 2.

	Treatments US	UG	UUFS	UUFG	
Kg dry matter per day					
— total	$6,16^{a}$	$5,83^{b}$	$5,87^{b}$	$6,11^{a}$	
— silage	$3,45^{a}$	$3,14^{b}$	$3,13^{b}$	$3,44^{a}$	
— barley	2,57	2,55	2,56	2,56	N.S.
— mineral mixture	0,14	0,14	0,18	0,18	
Crude protein, g per day					
— total	1329^{a}	1264^{b}	1253^{b}	1281^{b}	
— silage	1017^{a}	953^{b}	914^{b}	970^{b}	
— barley	313	312	313	313	N.S.
F.f.u (feed unit) per day					
— total	$5,13^{ac}$	$4,97^{ad}$	$5,02^{ad}$	$5,24^{b}$	
— silage	$2,25^{ac}$	$2,11^{ad}$	$2,14^{ad}$	$2,37^{b}$	
— barley	2,88	2,86	2,88	2,87	N.S.

N.S. = differences between means are insignificant ($p > 0,05$). Means in the same line not having the same superscript letter differ significantly ($p < 0,05$).

statistically significant ($p < 0,05$). The main part of the calculated energy intake came from barley in all groups.

It seems that for bulls the feed value of whole crop silage made from barley treated with urea or a urea + ureaphosphate mixture corresponds that of average quality grass silage. Due to the high content of butyric acid, whole crop silages treated with urea-based additives cannot be recommended for dairy cows. One of the greatest disadvantages of ammonia-based additives is the easy escape of ammonia from the silo during feeding resulting in the loss of nitrogen and mold growth in moist silage.

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SELOSTUS

Urea- tai urea + ureafosfaattiseoskäsittelyn vaikutus kokoviljasäiliörehun rehuarvoon

MARJA ALASPÄÄ

Maatalouden tutkimuskeskus

Kokoviljasäilörehu valmistettiin ohrasta kelasilppurilla ja säilöttiin laakasiiloihin. Kasvuasteeltaan ohra oli kelta- tai taikinatuleentumisasteella. Säilöntäaineena käytettiin nestemäistä ureaa (US), rakeista ureaa (UG), nestemäistä urea + ureafosfaatti (3 + 1)-seosta (UUFS), tai rakeista urea + ureafosfaatti (3 + 1)-seosta (UUFG).

Säilöntäaineen annostuksen tavoitteena oli lisätä 25 kg typpeä kuiva-ainetonnille, jolloin urean määrä kuiva-ainetonnille oli 54 kg ja urea + ureafosfaatti (3 + 1)-seoksen 63 kg. Ruokintakoe suoritettiin Ayrshire sonneilla. Eläimet saivat ryhmittäin kokoviljasäilörehua vapaasti ja 3 kg ilmakuivaa ohraa päivässä.

Ruokintakokeessa ei päivittäisessä lisäkasvussa eikä teuraspainoissa saatu merkitseviä eroja kokoviljasäilörehu-ryhmien välille. Kokoviljasäilörehun syönti oli ryhmittäin US 3,45, UG 3,14, UUFS 3,13 ja UUFG 3,44 kg KA päivässä. Kuiva-aineen syönti oli US ja UUFG säilötyllä kokoviljasäilörehuilla merkitsevästi suurempi kuin kahdella muulla. Sulavuus määritettiin pässeillä kokonaiskeruu-menetelmällä. Orgaanisen aineen sulavuudessa ei ollut merkitseviä eroja käsittelyjen eikä kasvuasteiden välillä.

Näitä ammoniakkipohjaisilla aineilla säilöttyjä kokoviljoja voidaan rehuarvoltaan verrata keskilaatuiseen ruohosäilörehuun. Lypsylehmille ei kuitenkaan voida suositella näitä ammoniakkipohjaisilla aineilla säilöttyjä kokoviljasäilörehuja, koska kaikki rehut sisälsivät runsaasti voihappoa.

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Seria ANIMALIA DOMESTICA N. 75 — Sarja KOTIELÄIMET n:o 75

COMPARISON OF TWO ANTICOCCIDIALS, CYGRO AND ELANCOBAN, IN BROILER DIETS

TUOMO KIISKINEN and PER ANDERSSON

KIISKINEN, T. & ANDERSSON, P. 1986. **Comparison of two anticoccidials, Cygro and Elancoban, in broiler diets.** Ann. Agric. Fenn. 25: 105—109. (Agric. Res. Centre, Dept. Anim. Husb., SF-31600 Jokioinen, Finland.)

An experiment consisting of 1950 broiler chicks was conducted to investigate and compare the effects of two anticoccidials Cygro (prinicin ammonium) and Elancoban (monensin). Cygro was used at a recommended level of 5 ppm and Elancoban at a level of 100 ppm. Contaminated litter was used to produce infection with *Eimeria*.

Each anticoccidial increased the body weight and improved the feed efficiency ($P>0,05$). The mean values of the feed conversion ratio were 2,01 (control), 1,90 (Cygro) and 1,87 (Elancoban). The higher ratio of males to females in the Elancoban group apparently favoured it in the comparison of the feed conversion ratio. No differences were ascertained in the mortality rate or occurrences with leg problems. The patological and parasitological investigation showed that each anticoccidial gave a good protection against coccidiosis, and that Cygro seemed to be at least as effective as Elancoban.

Index words: anticoccidials, prinicin ammonium, monensin, coccidiosis, broiler chick.

INTRODUCTION

Cygro (prinicin ammonium) is a new polyether ionophore anticoccidial that is produced via aerobic fermentation by a strain of *Actinomadura yumaensis*. It has been demonstrated to be effective against all six pathogen species of *Eimeria*, and the optimal treatment level has been shown to be 5 ppm (KANTOR and SCHENKEL 1984, KANTOR et al. 1984). The performance of the birds in the above mentioned studies was comparable with the results of 100—120 ppm of monensin.

Monensin or Elancoban has been used in Finland for ten years. Because of this duration, an alternative anticoccidial should be found, thus preventing the possibility of the birds developing a resistance. Therefore Cygro was tested as a possible alternative to Elancoban. This cooperative study was performed at the Department of Animal Husbandry of the Agricultural Research Centre and at the National Veterinary Institute.

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MATERIAL AND METHODS

Day-old, sexed broiler chicks (Pilch) were placed into 15 different floor pens (6 m^2) so that each pen contained 65 males and 65 females. The average initial weight was 36 g. Five pens were allotted for each dietary treatment. The housing conditions were maintained at the levels used in the broiler house of the Agricultural Research Centre (KIISKINEN 1983). The chicks were fed and watered *ad libitum*. Wood shavings were used as litter. Contaminated litter was mixed with the litter of each pen to produce natural infection with *Eimeria*.

The basic composition of the experimental diets was the same as in the commercial broiler feeds manufactured by Keskusosuusliike Hankkija. The starter which was used up to 1,5 weeks of age in this experiment contained according to the declaration: fish meal 9,5 %, soybean meal 11 %, plant oil 4 %, grain 66 %, corn gluten 4,5 %, Pekilo single cell product 2 %, minerals and vitamins. The finisher contained fish meal 6 %, soybean meal 12 %, plant oil 5 %, grain 69 %, corn gluten 4,5 %, minerals and vitamins. The declared protein and ME contents of the starter and finisher were 22 %, 12,8 MJ/kg and 20 %, 12,9 MJ/kg, respectively.

The dietary treatments:

Group 1. Basic feed, Avotan 10 ppm
,, 2. ,, ,, , ,, ,, , Cygro 5 ppm
,, 3. ,, ,, , ,, ,, , Elancoban 100 ppm

The individual weights of the birds at the age of 11 and 40 days were measured. The feed consumption was recorded for each pen between the weighings. In the slaughter house the total weight of the carcasses from each pen was measured. Mortality and non-classified leg weakness was recorded from each pen. The quality (wetness) of litter was controlled by visual survey and dry matter determination. The samples were taken at a distance of 20 cm from the watertrough.

After the first week all dead birds were sent to the National Veterinary Institute, for pathological and parasitological investigations. In the slaughter house, intestines of twenty birds from each pen were placed into plastic bags, refrigerated overnight, and then carried to the National Veterinary Institute for testing.

The proximate analysis for each diet was done at the Department of Animal Husbandry. The control of the concentrations of Avotan and the anticoccidials was arranged by the American Cyanamid Company.

The performance of birds (growth, feed intake, mortality) was statistically evaluated by using an analysis of variance. The differences between the treatment means were tested by the Tukey's test (STEEL and TORRIE 1960).

RESULTS AND DISCUSSION

According to the proximate analysis, the chemical composition of the diets was so close that the effect of this factor on the differences in the performance of the groups could be excluded (Table 1). The content of Cygro (4—4,5 ppm) was lower than the aim of 5 ppm, but it apparently did not affect the results.

The sexing of broiler chicks is more difficult than that of layer chicks. Therefore the result differed from the goal (50:50). In the control and Cygro groups the number of males was around 40 % and in the Elancoban group 46 % (Table 2). The difference in the ratio of the sexes slightly favored the Elancoban group in feed efficiency.

The average live weights of the groups at the age of 11 days were very uniform although the average weight of male chicks of the Cygro group

Table 1. Proximate analysis of the broiler diets and the analyzed contents of Avotan and the anticoccidials.

	Dry matter %	Crude protein %	Ether extract %	Crude fibre %	Ash %	Anticoccidial ppm	Avotan ppm
Starters							
Control	89,7	21,7	5,7	3,4	5,3	—	11,1
Cygro	89,6	22,6	5,6	3,7	5,3	4,0	10,1
Elancoban	89,7	21,7	5,7	3,5	5,2	99	12,0
Finishers							
Control	90,4	19,2	6,7	3,6	5,5		10,4
Cygro	89,8	19,7	6,7	3,7	4,9	4,5	10,2
Elancoban	90,2	19,6	6,8	3,7	5,1	97	11,5

Table 2. Performance of broiler chicks fed anticoccidials Cygro and Elanboban.

	Control	Cygro	Elancoban	SE
Avotan ppm	10	10	10	
Anticoccidial ppm	—	5	100	
Live weight:				
Male chicks				
n	249	245	282	
11 days g	247[a]	254[b]	248[a]	0,8
40 days g	1769	1791	1789	6,5
Relative	100	101	101	
Female chicks				
n	366	379	335	
11 days g	222	226	223	0,9
40 days g	1474[a]	1491[ab]	1510[b]	4,8
Relative	100	101	102	
Carcass weight:				
Males + females g	970[a]	992[ab]	1004[b]	5,1
Relative	100	102	103	
Mortality 1,5—6 weeks %	5,0	3,7	4,2	0,57
Leg problems %	3,4	3,8	4,3	0,40
Feed intake:				
g/day 0—11 days	23,7	24,4	23,4	0,30
,, 12—40 ,,	93,3[a]	90,6[b]	90,3[b]	0,52
kg/kg weightgain 0—40 days	2,01[a]	1,91[b]	1,87[b]	0,016
Relative	100	95,5	93,5	
kg/kg carcass weight	3,22[a]	3,04[b]	2,98[c]	0,028
Relative	100	94,5	92,5	

a-c $P<0,05$ Values with different superscript letters are significantly different. If no letters are used the differences are non-significant.
1) SE = standard error of mean.

was significantly ($P<0,05$) higher than that of the other groups. (Table 2). When compared to the control group each of the anticoccidial groups had higher final body weights. The increase was 1—2 % and was significant ($P<0,05$) only in the case of the female chicks of the Elancoban group. A significant interaction was not found between the use of an anticoccidial and the sex. KANTOR et al. (1984) reported an increase in weight of 0,5—4 % for Cygro (CL 259,971) and of 0—5 % for Elancoban (monensin). The slaughter weight increased by 2—3 % as a result of supplementing with the anticoccidials, and the difference between the Elancoban group and the control group was significant ($P<0,05$) as shown in Table 2.

There was no significant differences in mortality or in incidence of leg problems between the treatments.

As regards to the feed intake the chicks of the anticoccidial groups consumed during the last phase (11—40 days) around 3 g less feed daily per bird than the control group ($P<0,05$). Cygro improved feed efficiency on an average of 5 % ($P<0,05$) and Elancoban 7 % ($P<0,05$). Improvement of feed efficiency was 1—3 % for Gygro and 2—5 % for Elancoban in the study of KANTOR et al. (1984). No differences were observed in the wetness of litter between the treatments. This was also confirmed by the results of the dry matter determinations: 37,3 %, 34,7 % and 36,3 % for the control, Cygro and Elancoban, respectively.

According to the morphological determination the contamination included *Eimeria tenella* and at least two intestinal coccidia. The results show that the contamination was strong (Table 3). The most pathogen was *E. tenella.* Each anticoccidial gave a good protection against coccidiosis and the anticoccials Cygro and Elancoban seemed to be equal in this respect.

A total of 45 dead birds were submitted to post mortem examination and the counts of coccidia are presented in Table 4. The result is similar as in the case of the slaughtered birds. The actual cause of death was usually trauma.

Overall, it can be concluced that Cygro was an effective anticoccidial and its potency is equal to that of Elancoban. Each anticoccidial improved the performance of the broiler chick, and has especially improved the feed efficiency which will be economically important.

Table 3. Anticoccidial activity of Cygro and Elancoban

	Cage number	Number of samples	Duodenum			Jejunum			Caecum		
			+++	++	+	+++	++	+	+++	++	+
Control	1	20	1	1	10	—	—	14	—	12	7
	4	20	—	—	1	—	—	5	—	5	9
	7	19	—	—	1	—	—	8	—	10	6
	10	20	—	—	1	—	—	6	—	5	7
	13	19	—	2	4	—	—	7	—	10	3
			1	3	18	—	—	40	1	42	32
Cygro	2	20	—	—	—	—	—	—	—	—	1
	5	20	—	—	—	—	—	—	—	—	1
	8	20	—	—	—	—	—	—	—	—	1
	11	19	—	—	—	—	—	—	—	—	—
	14	19	—	—	—	—	—	—	—	—	—
			—	—	—	—	—	—	—	—	3
Elancoban	3	21	—	—	—	—	—	—	—	—	4
	6	19	—	—	—	—	—	—	—	—	3
	9	21	—	—	—	—	—	—	—	—	1
	12	20	—	—	—	—	—	—	—	—	4
	15	20	—	—	—	—	—	—	—	—	—
			—	—	—	—	—	—	—	—	12

+++ appearance of oocysts and schizonts, macroscopic tissue lesions
++ moderate appearance of oocysts and schizonts without tissue lesions
+ less than ten oocysts or schizonts

Table 4. Appearance of coccidia in dead birds examined post mortem.

	Duodenum			Jejunum			Caecum		
	+++	++	+	+++	++	+	+++	++	+
Control	—	1	—	—	3	—	1	1	7
Cygro	—	—	—	—	—	—	—	—	2
Elancoban	—	—	—	—	—	—	—	—	1

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SELOSTUS

Kahden kokkidiostaatin, Cygron ja Elancobanin, vertailu broilereilla

TUOMO KIISKINEN ja PER ANDERSSON

Maatalouden tutkimuskeskus ja Valtion eläinlääketieteellinen laitos

Cygro (prinicin ammonium) on uusi kokkidiostaatti, jonka ilmoitetaan tehoavan kaikkiin kuuteen patogeeniseen *Eimeria*-lajiin. Elancobania (monensin) on meillä käytetty jo noin 10 vuoden ajan, minkä vuoksi vastustuskyvyn kehittymistä voi ilmetä. Sen vuoksi näiden kokkidiostaattien tehoa tutkittiin ja verrattiin kokeessa, jossa oli 1950 Pilch-broilerpoikasta. Cygroa lisättiin 5 mg ja Elancobania 100 mg rehukiloon, mitkä ovat suositusten mukaiset käyttömäärät. Pehkun joukkoon lisättiin Eimeria-loisten saastuttamaa pehkua.

Kumpikin kokkidiostaatti paransi hieman (1—2 %) eläinten kasvua ja tuntuvammin rehun hyväksikäyttöä. Rehua kului lisäkasvukiloa ja teuraspainokiloa kohden seuraavasti: vertailuryhmä 2,01, 3,22, Cygro-ryhmä 1,91, 3,04, Elancoban-ryhmä 1,87, 2,98 kg. Erot vertailuryhmän ja kokkidiostaattiryhmien välillä olivat tilastollisesti merkitseviä. Elancoban-ryhmän muita suurempi kukkojen osuus ilmeisesti hieman paransi rehuhyötysuhdetta. Kuolleisuudessa ja jalkavikojen esiintymisessä ei todettu merkitseviä eroja.

Patologinen ja parasitologinen tutkimus osoitti kummankin valmisteen antavan hyvän suojan kokkidioosia vastaan ja että Cygro on vähintään yhtä tehokas kuin Elancoban.

ANNALES AGRICULTURAE FENNIAE, VOL. 25: 111—125 (1986)
Seria ANIMALIA DOMESTICA N. 76 — Sarja KOTIELÄIMET n:o 76

EFFECT OF SUPPLEMENTED CONCENTRATES ON THE MILK YIELDS OF COWS GRAZING GOOD PASTURE

ELSI ETTALA, KALLE RINNE, ERKKI VIRTANEN and HEIKKI RISSANEN

ETTALA, E., RINNE, K., VIRTANEN, E. & RISSANEN, H. 1986. **Effect of supplemented concentrates on the milk yields of cows grazing good pasture.** Ann. Agric. Fenn. 25: 111—125. (Agric. Res. Centre, North Savo Res. Sta., SF-71750 Maaninka, Finland.)

The effect of a concentrate supplement of the milk yields of dairy cows grazing good pasture was studied at the Agricultural Research Centre during 1969—1979. The experiments included 480 cows. This publication presents a summary of earlier experiments published separately.

The cows on the comparison treatment grazed only pasture grass while the cows on the concentrate supplement were fed mainly barley in addition to pasture grass. Oats, barley-oats and barley-dried molasses were compared to barley in a separate experiment. The amounts of barley varied from very small to amounts offered *ad libitum* at the milking time.

The 152 cows on the comparison treatment produced by grass-only 19,4 kg per day fat-corrected milk (FCM), on the best pastures 23,1 kg per day during the three-month grazing period.

The least amount of barley was equal to one third of the energy required for FCM production exceeding 10 kg per day. On average 1,3 kg barley per cow per day was fed to 87 cows. These cows produced 1,1 kg per day more FCM than cows on the same pasture without concentrate supplements. The increase in production was 846 g per kg barley. Fed twice that amount of barley on average 2,7 kg per day or 2/3 of their energy requirement exceeding 10 kg per day, the 87 cows produced 1,6 kg per day more FCM than cows in the comparison groups on the same pasture. The increase in production was 593 g per kg barley.

Larger barley rations of 4—6 kg per cow per day or *ad libitum* at milking time increased production less than the above rations. With an oats and barley-oats mixture the increase in production was slightly better than with barley alone, but the results were variable in different years. A mixture of barley-dried molasses was not better than barley as a concentrate supplement.

The grain supplement increased the milk protein content as well as lactose content to some extent but had no effect on milk fat content. The live weights of the cows on the concentrate treatment increased more than those of cows in the comparison groups during the pasture period.

The experiments demonstrate that cows can produce 22—25 kg milk per day without concentrates grazing good pasture and can achieve average daily milk yields of 20 kg during the summer. The poor effect of concentrate supplements was probably due to the fact that grass consumption decreased by nearly the same amount as the energy value of the concentrates.

Index words: dairy cow, pasture, concentrate feeding, grazing.

INTRODUCTION

In Finland, the growth and protein content of pasture grass has improved remarkably due to increased nitrogen fertilization in the 1970's. Thus there was reason to study how much milk cows could produce grazing pasture grass alone and to what extent concentrate supplements could increase production.

This problem has been widely studied in countries where the grazing period is longer. Most of these experiments have shown that concentrate supplement fed to cows grazing good pasture has not yielded significant or profitable increases in milk production (CASTLE et al. 1960, 1964, 1968, GORDON 1976, SHEPHERD 1962, WOOD 1966), or that a notable increase has only been obtained at the peak of lactation (LAIRD and WALKER-LOVE 1962). An explanation might be the eating behaviour of the cows. CASTLE et al. 1968 and HANCOCK 1958 found that consumption of a concentrate decreased grazing time. HANCOCK's 1958 studies reported that the dry matter intake of cows consuming concentrates was greater and the milk yield was higher than that from pasture grass-only. In addition the stocking rate on pasture has had an effect on the results (HANCOCK 1958, CASTLE et al. 1968, 1972, GORDON 1976). SJOLLEMA (1950) concluded that a concentrate with a poor protein content was an important factor for balancing an excessive protein content in grass.

A series of pasture experiments were conducted as a cooperation between the Departments of Animal Husbandry and Plant Husbandry and the North Savo Research Station at the Agricultural Research Centre. This publication includes results of these experiments (ETTALA et al. 1971, ETTALA and RINNE 1982, ETTALA and VIRTANEN 1982, RINNE and ETTALA 1981). The effect of low and moderate grain rations as well as the effect of high rations were studied.

RESULTS

Low and moderate barley rations

Barley was supplemented according to the energy required for milk production. The energy requirement was calculated to be 0,4 f.u. per kg of 4 % fat-corrected milk. The daily barley ration was 1/3 or 2/3 of the energy required for FCM production exceeding 10 kg per day. The barley supplement was given at the morning and evening milking. The cows in the comparison group were fed only grass. Cows had free access to mineral supplement.

1. The effect of barley supplement on the milk yields of cows grazing pastures fertilized by different amounts of nitrogen

By a 3 × 3 factor analytical experiment the balancing effect of a concentrate with a poor protein content and grazing high nitrogen was studied. The experiment included three levels of concentrates and three levels of nitrogen fertilizers. The total number of cows was 108. Nitrogen applications of 100, 200 and 300 kg N per ha were spread in three dressings during the summer and pasture area was decreased as the nitrogen application increased. The ratio was 7:6:5 or 0,49, 0,41 and 0,36 ha per cow per summer.

Each fertilization level included cows without barley supplement and cows supplemented barley according to either the 1/3 or 2/3-level. On the 1/3-level the cows were supplemented 1,2 kg per day and on the 2/3-level they were fed 2,5 kg supplement per day. The grain rations were 11 and 21 % of their energy requirement.

The animals produced 17,2 kg milk per day grazing pasture grass-only and 18,7 kg per day

on both concentrate levels. The increase in milk yield was 1 250 g per kg barley on the lower barley supplement and 600 g per kg barley on the higher ration. Milk protein content and live weight gain increased on the barley treatments (Table 1).

The interaction between pasture grass and barley supplement in milk production was studied by stepwise regression analyses. Other pasture experiments (ETTALA et al. 1971) were also included. The regressors chosen were: the crude protein, crude fibre and nitrogen free extracts contents, the amount of grass dry matter per ha, and the amounts of barley. The three best regressors were the following.

Step 1 Crude fibre of grass of the previous period —8,15**
Step 2 Crude protein of grass of the same period +8,00**
Step 3 Barley supplement +6,14*

The amount of grass per ha had a curvilinear effect on the variations in milk yields. To a certain extent the growth rate of grass increased yields, but an abundant amount of grass evidently contained more fibre and milk production reduced. The results shows that barley was needed mainly as a concentrate supplement to feeding. Because the crude protein content of grass had a positive effect on milk yields it was not excessively high, and therefore barley was not required for balancing the supply of protein and carbohydrates.

Table 1. Effect of barley supplement with grazing on milk yields, Jokioinen 1969—71.

Levels of concentrates	No. of cows	Barley, kg per day	Milk yield, kg per day	4 % fat-corrected milk, kg per day
1969				
0-level	12—9[1)]	—	16,2	16,6
1/3-level	12—9	1,1	18,5	18,6
2/3-level	12—9	2,2	17,4	18,2
1970				
0-level	9	—	18,2	19,1
1/3-level	9	1,3	19,1	19,5
2/-level	9	2,6	19,2	20,2
1971				
0-level	15	—	16,9	17,0
1/3-level	15	1,2	18,4	18,6
2/3-level	15	2,6	19,1	19,3
Mean				
0-level	36—33[1)]	—	17,2	17,6
1/3-level	36—33	1,2	18,7	18,9
2/3-level	36—33	2,5	18,7	19,3

Levels of concentrates	Milk fat, %	Milk protein, %	Change in live weight, kg
1969			
0-level	4,21	3,36	+ 9
1/3-level	4,07	3,35	+ 8
2/3-level	4,35	3,46	+ 11
1970			
0-level	4,40	3,34	+ 9
1/3-level	4,18	3,35	+ 20
2/3-level	4,44	3,48	+ 20
1971			
0-level	4,04	3,40	+ 21
1/3-level	4,11	3,46	+ 31
2/3-level	4,09	3,50	+ 35
Mean			
0-level	4,20	3,37	+ 14
1/3-level	4,12	3,40	+ 22
2/3-level	4,27	3,48	+ 24

[1)] In 1969 three cows were transferred from each treatment in the middle of the experiment because of the weak growth of grass due to prolonged drought.

2. The effect of barley supplement on the milk yields of cows grazing pastures with different stocking rates

The effect of barley supplement on the milk yields of cows with three different stocking rates was studied during 1972—74. The purpose was to determine if it would be possible to save pasture area by using grain supplements. The total number of cows in the experiment was 162 (RINNE and ETTALA 1981, ETTALA and RINNE 1982). The pasture areas and the number of cows per ha were as follows:

1972		1973—74	
ha per cow	cows per ha	ha per cow	cows per ha
0,25	4,0	0,31	3,2
0,30	3,3	0,37	2,7
0,35	2,9	0,43	2,3

The levels of concentrates were the same as those of the previous experiments: 0, 1/3 and 2/3. Each concentrate group was on its own sward. The arrangement of the experiment was 3 × 3 factor analytical where there were three levels of

concentrates and three pasture areas. Each of the nine groups included six cows. The number of pasture paddocks was 36 in 1972 and 45 in 1973—74. The spring crop was ensiled from nine paddocks. Nitrogen application was 200 kg per pasture ha spread in three dressings during the summer.

The cows were high-yielding. During the preparation period the average daily milk yield of the 162 cows was 28—29 kg (Fig. 1—3): at the lower 1/3-concentrate level the cows were offered 1,4 kg barley per day (0,6—2,3 kg) and the higher 2/3-level 2,9 kg per day (1,3—5,2 kg) on average. Milk production was 19,0 kg per day on the grass-only treatment, 20,3 at the 1/3-concentrate level and 20,7 kg at the 2/3-concentrate level as the mean value of the three years (Table 2, Fig. 1).

The increase in milk yield was 642 g and 586 g per kg barley. As 4 % fat-corrected milk the corresponding values were 714 g and 552 g. The mean supply of grass per cow was almost the same at different levels (29—30 kg dry matter per day). When the pasture area per cow increased, the available amount of grass increased significantly ($P<0,001$) (RINNE and ETTALA 1981). Milk production did not increase respectively, but the daily milk yield on the medium size pasture areas was even higher than on the largest areas (Table 3). Statistical calculation was performed by the analysis of variance of the least squares (HARVEY 1966), where it was possible to eliminate differences between animals and years. There was a statistically significant difference ($P<0,01$) between the extreme average yields (19,1, 2,6 and 21,9, Table 3).

The milk yield per ha increased significantly ($P<0,001$) when the area per cow reduced (Table 4). There was a significant difference ($P<0,01$) in milk yields per ha between different concentrate levels only in the third year when the available amount of grass was the least (RINNE and ETTALA 1981). The concentrate level and pasture area did not significantly influence milk production per ha.

Table 2. Effect of barley supplement with grazing on milk yields, Jokioinen 1972—74.

Levels of concentrates	No. of cows	Barley, kg per day	Milk yield, kg per day	4 % fat-corrected milk, kg per day
1972				
0-level	18	—	19,7	19,7
1/3-level	18	1,4	21,5	21,3
2/3-level	18	2,7	20,8	20,7
1973				
0-level	18	—	18,0	18,6
1/3-level	18	1,3	18,5	19,2
2/3-level	18	3,0	20,3	20,7
1974				
0-level	18	—	19,4	19,5
1/3-level	18	1,4	19,9	20,3
2/3-level	18	3,0	21,0	21,4
Mean				
0-level	54	—	19,0	19,3
1/3-level	54	1,4	19,9	20,3
2/3-level	54	2,9	20,7	20,9

Levels of concentrates	Milk fat, %	Milk protein, %	Lactose, %	Change in live weight, kg
1972				
0-level	4,00	3,24	4,47	+ 12
1/3-level	3,93	3,26	4,46	+ 17
2/3-level	3,95	3,36	4,59	+ 17
1973				
0-level	4,25	3,26	4,51	— 1
1/3-level	4,33	3,38	4,58	— 6
2/3-level	4,17	3,42	4,63	+ 19
1974				
0-level	4,05	3,49	4,71	+ 9
1/3-level	4,18	3,57	4,72	+ 11
2/3-level	4,14	3,68	4,82	+ 13
Mean				
0-level	4,10	3,33	4,56	+ 7
1/3-level	4,15	3,40	4,58	+ 7
2/3-level	4,09	3,49	4,68	+ 16

Table 3. Effect of barley supplement on average daily milk yields per cow grazing different pasture areas, Jokioinen 1972—74.

Pasture area ha per cow		4 % fat-corrected milk kg per day 0-level	1/3-level	2/3-level	Mean
1972	1973—74				
0,25	0,31	19,1	20,7	19,3	19,7
0,30	0,37	19,7	20,4	21,9	20,7
0,35	0,43	19,1	19,7	21,6	20,1
Mean		19,3	20,3	20,9	20,2

Table 4. Effect of barley supplement and grazing different pasture areas on milk yields per ha, Jokioinen 1971—74.

Pasture area ha-per cow		4 % fat-corrected milk per day 0-level	1/3-level	2/3-level	Mean
1972	1973—74				
0,25	0,31	6704	7123	6748	6859
0,30	0,37	5760	5975	6357	6031
0,35	0,43	4806	4939	5359	5035

The effect of a concentrate was unexpectedly small considering the very high-yielding cows included in the experiment. Typical of the experiment was that the variation in the yields of the groups offered barley was identical to that of grass-only groups (Fig. 2 and 3). The quality of pasture grass was therefore decisive. This can clearly be seen in 1973 when the fibre content of grass was first high because of the rapid growth of cocksfoot (Fig. 2). When the quality of grass was kept quite steady in 1974, production was also more regular (Fig. 3). The greatest profit from the higher concentrate level was when the pasture area was of medium size in both years. Obviously, grass was then sufficiently available and palatable, so that the cows consumed it willingly in spite of the grain fed at milking time. On the smallest pasture areas weighed additional grass sometimes had to be provided. Most excess grass was left on the largest pasture areas (RINNE and ETTALA 1981). The cows received 11,5 % of their average energy requirement from barley at the lower concentrate level and 23,5 % at the higher level.

The grain supplement had no effect on milk fat content (Table 2). Milk protein and lactose

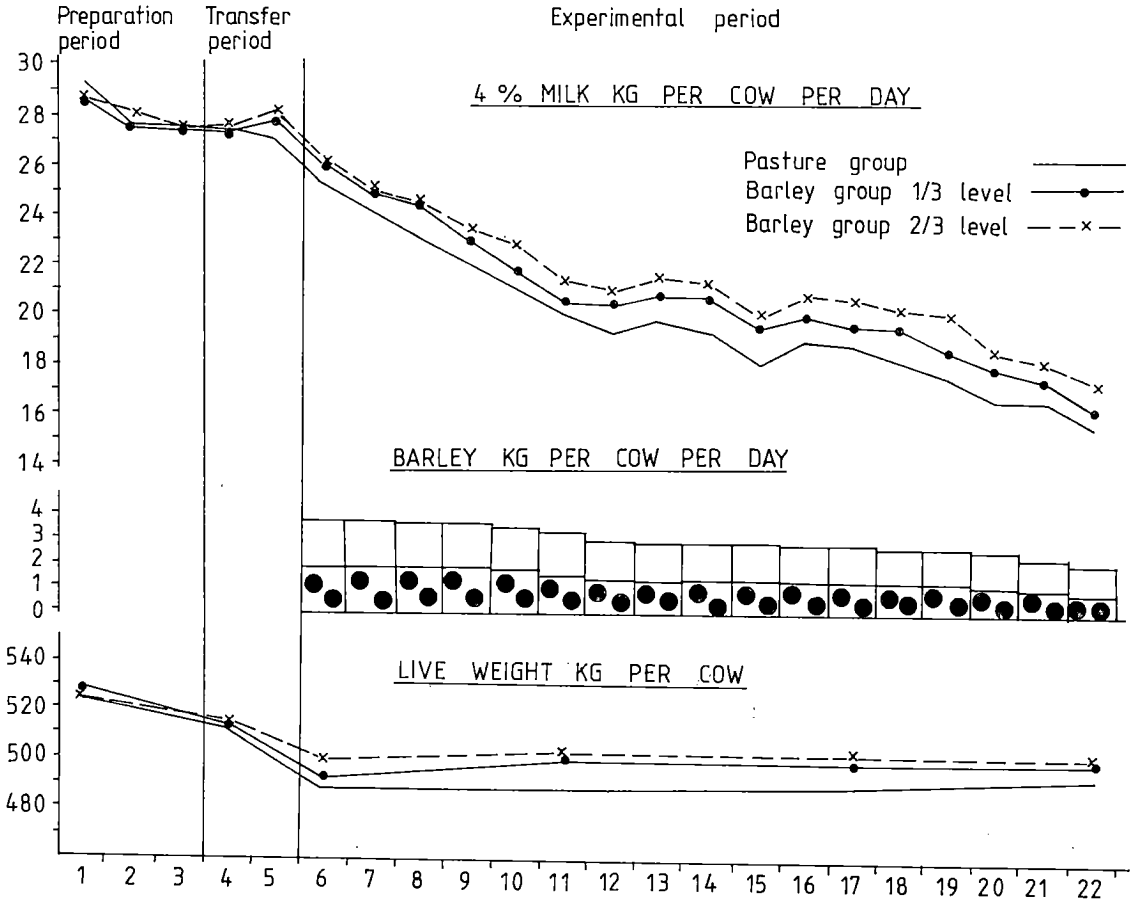

Fig. 1. Change in milk yields and live weights of cows fed grass-only and fed different amounts of barley together with grazing, Jokioinen 1972—74 (162 cows).

content increased with the larger ration of grain. The increase between extreme cases was significant ($P<0,05$, $P<0,01$). The live weight of the cows fed the larger barley supplements increased most (Table 2).

Large barley rations

The purpose of using grain rations was to determine if it would be possible to balance the supply of nutrients by feeding grain when the quantity or the quality of grass was poor. These experiments were conducted at the North Savo Research Station during 1975—79.

In the first two summers barley was offered freely at milking time, during the following two summers 3 kg in the morning and evening, and in the last summer 2 kg in the morning and evening. The comparison group grazed only

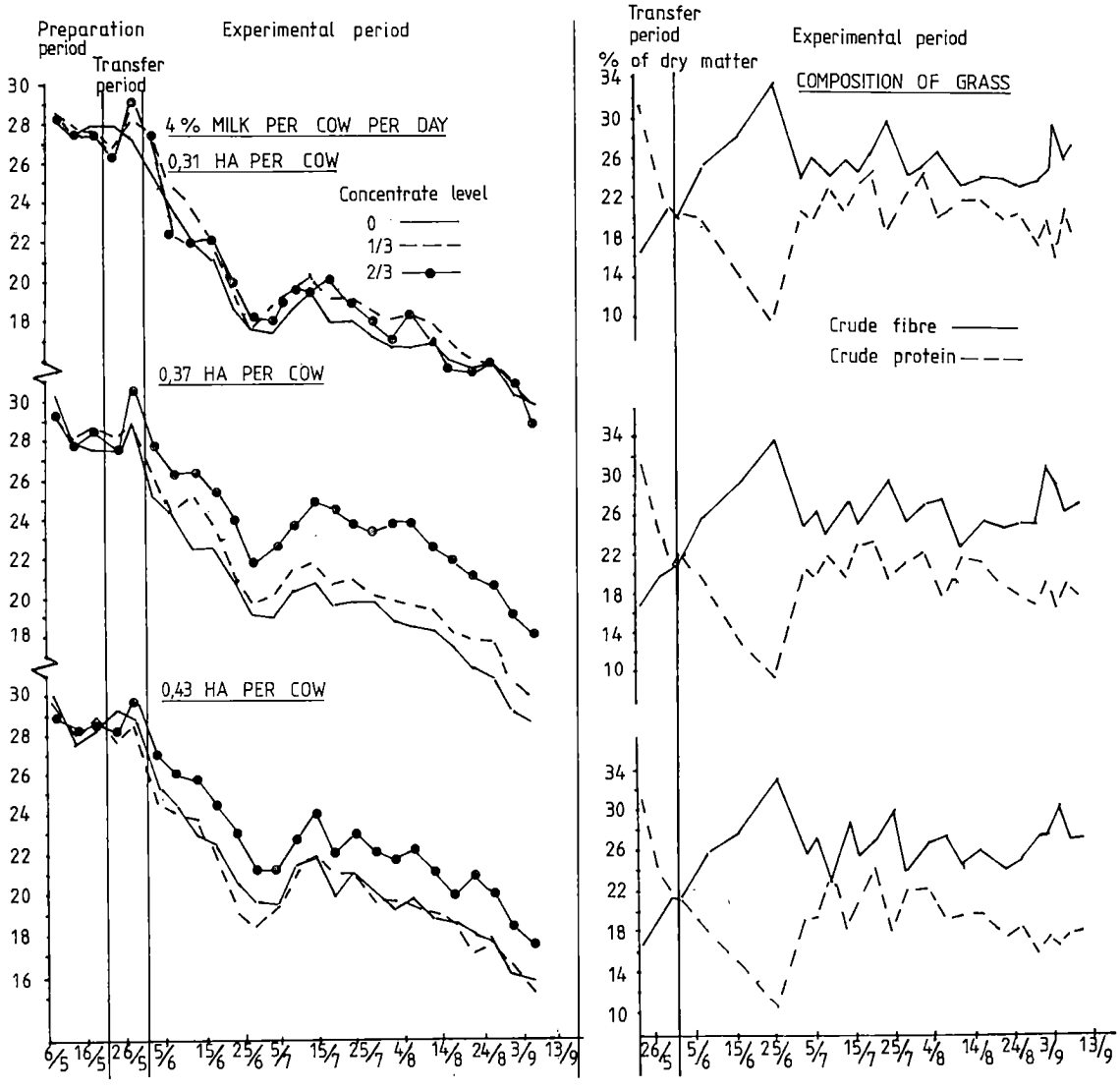

Fig. 2. Milk yields and composition of grazing grass with different pasture areas and feeding different amounts of concentrates, Jokioinen 1973.

pasture grass. Nitrogen application was 100—110 kg per ha for the spring crop and 50—80 kg for the other crops.

In early summer 1975 it seemed that the cows increased the intake of barley when the crude fibre content of grass increased and production was higher than that of the comparison group (Fig. 4). In the autumn the intake of barley continued to increase but production was not very much different from that of the pasture group. Only 800 g per cow per day more milk per day with the consequence of disturbances. The average intake of barley was 5,8 kg per day from which about half of the nutrient requirement of the cows was satisfied. Obviously the (18,9, 19,7) was the average increase during the

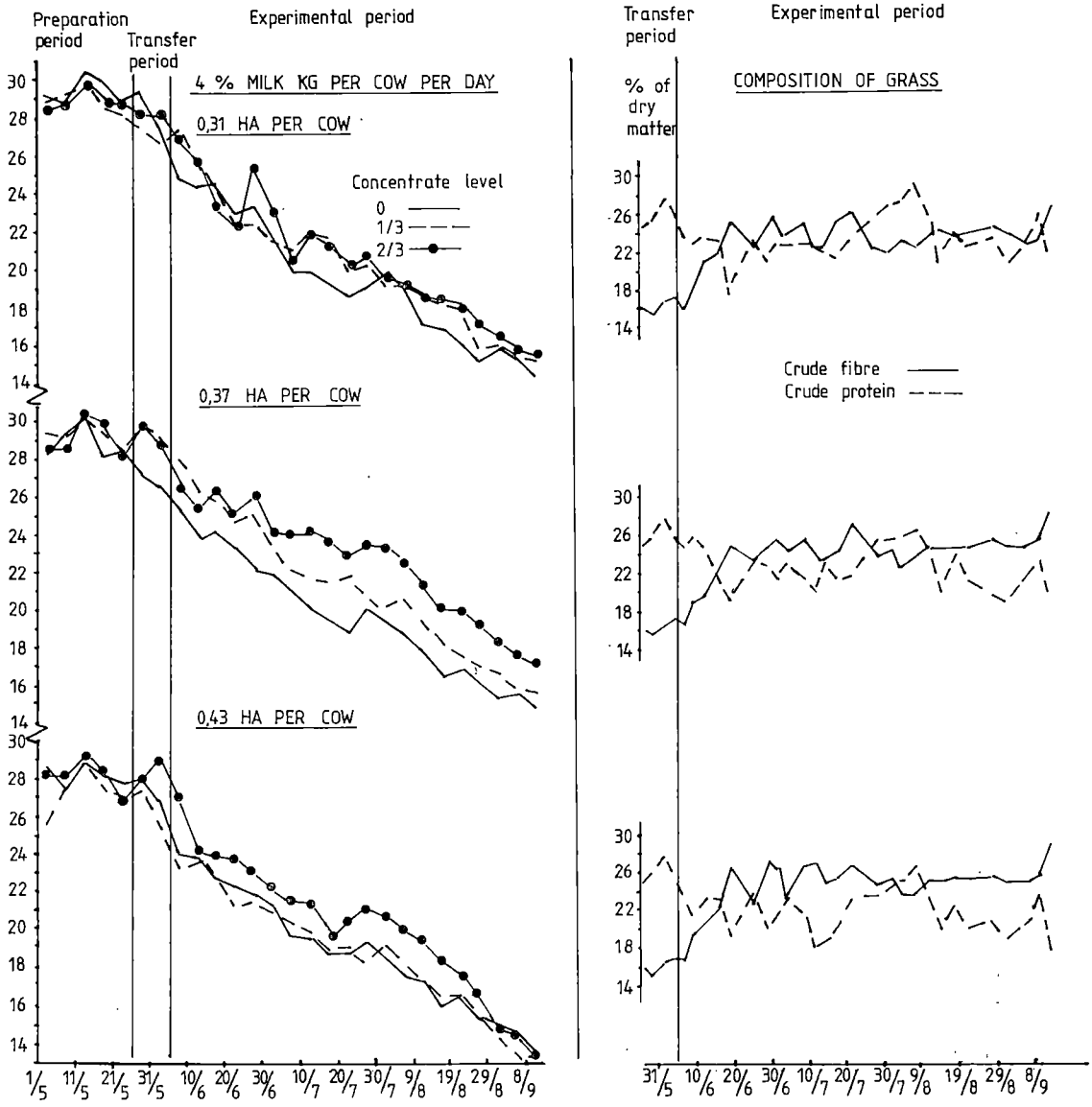

Fig. 3. Milk yields and composition of grazing grass with different pasture areas and feeding different amounts of concentrates, Jokioinen 1974.

whole summer although 5,1 kg barley per day was consumed (Table 5).

In 1976 the intake of barley was not at all logical. Some cows consumed 9—13 kg barley per day with the consequence of disturbances. The average intake of barley was 5,8 kg per day from which about half of the nutrient requirement of the cows was satisfied. Obviously the intake on grass decreased respectively and the supply of protein was thus reduced. In every case the production of the barley group was 800 g per cow per day lower than that of the pasture group (18,5, 17,7) in this experiment (Fig. 5, Table 5). The gain in live weight was equal in both groups.

When the barley ration was restricted to three

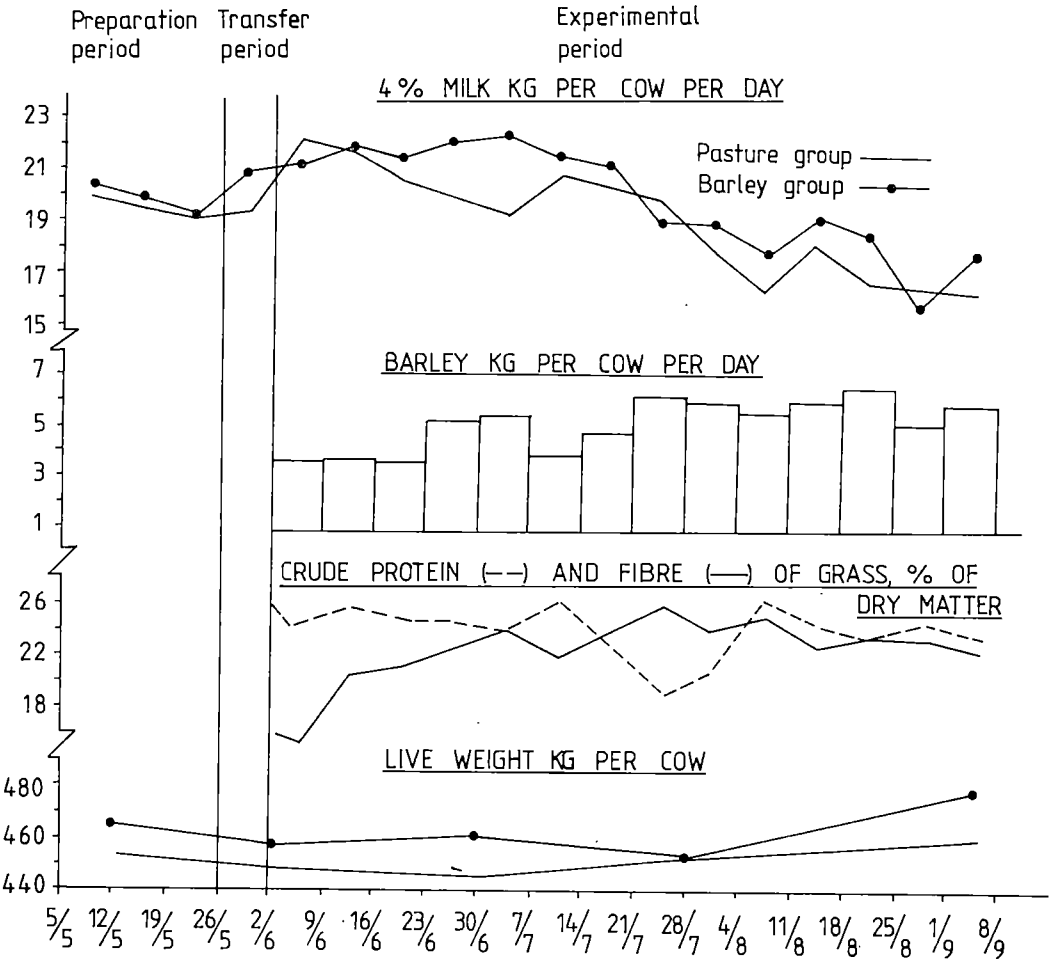

Fig. 4. Change in milk yields and live weights of cows fed grass-only and fed *ad libitum* barley at milking time together with grazing, North Savo Research Station 1975.

kg in the morning and evening consumption averaged 4,7—4,8 kg barley per day (Table 5). Thus the increase in daily milk yield was 1,2—1,3 kg with the barley supplement (19,6, 20,8 kg and 22,5, 23,8 kg) (Table 5, Figs. 6 and 7). However, this increase amounted to only 225 g and 270 g per kg barley. When the morning and evening barley ration was 2 kg the increase in milk yield was again 1,2 kg per day (19,2, 20,4 kg) and 300 g per kg barley (Table 5, Fig. 8).

The production level was essentially dependent on grass quality. In 1978 especially pasture grass was rich in nutrients. Its average crude protein content was 22,7 % and average crude fibre content was 20,7 % of dry matter. With pasture

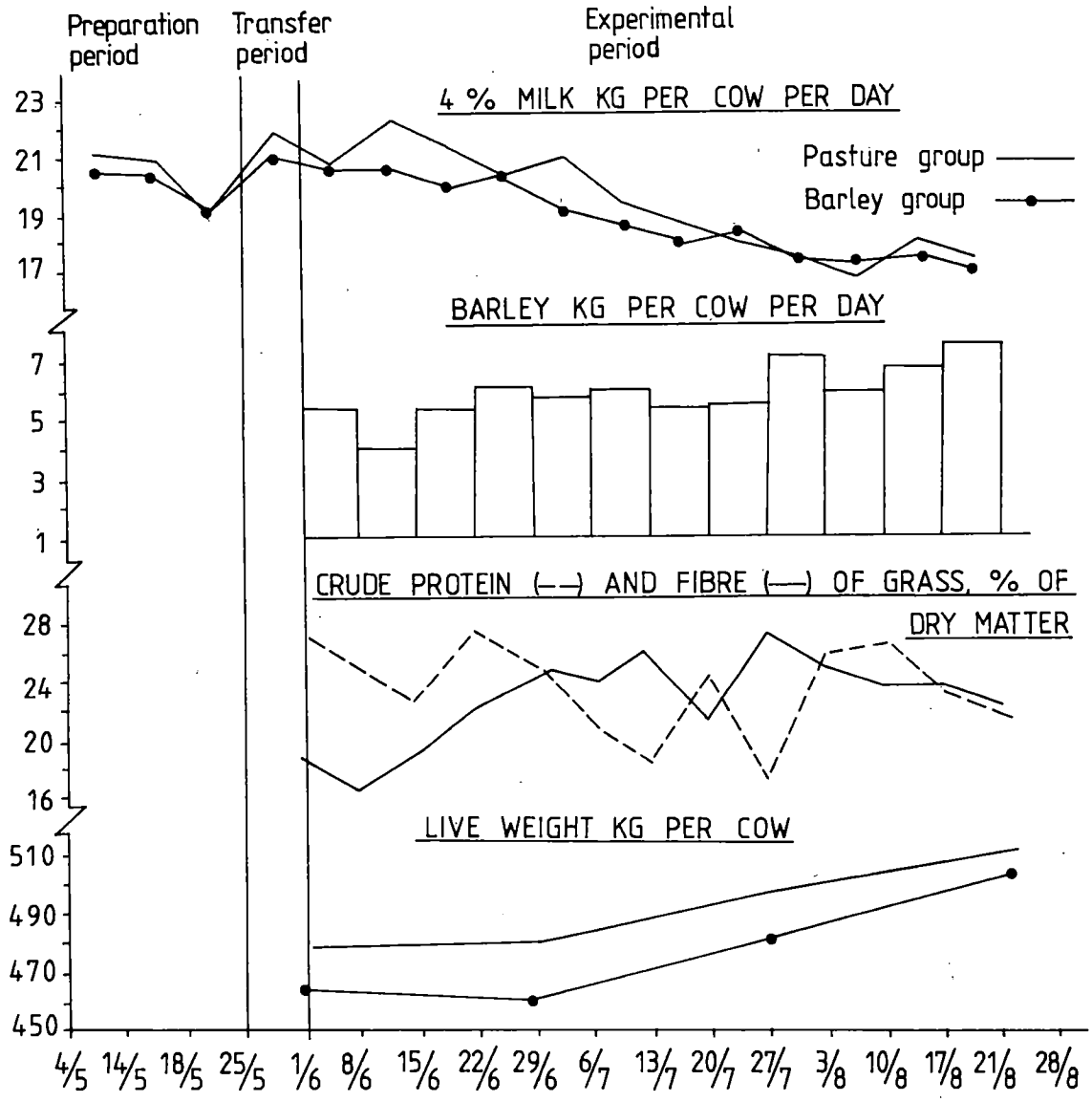

Fig. 5. Change in milk yields and live weights of cows fed grass-only and fed *ad libitum* barley at milking time together with grazing, North Savo Research Station 1976.

Table 5. Effect of high amounts of barley supplement and grazing good pasture on milk yields, North Savo Research Station 1975—1977.

	Number of cows	days	kg per cow per day barley	milk	4 % milk	Milk fat %	Milk protein %	Milk lactose %	Change in live weight kg
1975									
pasture group	7	98	—	18,9	19,3	4,24	3,35	4,78	+ 12
barley group	7	98	5,1	19,7	20,2	4,21	3,49	4,83	+ 22
1976									
pasture group	7	84	—	18,5	19,3	4,36	3,46	4,81	+ 31
barley group	7	84	5,8	17,7	18,9	4,47	3,67	4,97	+ 39
1977									
pasture group	7	98	—	19,6	20,4	4,25	3,48	4,90	— 8
barley group	7	98	4,7	20,8	21,2	4,16	3,63	4,94	— 1
1978									
pasture group	7	105	—	22,5	23,1	4,24	3,25	—	—11
barley group	7	105	4,8	23,8	23,9	4,07	3,46	—	+ 37
1979									
pasture group	7	84	—	19,2	19,9	4,27	3,36	—	+ 16
barley group	7	84	4,0	20,4	21,1	4,26	3,39	—	+ 9
Mean									
pasture group	35	94	—	19,7	20,4	4,27	3,38	4,83	+ 8
barley group	35	94	4,9	20,5	21,1	4,22	3,52	4,91	+ 21

Table 6. Sugar content of pasture grass, Jokioinen 1976—77.

	Glucose per dry matter, % June	July	August	September	mean	
1976	13,2	10,3	8,3	12,6	11,1	Morning sample
	14,7	10,4	10,3	16,4	12,9	Evening sample
1977	11,8	5,3	7,4	6,6	7,5	Morning sample
	13,0	7,4	9,9	10,2	9,9	Evening sample

grass-only production was 23,1 kg FCM per day and the average production of seven cows was 21 kg per day in the autumn (Fig. 7).

Milk fat content was practically the same on the pasture and barley treatments (Table 5).

Table 7. Effect of different concentrates with grazing on milk yields, Jokioinen 1975—77. Rissanen 1984.

Group	Number of cows	kg per cow per day concentrate	milk	4 % milk	Milk fat %	Milk protein %	Milk lactose %	Change in live weight, kg
1975								
pasture group	10	—	19,0	19,4	4,15	3,09	4,82	+ 6
barley group	10	2,1	19,7	19,7	4,01	3,17	4,75	+ 16
oats group	10	2,0	18,8	18,9	4,07	3,19	4,83	— 7
barley-oats group	10	2,3	20,1	20,5	4,19	3,21	4,80	— 1
barley-dried molasses group	10	1,2 + 1,0	19,1	19,4	4,17	3,25	4,90	± 0
1976								
pasture group	10	—	20,9	21,5	4,23	3,32	5,11	+ 26
barley group	10	2,4	20,7	21,3	4,24	3,35	5,05	+ 32
oats group	10	2,9	22,5	23,2	4,26	3,34	5,25	+ 28
barley-oats-group	10	2,7	22,5	22,9	4,13	3,36	5,09	+ 30
barley-dried molasses group	10	1,6 + 1,0	21,0	21,6	4,27	3,50	5,22	+ 26
1977								
pasture group	10	—	20,8	21,7	4,29	3,18	4,91	+ 7
barley group	10	2,7	22,3	23,1	4,22	3,14	4,95	+ 22
oats group	10	3,0	23,4	24,1	4,24	3,12	5,02	+ 6
barley-oats group	10	2,6	21,3	22,2	4,35	3,34	4,89	+ 24
barley-dried molasses group	10	1,5 + 1,0	21,8	22,7	4,31	3,29	4,91	+ 27
Mean								
pasture group	30	—	20,2	20,9	4,22	3,20	4,95	+ 13
barley group	30	2,4	20,9	21,4	4,16	3,22	4,92	+ 23
oats group	30	2,6	21,6	22,1	4,20	3,22	5,04	+ 9
barley-oats group	30	2,5	21,3	21,9	4,22	3,31	4,93	+ 18
barley-dried molasses group	30	1,4 + 1,0	20,6	21,2	4,25	3,35	5,01	+ 18

Instead, the milk protein content increased with the barley supplement as in the previous experiments. The live weights of the cows also rose on the pasture treatment in most of the experiments (Table 5). Energy requirement was supplied by grass only. The increase in live weight of the barley group was, however, higher than that of the comparison group. A part of the energy supplied by barley was used for fattening.

Different concentrates on pasture grazing

A comparison of barley and other concentrates on pasture grazing was conducted during 1975—77 at Jokioinen. The concentrates compared were: barley, oats, a barley-oats mixture (1/2—1/2) and barley-dried molasses. The concentrate was given 0,2 f.u. per kg FCM for productions exceeding 10 kg per day. The barley-dried molasses group was fed 1,0 kg dried

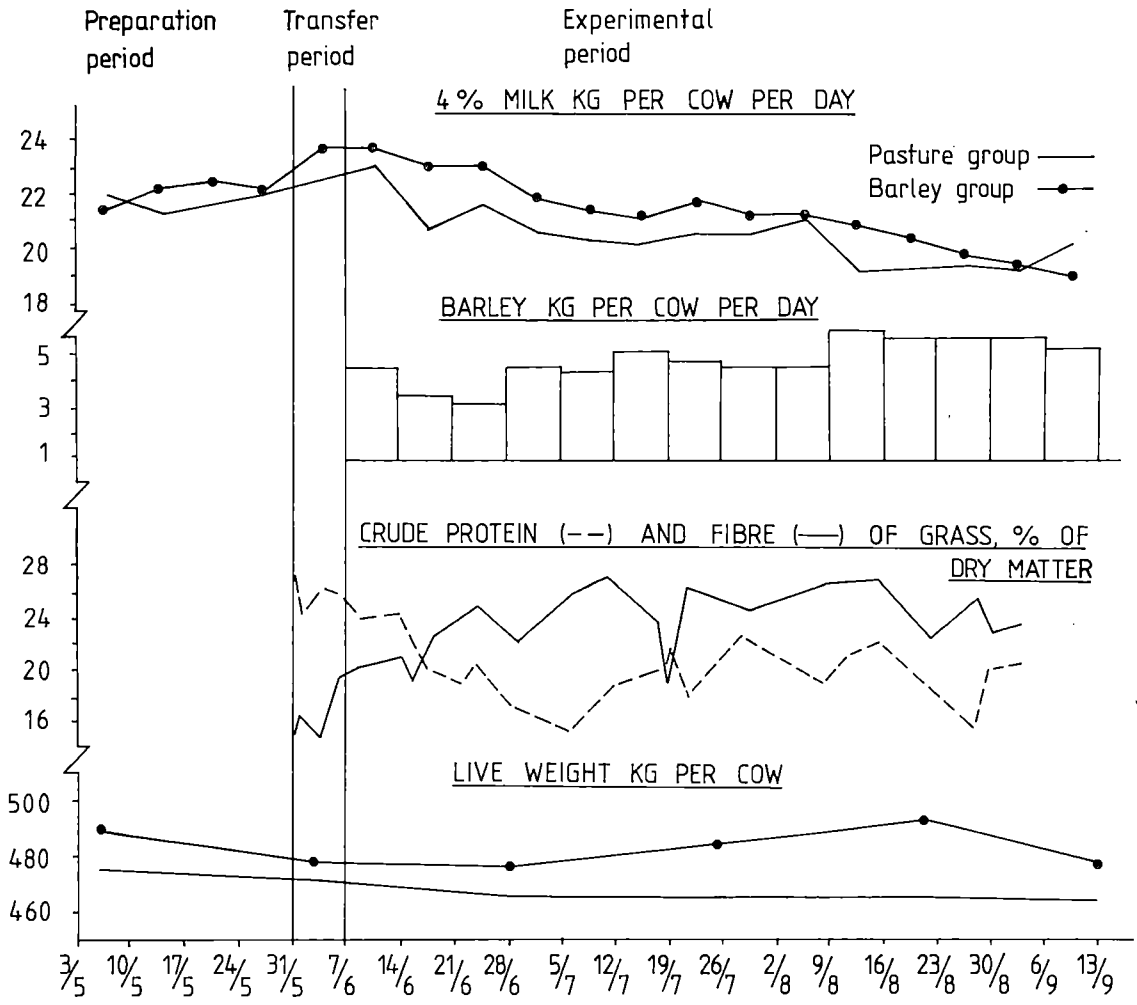

Fig. 6. Change in milk yields and live weights of cows fed grass-only and fed 3 kg barley in the morning and evening together with grazing, North Savo Research Station 1977.

molasses per day and the rest of the calculated concentrate requirement was supplied by barley. Pasture was predominantly cocksfoot. Nitrogen applications of 300 kg per ha in three equal dressings were spread during the summer.

Because nitrogen application was high the sugar content of the grass was analysed during two following summers. Samples were collected in the morning and evening before changing to a new sward. Especially in the early summer and in the autumn the sugar content in pasture grass proved to be very high (Table 6).

The average amount of a concentrate consumed by the cows was 2,4—2,6 kg per day which covered about 20 % of their calculated energy requirement (Table 7). The average milk yield of the pasture group was 20,2 kg per day.

The barley or the barley-dried molasses supple-

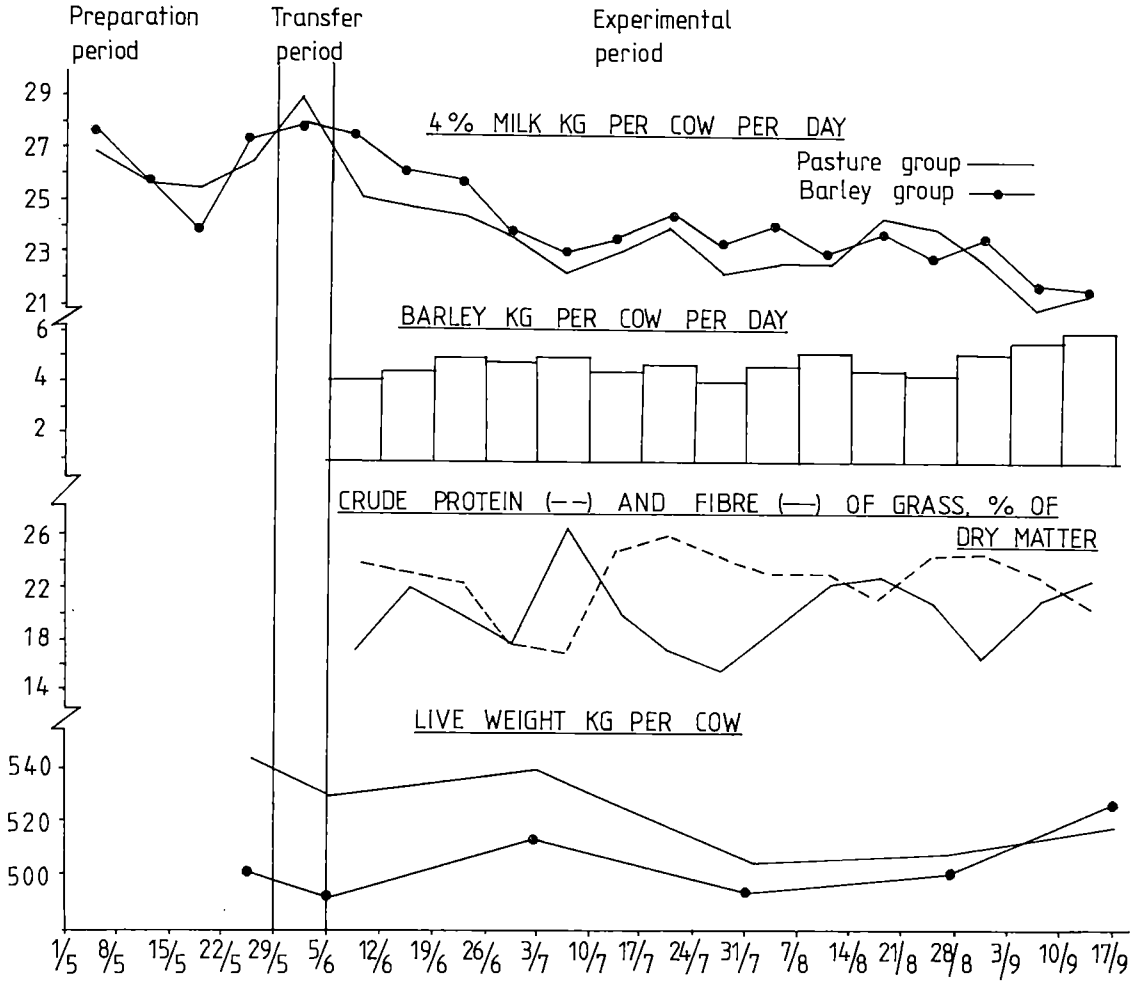

Fig. 7. Change in milk yields and live weights of cows fed grass-only and fed 3 kg barley in the morning and evening together with grazing, North Savo Research Station 1978.

ment yielded only a small increase in milk production. The oats and barley-oats supplement increased production more than barley alone but the results varied from year to year. Oats increased milk yield by 1,4 kg per day and 424 g per f.u. on average. The concentrate supplements had no effect on milk fat or lactose content (Table 7). The milk protein content increased slightly with concentrates. The cows gained weight on all treatments.

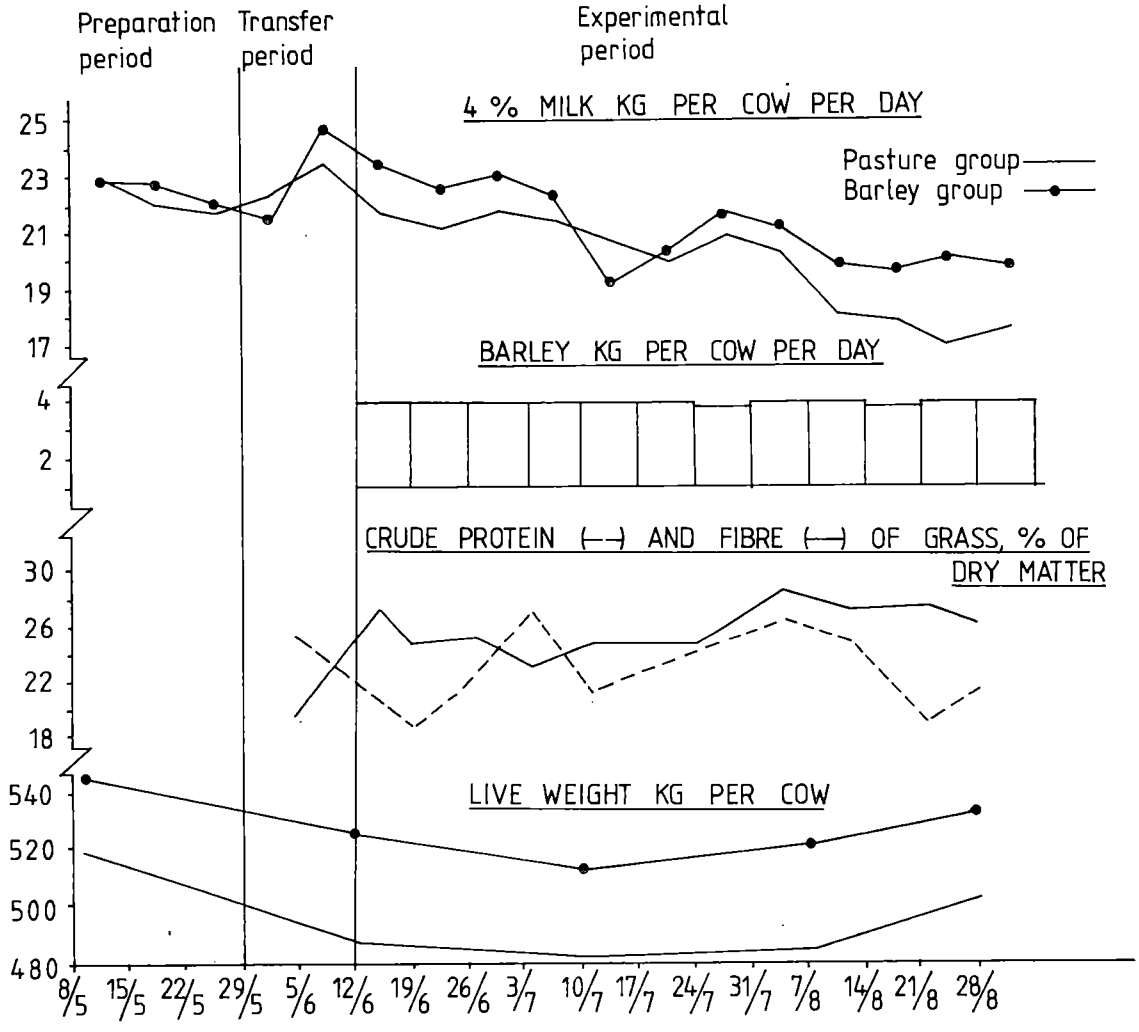

Fig. 8. Change in milk yields and live weights of cows fed grass-only and fed 2 kg barley in the morning and evening together with grazing, North Savo Research Station 1979.

CONCLUSIONS

From the experiments it can be concluded that cows can produce 22—25 kg milk per day without a concentrate supplement grazing good pasture and achieve daily milk yields of 20 kg during summer. This result corresponds well to results obtained elsewhere (DONKER et al. 1968) and is in agreement with results of other countries that the effect of a concentrate is minimal on good pasture (e.g. CASTLE 1960, 1964, 1968, DONKER et al. 1968, SHEPHERD 1962, WOOD 1966). Cows obviously decrease the intake of grass almost equally to the energy value of a concentrate. The best result with a supplement was achieved when the grass was in a late stage of growth. This is important especially for high-yielding cows. A problem of pasture feeding is actually how maintain the quality of grass good through the entire summer.

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SELOSTUS

Väkirehun vaikutus lehmien tuotantoon hyvällä laitumella

ELSI ETTALA, KALLE RINNE, ERKKI VIRTANEN ja HEIKKI RISSANEN

Maatalouden tutkimuskeskus

Tutkimuksen tarkoituksena oli selvittää, millaisiin tuotoksiin lehmät pääsisivät hyvällä laitumella ilman väkirehulisää ja minkä verran väkirehun käyttö lisäisi tuotosta. Kokeet tehtiin vuosina 1969—1979 Maatalouden tutkimuskeskuksen kotieläinhoito-osaston, kasvinviljelyosaston ja Pohjois-Savon tutkimusaseman yhteistyönä. Kokeissa oli mukana yhteensä 480 lehmää. Tässä julkaisussa on esitetty yhteenveto aikaisemmin erikseen julkaistuista kokeista.

Vertailuryhmien lehmät saivat pelkästään laidunruohoa. Väkirehuryhmien lehmät saivat laidunruohon lisäksi pääasiassa ohraa. Kauraa, ohra-kauraa ja ohra-melassileikettä verrattiin erillisessä kokeessa ohraan. Ohramäärät vaihtelivat hyvin pienistä määristä runsaisiin, vapaasti lypsyn yhteydessä annettaviin.

Vertailuryhmien lehmät (152) tuottivat pelkällä laidunruoholla noin kolmen kuukauden pituisen laidunkauden aikana keskimäärin 19,4 kg 4-prosenttista maitoa päivässä, parhailla laitumilla 23,1 kg.

Pienin ohramäärä vastasi 1/3 siitä energiamäärästä, jonka lehmät tarvitsivat yli 10 maitokilon tuotantoonsa (4 %). Tätä ohralisää, keskimäärin 1,3 kg/lehmä/d sai 87 lehmää. Ne tuottivat keskimäärin 1,1 kg enemmän 4-prosenttista maitoa päivässä kuin lehmät samoilla laitumilla ilman väkirehulisää. Lisätuotos oli 846 g yhtä ohrakiloa kohti. Kaksinkertaisella ohramäärällä (2/3 vastaavasta energiatarpeesta), keskimäärin 2,7 kg:lla päivässä, lehmät (87) tuottivat 1,6 kg 4-prosenttista maitoa enemmän kuin vertailuryhmien lehmät vastaavilla laitumilla. Lisätuotos oli 593 g yhtä ohrakiloa kohti.

Runsaat ohramäärät, 4—6 kg lehmää kohti päivässä tai vapaasti lypsyn yhteydessä annetut ohra-annokset antoivat pienemmän tuotoslisän kuin edellämainitut.

Kaura ja ohra-kauraseos antoivat vähän paremman lisätuotoksen kuin ohra yksin, joskin tulokset vaihtelivat eri vuosina. Ohra-melassiseos ei ollut lisärehuna ohraa parempi.

Viljalisä kohotti maidon proteiinipitoisuutta ja jossain määrin maitosokeripitoisuutta, mutta ei vaikuttanut rasvapitoisuuteen. Väkirehua saaneiden lehmien paino nousi laidunkaudella enemmän kuin vertailuryhmien.

Kokeista voidaan tehdä se johtopäätös, että lehmät voivat hyvällä laitumella ilman väkirehua tuottaa 22—25 kg maitoa päivässä ja päästä kesän aikana 20 kg:n keskimääräisiin päivätuotoksiin. Väkirehun vähäinen vaikutus ilmeisesti johtui siitä, että lehmät vähensivät ruohon syöntiä lähes väkirehun energia-arvoa vastaavasti. Paras tulos väkirehulisällä saatiin ruokinnan väkevöittäjänä, kun ruoho oli myöhäisellä kasvuasteella.

ANNALES AGRICULTURAE FENNIAE, VOL. 25: 127—133 (1986)
Seria AGRICULTURA N. 76 — Sarja PELTOVILJELY n:o 76

THE EFFECT OF STARCH GELATINIZED NUTRIENT MEDIA IN BARLEY ANTHER CULTURES

SEPPO SORVARI

SORVARI, S. 1986. **The effect of starch gelatinized nutrient media in barley anther cultures.** Ann. Agric. Fenn. 25: 127—133. (Agric. Res. Centre, Dept. Pl. Breed., SF-31600 Jokioinen, Finland.)

The response of barley anthers cultured in barley-, corn-, potato-, rice- and wheat-starch gelatinized media was compared to the response in agar gelatinized media.

Embryoid/callus formation was in all starch media better than in agar media. The highest response per 1000 cultured anthers was in barley-starch and in wheat-starch media, in barley-starch 49,2 and in wheat-starch 38,4. In the agar based media the corresponding response was 9,9.

The barley-, potato-, rice- and wheat-starches were also better for the differentiation of green plantlets than agar media. Per 1 000 cultured anthers barley-starch produced 16,7, potato-starch 1,7, rice-starch 1,5, wheat-starch 7,4 and agar media 0,8 green plantlets.

A remarkable feature of the starch media was the formation of embryoids. The emerging embryoids had dense globular structure and nondormant types were able to germinate of the size of less than 1 mm.

Index words: *Hordeum vulgare*, barley, anther culture, androgenesis, differentiation, haploids, gelatine agent, barley-starch, corn-starch, potato-starch, rice-starch, wheat-starch, agar.

INTRODUCTION

One of the most utilized gelatine agents in the nutrient media of cell- and tissue cultures is agar. It is very handy and in many cases it is difficult to replace by other gelatine agents. However, there is some evidence that agar contains inhibitory substances. According to KOHLENBACH and WERNICKE (1978) those substances cause premature abortions of embryoids in the anther cultures of *Nicotiana tabacum*.

Agar omitted liquid media have often proved more satisfactory in the callus formation (WILSON 1977, SUNDERLAND et al. 1979). A low differentiation capacity, and sinking of the calli to the bottom of culture vessel have limited the use of liquid culture systems (KAO 1981).

To avoid the negative effects of low density liquid culture systems KAO (1981) has suggested the use of Ficoll 400 for increasing the density of

liquid media. There are also reports on successful use of active carbon in agar-gelatinized media in tobacco (NAKAMURA and ITÄKAGI 1973, ANAGNOSTAKIS 1974, WERNICKE and KOHLENBACH 1976, KOHLENBACH and WERNICKE 1978) and in anemone (JOHANSSON and ERIKSSON 1977).

This study was initiated to search for gelatine agents with advantages of solid medium without inhibitory influence of agar. In this report the effects of five different starch sorts as gelatine agents used in the nutrient media of barley anther cultures are described.

MATERIAL AND METHODS

Donor plant materials

For donor plant materials, two barley varieties of spring type cvs. 'Dissa' and 'Ingrid' were used. The six-rowed 'Dissa' was kindly supplied by Dr. Bärbel Foroughi-Wehr in Grünbach (FRG). The two-rowed 'Ingrid' is commercially available malting barley variety from Weibull (Sweden).

Starch sorts

Corn-, potato-, rice- and wheat-starches were from Sigma, but the poor quality potato starch from Sigma was substituted by normal supermarket starch. Barley starch was kindly supplied by M.Sc. Aino Mansikkamäki in Nestesokeri Oy. "Agar Noble" (Difco) was used as a control. Rawprotein content (in dry matter) was in agar 0,40 %, barley 0,53 %, corn 0,42 %, potato 0,14 %, rice 0,52 %, and wheat 0,42 %.

Gelatinization with starches

For the gelatinization, the following starch concentrations were used: barley-starch 60 g/l, corn-starch 50 g/l, potato-starch 60 g/l, rice-starch 50 g/l and wheat-starch 60 g/l nutrient medium. The control medium was gelatinized with 0,7 % (w/v) "Difco Noble Agar" (Table 1).

The gelatinization of the nutrient medium was made by mixing the starch in cold destilled water and pouring the starch-water mixture slowly into hot (> 95 °C) nutrient medium, mixing vigorously all the time. The gelatinized medium was autoclaved at 121 °C for 15 minutes and then poured into ∅ 35 mm petridishes.

Because of the soft texture of the starch media, polyester nets were laid on the nutrient media to prevent sinking of the cultures.

Growth conditions for donor plants

Donor plant materials were grown in growth chambers. By day the temperature was 18 °C (80 % RH) and by night 12 °C (80 % RH). Plants were illuminated by Osram "Power Star" HQJ-T 1 000 W/D lamps with light intensity of 50 klux measured in the tops of the plants. Photoperiod was set on 18 h.

Plants were fertilized once a week with N-P-K-fertilizer (6-7-17).

Removal of anthers

Anthers with microspores at the uninucleate stage were removed aseptically from the florets. The stage was checked by squashing one anther from the center and the third or the fourth anther from the distal parts of the florets in acetic-orcein. From two to four anthers in the distal ends of the florets were discarded. Anthers were placed in ∅ 35 mm petridishes.

In order to minimize the influences of genotypes between the treatments, anthers from a single floret were always distributed in the control, and in the five starch based media. About 30 anthers were placed in each petridish.

Table 1. Compositions of nutrient media used in the combinations of five different starch sorts.

Basic medium for all experiments
MURASHIGE and SKOOG (1962), LINSMAIER and SKOOG (1965) and FOROUGHI-WEHR et al. (1976)

Macro Nutrients	mg/l	mM	Micro Nutrients	mg/l	uM
KNO_3	1900	18,8	H_3BO_3	6,2	100,0
KH_2PO_4	170	1,3	$MnSO_4 \cdot 4H_2O$	22,3	100,0
NH_4NO_3	1650	20,6	$FeSO_4 \cdot 7H_2O$	27,8	100,0
$MgSO_4 \cdot 7H_2O$	370	1,5	$Na_2EDTA \cdot 2H_2O$	37,3	100,0
$CaCl_2 \cdot 2H_2O$	440	3,0	$CoCl_2 \cdot 6H_2O$	—	—
			$CuSO_4 \cdot 5H_2O$	0,025	0,1
			$ZnSO_4 \cdot 7H_2O$	8,6	30,0
			$Na_2Mo0_4 \cdot 2H_2O$	0,25	1,0
			KJ	0,83	5,0
Organic Supplements	mg/l	mM	Organic Supplements	mg/l	uM
m-Inositol	100,0	0,6	Thiamine HCl	0,4	1,2

pH 5,8

Modifications made in different treatments

LSH		A		B		C		P		R		W	
		I	II	I	II	I	II	I	II	I	II	I	II
IAA	mg/l	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4
BAP	mg/l	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4	1,0	0,4
Sucrose	g/l	60	30	60	30	60	30	60	30	60	30	60	30
Agar (Noble, Difco)	g/l	7	7										
Barley Starch	g/l			60	60								
Corn Starch	g/l					50	50						
Potato Starch	g/l							60	60				
Rice Starch	g/l									50	50		
Wheat Starch	g/l											60	60

Callus induction and differentiation

Inorganic and organic nutrients used for embryoid/callus induction was based on the studies by MURASHIGE and SKOOG (1962) and LINSMAIER and SKOOG (1965) and the modifications of CLAPHAM (1973) and FOROUGHI-WEHR et al. (1976). The final compositions of the media are given in Table 1 under LSH I:s.

Anthers on the embryoid/callus induction media were incubated in the dark at 25 °C until embryoids/calli appeared. The harvesting period for the embryoids/calli lasted from the second to the fifth week. Once a week embryoids/calli were harvested and laid on the differentiation media in ∅ 35 mm petridishes with the same starch sorts as in the induction media. The final compositions of the differentiation media are given in Table 1 under LSH II:s. Cultures for differentiation were kept in the phytotrons under low intensity fluorescent light (3 000 lux) for an 18 h photoperiod at constant temperature of 25 °C. °C.

Potting and cytological analysis

Differentiated plantlets were transferred in culture tubes (∅ 24 mm). The medium for the differentiated plantlets was similar to the LSH II with or without hormones. After the root formation, the plants were potted and hardened for normal growth conditions.

Chromosome counts were made from root tips fixed in (1:3) glacial acetic acid: alcohol, and then stained by acetic orcein.

RESULTS

The effects of the starches in the nutrient media varied depending on the starch sources. The highest amounts of anthers producing embryoids/calli (AAPEC) were observed in the media gelatinized with starches originated from monocots barley (49,2/1 000), wheat (38,4/1 000) and rice (18,2/1 000). Among the starch gelatinized media potato- (15,7/1 000) and corn-starch (12,2/1 000) had the lowest AAPEC, but was still higher than the agar based media (9,9/1 000)(Table 2).

The superiority of barley- and wheat-starch, compared to agar, was even clearer in the regeneration of plantlets from the embryoids/calli. The amount of regenerated green plantlets in the barley-starch media was about twenty times higher (16,7/1 000) and in the wheat-starch media about nine times higher (7,4/1 000) than in the agar media (0,8/1 000). Although the AAPEC in the corn starch media was higher (12,2/1 000) than in the agar media, the regeneration of green plantlets in the corn starch media was not successful. In the potato- (1,7/1 000) and the rice-starch (1,5/1 000) media the regeneration of green plantlets was about two times higher than in the agar media.

In all of the media used, the regeneration of chlorophyll deficient plantlets was unavoidable. The ratio of green plantlets to albinos was higher both in the barley starch and the wheat-starch media, than in the agar media. The ratios being in the barley-starch 1/1,5, in the wheat-starch 1/1,4, and in the agar 1/4,1 (Table 3).

Within the experiment, the variety 'Ingrid' produced in the barley- and in the wheat-starch media more green, than albino plantlets. The ratio of green plantlets to albinos in the barley-starch media was 1/0,9 and in the wheat-starch media 1/0,7. In the potato-starch media the

Table 3. Ratio of green plantlets to albino plantlets in agar (A), barley- (B), corn- (C), potato- (P), rice- (R) and wheat-starch (W) gelatinized media.

	Ratio of Green Plantlets/Albino Plantlets					
Gelatinizing Agent	A	B	C	P	R	W
Whole Experiment	1/4,1	1/1,5	0/2,6	1/2,4	1/4,1	1/1,4
Dissa	0/3,9	1/2,2	0/5,9	0/11,8	1/5,9	1/4,0
Ingrid	1/2,0	1/0,9	0/0,0	1/1,0	1/1,8	1/0,7

Table 2. Response of anthers of barley cvs Dissa and Ingrid in the agar (A), barley- (B), corn- (C), potato- (P), rice- (R) and wheat-starch (W) gelatinized media.

Gelatinizing Agent		Numbers of Anthers Inoculated						Anthers Producing embryoids/calli					
		A	B	C	P	R	W	A	B	C	P	R	W
Donor Plant Material	Dissa	510	510	510	510	510	510	5	24	6	7	15	22
	Ingrid	708	811	642	702	811	714	7	41	8	12	9	25
Total Numbers		1218	1321	1152	1212	1321	1224	12	65	14	19	24	47
Response per 1000 Inoculated Anthers	Total							9,9	49,2	12,2	15,7	18,2	38,4
	Dissa							9,8	47,7	11,8	13,7	29,4	43,1
	Ingrid							9,9	50,6	12,5	17,1	11,1	35,0
Gelatinizing Agent		Green Plants Regenerated						Albino Plants Regenerated					
		A	B	C	P	R	W	A	B	C	P	R	W
Donor Plant Material	Dissa	—	10	—	—	1	2	2	22	3	3	6	8
	Ingrid	1	12	—	2	1	7	2	11	—	2	2	5
Total Numbers		1	22	—	2	2	9	4	33	3	5	8	13
Response per 1000 Inoculated Anthers	Total	0,8	16,7	—	1,7	1,5	7,4	3,3	25,0	2,6	4,1	6,1	10,6
	Dissa	—	19,6	—	—	2,0	3,9	3,9	43,1	5,9	5,9	11,8	15,7
	Ingrid	1,4	14,8	—	2,8	1,4	9,8	2,8	13,6	—	2,8	2,5	7,0
Regeneration capacity in % of Androgenetic Anthers		8,1	33,9	—	10,8	8,2	19,3	33,3	50,8	21,3	26,1	33,5	27,6

amounts of green plantlets and albinos were fifty-fifty thus the ratio was exactly 1/1,0, (Table 3).

The regeneration capacities of the embryoids/calli formed in the barley-starch and in the wheat-starch media were also higher. Because it was often difficult to see the number of single embryoids/calli in an anther, the regeneration capacities was expressed in percentage of the androgenetic anthers.

Thus the percent of green plantlets regenerated in the barley starch media was 33,9 % and in the wheat-starch media 19,3 %. The percentages of green plantlets regenerated in potato-, rice- and agar-media were in the same ranges, being in potato-starch 10,8 %, in rice-starch 8,2 % and in agar media 8,1 % (Table 2).

In the barley-starch media, the total plant production (green + albino plantlets) from the AAPEC was better than 84 %, but in the others it was less than 45 % (Table 2). The ratio of green diploids to green haploids was in agar- 10/1, in barley- 2,7/1, in corn- 0/0, in potato- 1/1, in rice- 2/0 and in wheat-starch media 2/1.

The consistency of the media depended on the origin of the starch. Developing cultures in potato starch media (which remained soft and pasty) without polyester nets was rather difficult because of the tissues sinking. Although barley-, corn-, rice-, and wheat-starch gelatinized media are in the beginning relatively hard, the softening of the media takes place during the culture procedure, thus polyester nets are also advisable.

DISCUSSION

The existence of inhibitory components in agar have been known for a long time (KOHLENBACH and WERNICKE 1978), but it has been difficult to substitute agar based media for other culture systems which are similar to agar, but don't contain inhibitory compounds of agar. Liquid culture systems used by WILSON (1977), SUNDERLAND et al. (1979) was good in callus production, but failed in differentiation (KAO 1981). Barley-, potato-, rice- and wheat-starch promoted both embryoid/callus formation and differentiation. The response of anthers in the corn-starch medium was similar to the response in agar, but the differentiation of green plantlets in corn-starch failed. Corn-starch seems to contain compounds that inhibit the differentiation of green plantlets, but it is unclear if those compounds are similar to the inhibitors of agar.

The most remarkable feature in the starch media was the embryoid formation. The emerging embryoids had dense globular structure, and if they were not dormant they were able to germinate already of the size of less than 1 mm. The direct callus formation was rather rare in the starch media. In the case of callus formation the plantlets were differentiated either via organogenesis or embryogenesis.

In the embryoid/callus formation the best results were achieved in the barley- and wheat-starch media. The amount of anthers producing embryoids/calli in the barley-starch media was about five times higher (49,2/1 000) and in the wheat-starch media about four times higher (38,4/1 000) than in the agar based media (9,9/1 000). The trend in the regeneration of green plantlets was similar being in the barley-starch media about 20 times (16,7/1 000) and in the wheat-starch media about 9 times (7,4/1 000) higher than in the agar based media (0,8/1 000). But the increase in the production of green plantlets does not due only to the increased number androgenic anthers, but also the number of embryoids per anther was markedly increased. The amount of green plantlets did not increase proportional to the amount of embryoids but most embryoids remained

dormant or they formed non-morphogenic calli after transfer into differentiation medium.

The occurrence of albinos in cell cultures of *Gramineae* is a common phenomenon. After SUN et al. (1979) albino rice plants are lacking fraction I protein and 23S and 16S RNA of plastid ribosomes are absent or rare. After these studies the occurrence of albino forms may due to changes in plastid or nuclear genome.

WANG et al. (1977) found that temperature was the only cultural factor *in vitro* effecting albino freguency of rice. The earlier studies of CLAPHAM with barley (1973) showed however that frequency of albino and green plantlets could be altered by changing the compositions of culture media. The results from this experiment also showed evidence of the influence of the nutrient medium on the ratio of green to albino plantlets. The highest ratio of green to albino plantlets was in barley-starch (1/1,5) and the lowest in corn-starch (0/2,6) with no green plantlets.

Starch is a natural nutritive component in the seed endosperm, making about 60—70 % of the dry weight of the seed. If there are no unnatural changes in the starch during processing from the seed it can be assumed that starch offers ideal circumstances for the development and differentiation of microspores into plantlets. In these experiments the best results were achieved in the barley starch gelatinized nutrient media. The nutritive role of the starches in the media is at the moment obscure, but the softening of the starch-gelatine indicated possible enzymatic activity of the tissues during the culture procedure.

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SELOSTUS

Tärkkelys ravintoalustan kiinteyttäjänä ja sen vaikutus ohran ponsiviljelmiin

SEPPO SORVARI

Maatalouden tutkimuskeskus

Erilaistumisprosessi epäkypsästä siitepölyhiukkasesta, mikrospoorista, haploidiksi kasviksi on äärimmäisen herkkä ulkopuolisille ärsykkeille. Tupakkakasvien solukkoviljelmillä tehdyissä tutkimuksissa on voitu havaita, että ravintoalustan kiinteyttämiseen käytetty agar sisältää inhibiittoreita, jotka voivat estää erilaistumisprosessin tai aiheuttaa siinä häiriöitä.

Ongelmaa on yritetty ratkoa mm. nestemäisillä ravintoalustoilla, mutta tulokset eivät ole olleet tyydyttäviä. Tämän tutkimuksen tarkoituksena oli selvittää ohran, maissin, perunan, riisin ja vehnän tärkkelysten soveltuvuutta ravintoalustan kiinteytykseen ja niiden vaikutuksia ohran ponsiviljelmiin.

Alkioiden/kalluksien muodostuksessa olivat kaikki tärkkelykset parempia kuin agar. Erikoisesti ohran tärkkelyksellä oli alkioiden/kalluksien muodostusta edistävä vaikutus. Tällaisten (androgeenisten) ponsien määrä oli ohran tärkkelysalustalla kymmenisen kertaa korkeampi kuin agarilla.

Vihreiden kasvien muodostuksessa oli ohran tärkkelysalusta parikymmentä kertaa tuottoisampi kuin agaralusta. Vihreiden kasvien erilaistumisessa oli erityyppisillä tärkkelyksillä kuitenkin huomattavia eroja. Maissin tärkkelysalustalle ei vihreiden kasvien regeneraatio onnistunut lainkaan. Mahdollisesti maissin tärkkelys sisältää inhibiittoreita, jotka estävät nimenomaan vihreiden kasvien erilaistumisen sillä albinoja, klorofyllittomia, ohria saatiin myös maissin tärkkelysalustoilla.

Tärkkelys on eräs oleellisimmista komponenteista kasvin solun metaboliassa. On luonollista, että ohran tärkkelys osoittautui parhaimmaksi ohran ponsiviljelmissä, koska se on ohran oman aineenvaihdunnan tuote.

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Seria AGRICULTURA N. 77 — Sarja PELTOVILJELY n:o 77

DIFFERENTIATION OF POTATO TUBER DISCS IN BARLEY STARCH GELATINIZED NUTRIENT MEDIA

SEPPO SORVARI

SORVARI, S. 1986. **Differentiation of potato tuber discs in barley starch gelatinized nutrient media.** Ann. Agric. Fenn. 25: 135—138. (Agric. Res. Centre, Inst. Pl. Breed., SF-31600 Jokioinen, Finland.)

Differentiation of potato tuber discs of cultivar 'Pito' was studied in barley starch gelatinized nutrient media. In this type of media very high and rapid differentiation rates were achieved. The first shoots appeared in the 3rd week after inoculation, and discs that originated from different sites of tubers had all very good regeneration capacity. The results from this study shows that agar can be completely replaced by barley starch in potato tuber disc cultures.

Index words: differentiation, barley starch, gelatinization agent, tuber disc, potato.

INTRODUCTION

Somaclonal variation occurring in the cell and tissue culture of regenerated plants is a novel tool in crop improvement. SHEPARD et al. (1980) reported that potatoes regenerated from leaf protoplasts of Russet Burbank were highly variable, but the variations remained stable.

However, when using screening procedures at the cellular level, the regeneration capacity often decreased or was inhibited. Although potato tuber discs generally have a relatively good regeneration capacity in the agar based medium (FELLENBERG 1963, BRAGDÖ-ÅS 1977, LAM 1977 and JARRET et al. 1980), under critical conditions the inhibitory factors of agar can stop the regeneration.

In *Nicotiana tabacum* anther cultures the presence of inhibitory in agar is shown by KOHLENBACH and WERNICKE (1978). In barley anther cultures, barley starch gelatinized media was proven to be better than an agar based media (SORVARI 1986).

The purpose of this work was to study the regeneration of potato tuber discs in the nutrient medium gelatinized with barley starch.

MATERIAL AND METHODS

Tubers of *S. tuberosum* L. cv. 'Pito' were sterilized in 5 % Na-hypochlorite solution with 2 drops of Tween 80 for 15 minutes and then rinsed two times with sterile destilled water. Using a ∅ 7 mm cork borer, cylinders of tuber tissues were excised from the mid-section in the right angle to the proximal distal acis. The cylinders were sliced into 2 mm thick discs. Each disc was placed in a 35 mm petridish that contained 5,5 ml of the nutrient media.

Discs were numbered from 1 to 11, so that the increasing number means distance from the epidermis. Number 1 was derived from the periderm site and number 11 from the pith site.

The cultures were kept under fluorescent light (3000 lux), with 18 h photoperiods at 25 °C, and every 4th week the cultures were transferred to fresh medium.

With some modifications in the organic component (Table 1), the composition of the nutrient medium was based on that of MURASHIGE and SKOOG (1962). The gelatinization with barley starch was made according to the method described earlier (SORVARI 1986). Because of the softening of the nutrient medium during the culture procedure, polyester nets were used to prevent the discs from sinking.

Table 1. Modified MURASHIGE and SKOOG (1962) medium used in the potato tuber disc cultures.

Macro Nutrients	mg/l	Micro Nutrients	mg/l
KNO_3	1900	H_3BO_3	6,2
KH_2PO_4	170	$MnSO_4 \cdot 4H_20$	22,3
NH_4NO_3	1650	$FeSO_4 \cdot 7H_20$	27,8
$MgSO_4 \cdot 7H_20$	370	$Na_2EDTA \cdot 2H_20$	37,3
$CaCl_2 \cdot 2H_20$	440	$CoCl_2 \cdot 6H_20$	0,025
		$CuSO_4 \cdot 5H_20$	0,025
		$ZnSO_4 \cdot 7H_20$	8,6
		$Na_2Mo0_4 \cdot 2H_20$	0,25
		KJ	0,83
Organic Supplements	**mg/l**	**Organic Supplements**	**mg/l**
m-Inositol	100,0	GA_3	0,5
Glycine	2,0	NAA	0,1
Thiamine HCl	0,1	IAA	0,1
Pyridoxine HCl	0,5	Kin	0,5
Nicotinic Acid	0,5	BAP	3,0
Casein-Hydrolysate	10^3	Barley Starch	6×10^4
Saccharose	2×10^4		
pH 5,8			

RESULTS

After 1—2 weeks the healthy discs displayed a clear green colour, and the formation of protuberances started. Protuberances were mostly formed in the lateral faces of the discs. The differentiation of the shoots was preceded by the formation of protuberances (Fig. 1).

Between the 2nd and 4th week, 7,7 % of the discs Nr 2 from the periderm site, and Nr:s 5, 6 and 7 from perimedullary tissue showed shoot formation. Discs Nr 8 also from the perimedullary tissue showed 16,7 % (Fig. 2).

After 12 weeks of cultivation, more than 90 % of the discs 2, 3, 4, 7, 8, 9 and 11 had shoot

Fig. 1. Potato tuber disc of cv. 'Pito' with protuberances and differentiated shoots after the culture of four weeks.

formation (Fig. 2). The low shoot formation from the periderm in the discs of number 1 was due either to the contamination of the peridermal tissue towards epidermis during storage or to the damages caused by disinfection.

After the culture of 16 weeks almost 100 % of the discs formed shoots. Only a part of the discs did not form shoots in the discs Nr 1, 5 and 6, because of contamination or other difficulties during the culture procedure.

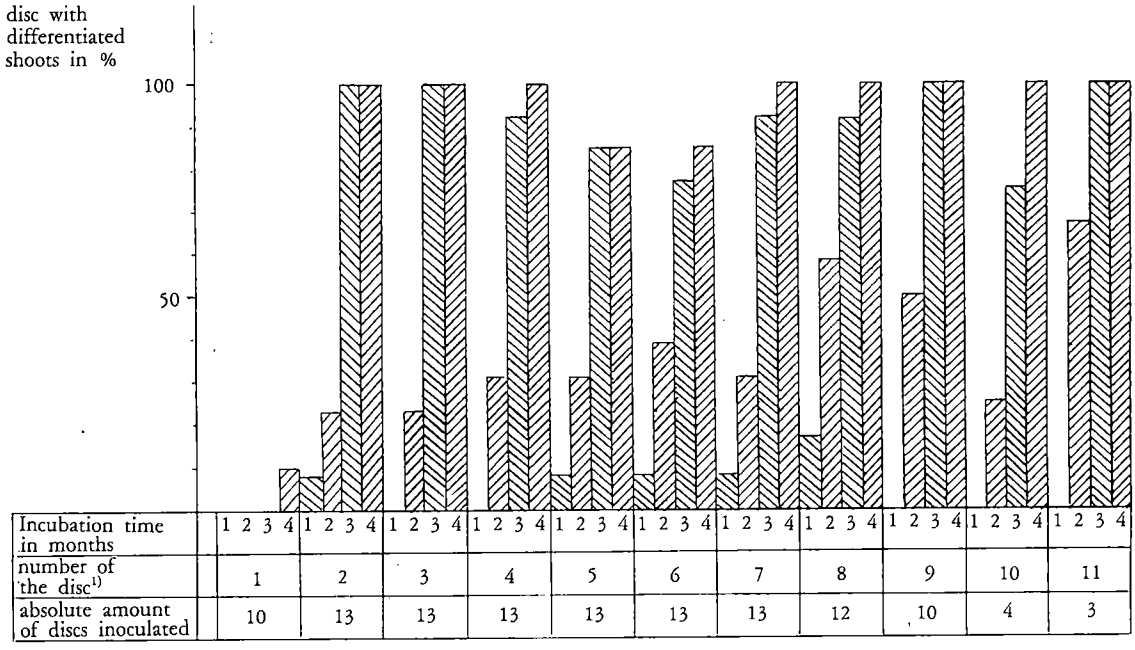

Fig. 2. Emerging of shoots in potato tuber discs during the culture of 4 months.

[1)] Increasing disc number means the distance of the disc from the epidermis. Each disc is 2 mm thick and discs Nr 1 are dissected from the epidermal site and 11 from the pith site.

DISCUSSION

One of the most remarkable features of the medium with barley starch as a gelatinization agent was the rapid differentiation of shoots. The first shoots appeared already in the third week, and after the 12th week, 7 cultures of 11 had more than 90 % discs with shoots.

LAM (1977) observed the first shoots after 14 weeks of culture and JARRET et al. (1980) found the first shoot primordia after 5 weeks.

Except for discs Nr 1, the site of the tissue did not influence very much the differentiation of the discs. Both in the pith site and periderm site 100 % differentiation rates was achieved. After 2—3 weeks of culture the discs towards the epidermis turned necrotic and no or very little growth was observed. This was due probably to damage in the disinfection process, or contamination in the epidermal tissue began during the storage of tubers.

In this experiment only barley starch was used. In the earlier studies with various starch sorts potato starch was found inadequate (SORVARI 1986). The structure of barley starch was more solid than the potato starch which was pasty.

However, it is advisable to use polyester nets also in the nutrient media gelatinized with barley starch because it gets soft during the culture procedure.

The effect of barley starch is still hypothetical, and probably there are more factors involved. One of these factors could be the absence of inhibitors in agar. On the other hand, barley starch could contain impurities favouring differentiation in cell, and tissue culture, or/and the starch itself can function as a nutritive component during the culture procedure.

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SELOSTUS

Perunan mukulan varastosolukon erilaistuminen ohran tärkkelysalustalla.

SEPPO SORVARI

Maatalouden tutkimuskeskus

Perunan solukkoviljelmistä regeneroiduissa kasveissa on voitu osoittaa huomattavaa vaihtelua. Tällaista somaklonaalista vaihtelua voidaan hyödyntää jo solukkoviljelmilläkin asettamalla viljelmän miljoonat solut alttiiksi jollekin rasitustekijälle ja regeneroimalla rasituksen kestävistä soluista uusia kasveja. Tällä menetelmällä voidaan tehdä valintaa lukemattomista vaihtoehdoista erittäin pienessä tilassa, petrimaljassa.

Solukkoviljelmissä rasitus vähentää kuitenkin usein jakautusmiskykyä ja alentaa regeneraatiokapasiteettia.

Jotta solut saataisiin erilaistumaan myös rasitettuina, on erittäin tärkeää, että olosuhteet muuten voidaan optimoida niin hyviksi kuin mahdollista. Regeneraatiota haittaavat mm. agarin sisältämät inhibiittorit. Inhibiittoreita ei ole voitu määrittää, mutta niiden olemassa olo on voitu osoittaa.

Tässä tutkimuksessa on selvitetty mhdollisuuksia korvata agar tärkkelyksellä, jolla on lähes vastaavat geelinmuodostusominaisuudet kuin agarillakin. Tutkimuksessa käytettiin yksinomaan ohran tärkkelystä, koska sitä on saatavissa erittäin puhtaana.

Kirjoittajan aikaisemmissa tutkimuksissa ohran tärkkelysgeeli osoittautui ohran ponsiviljelmillä ylivoimaisesti paremmaksi kuin agar geeli. Myös perunan varastosolukon regeneraatiossa ohran tärkkelysgeelialustalla päästiin hyvään tulokseen. Tulokset osoittavat, että perunan varastosolukkoviljelmissä agar voidaan täysin korvata ohran tärkkelyksellä.

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Seria ANIMALIA NOCENTIA N. 122 — Sarja TUHOELÄIMET n:o 122

SPECIFICITY OF PHEROMONE PREPARATES FOR LEPIDOPTEROUS PESTS

PEKKA PELTOTALO and TUOMO TUOVINEN

PELTOTALO, P. & TUOVINEN, T. 1986. **Specificity of pheromone preparates for lepidopterous pests.** Ann. Agric. Fenn. 25: 139—146. (Agric. Res. Centre, Dept. Pest Inv., SF-31600 Jokioinen, Finland.)

The specificity of 15 commercial pheromone preparates for monitoring lepidopterous pests was studied. The preparates for *Phyllonorycter blancardellus, Plutella xylostella, Archips rosanus, Adoxophyes orana, Hedya nubiferana* and *Spilonota ocellana* proved to be specific enough for use by growers in Finland.

The preparate for *Archips podanus* attracted males of *Euhyponomeutoides rufella*, for *Archips xylosteanus* males of *Aphelia paleana*, for *A. rosanus* males of *Ptycholoma lecheanum* and three preparates for *Archips*-species males of the species complex *Yponomeuta padellus/malinellus*. The preparate for *Synanthedon exitiosa* did not attract males of *S. tipuliformis*.

Index words: pheromones, specificity of pheromones, *Phyllonorycter blancardellus, Plutella xylostella, Archips rosanus, Adoxophyes orana, Hedya nubiferana, Spilonota ocellana, Euhyponomeutoides rufella, Aphelia paleana, Ptycholoma lecheanum, Yponomeuta* spp., *Synanthedon tipuliformis*.

INTRODUCTION

Synthetic insect sex pheromones have widely been used in pest control. The most common use of pheromones is to bait traps with pheromone capsules and monitor the flight of target insects. According to the number of males trapped, the necessity of insecticide application may be evaluated and the proper time of application may be determined following the peak flight of the pest species. KLASSEN et al. (1982) lists 81 species of *Lepidoptera* which have been monitored or surveyed using pheromones or other attractants.

In Finland, pheromone traps are applied in practical farming to monitor the codling moth *Cydia pomonella* (L) (HEIKINHEIMO 1981) and the pea moth *Cydia nigricana* (Fabr.) (TUOVINEN 1982). Pheromone traps have proved useful in determining the date of insecticide application as well as in evaluating the need for sprays. Both methods are applicable for independent use by farmers.

The species specificity of pheromone traps is important when traps are used in practice by growers. Ideally a pheromone trap attracts only the target species to be monitored. However, in practice many other insects are trapped either randomly or are attracted by the white traps alone. The pheromone preparate itself attracts

other, often near-related species. The target species should be, in any case, easily recognizable and distinguishable from other insects caught by the traps. Alternatively, a trap may attract more than just one species and they may be simultaneously monitored by this one trap.

In summer 1984, pheromone traps for 15 lepidopterous pest species were used in Southern Finland in order to study the specificity and efficiency of the preparates. The preparates were selected from the list of available monitoring systems produced by Hoechst AG (Germany) and Albany International (USA).

MATERIAL AND METHODS

Pheromone traps and preparates were supplied by Hoechst AG (H) and Albany International (A). Traps were either delta traps with a piece of rubber tube impregnated with pheromone (H) or wing style traps with microvials or hollow fibres as the pheromone source (A). The target species and the attracting chemicals are listed in Table 1.

One trap for each species was placed in three localities near plantations of the host plants of the target species. However, the host plants of *Cydia funebrana* and *Argyresthia ephippiella*, plum and cherry trees, were available only in Viikki, Helsinki. *Synanthedon exitiosa* (Say) traps were situated nearby blackcurrant bushes in four additional localities, where the currant clearwing *S. tipuliformis* (Clerck) was known to occur. The trapping period lasted three months, from the end of May to the end of August.

The traps were checked on an average twice a month, the shortest interval being three days and the longest four weeks. At the same time the glue bottoms of the traps containing insects were changed. Insect species were determined using genital preparates, when needed.

Table 1. The target species and pheromone components. Information from Hoechst AG, Germany (H) or from the literature (references).

Phyllonorycter blancardellus (H)	trans-10-dodecenylacetate
Synanthedon exitiosa (A)	cis-3, cis-13-octadecadien-1-ol-acetate and trans, cis-isomere (TUMLINSON et al. 1974)
Argyresthia ephippiella (H)	cis-11-hexadecenal
Plutella xylostella (H)	cis-11-tetradecenol cis-9-tetradecenol
Choristoneura hebenstreitella (H)	cis-11-tetradecadienol trans-11-tetradecadienol (FREROT et al. 1979)
Archips podanus (H)	cis-11-tetradecenylacetate trans-11-tetradecenylacetate
Archips xylosteanus (H)	cis-11-tetradecenylacetate trans-11-tetradecenylacetate
Archips rosanus (H)	cis-11-tetradecenylacetate
Adoxophyes orana (H)	cis-9-tetradecenylacetate cis-11-tetradecenylacetate (TAMAKI et al. 1971)
Hedya nubiferana (H)	trans-8, trans-10-dodecadienylacetate
Spilonota ocellana (H)	cis-8-tetradecenylacetate
Pammene rhediella (H)	cis-8, trans-10-dodecadienol
Cydia funebrana (H)	cis-8-dodecenylacetate (GRANGES and BAGGIOLINI 1971)
Orgyia antiqua (H)	cis-6-heneicosen-11-on
Agrotis ipsilon (H)	cis-7-dodecenylacetate cis-9-tetradecenylacetate

RESULTS

Pheromones were judged to attract a special species if males were caught in the three localities, or if the species was related to the target species and several specimens were trapped. The species are classified in two groups, first the species judged to be attracted by the pheromone (= main species) and secondly, the species group in which attractivity is considered to be questionable, or which have been trapped by mere chance (= other species) (Table 2).

A random sample of 241 specimens from the catch of the tentiform leaf miner *Phyllonorycter blancardellus* (F.) were revealed to be males. In addition, the other specimens listed in Table 2 are males if not marked otherwise.

Most of the traps caught an abundance of flies, mainly blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*). The traps for *S. exitiosa, Choristoneura hebenstreitella* (Müll.), *Argyresthia pruniella* L., *Cydia funebrana* (Tr.), *Orgyia antiqua* (L.) and *Agrotis ipsilon* (Hufn.) did not attract the target species. *S. exitiosa* does not occur in Finland and the species *C. hebenstreitella, A. pruniella, C. funebrana* and *A. ipsilon* may have been so rare that they did not occur in the trapping localities.

The flying periods of the species attracted by pheromone preparates could not be determined exactly because of the varying and in many cases long checking intervals. However, *P. blancardellus* had two generations and *Spilonota ocellana* (Den. and Shiff.) a partial second generation according to the trapping results. All the other target species seem to have had only one generation in 1984.

Table 2. Pheromone trap catches in 1984. Number of specimens from three traps in three places. H = Hoechst AG, A = Albany International.

Target species	Main species		Other species	
Phyllonorycter blancardellus (H)	*P. blancardellus*	1 350	*Argyresthia* spp.	1
	Cydia tenebrosana	16	*Gelechia* spp. ♀	1
Plutella xylostella (H)	*P. xylostella*	12	*Agriophila staminella*	1
	Cerapteryx graminis	19	*Agrotis exclamationis*	1
			Coleophora spp.	1
			Crambus spp.	3
			Cydia tenebrosana	2
			Mesoligia furungula	7
			Noctuidae, spp	6
			Xantorhoe montanata	1
Synanthedon exitiosa (A)	*Hoplodrina alsines*	64	*Euplixea lucipara*	1
			Itame wauaria	1
			Synanthedon culiciformis	1
			Synanthedon tipuliformis ♀	1
Archips podanus (H)	*A. podanus*	35	*Aleimma loeflingianum*	1
	Euhyponomeutoides rufella	25	*Argyresthia arcella*	1
	Yponomeuta spp.	9	*Callisto denticulella*	1
	Acompsia cinerella	5	*Coleophora* spp.	1
			Colocasia coryli	1
			Cydia pomonella	1
			Gelechia rhombella	1
			Hydraecia micacea	1
			Mythimna pallens	1

Target species	Main species		Other species	
Archips xylosteanus (H)	*Aphelia paleana*	153	*Acleris rhombana*	1
	Yponomeuta spp.	10	*Argyrestiha arcella*	3
	Archips oporanus	1	*Gelechia rhombella*	2
			Pandemis spp.	3
Archips rosanus (H)	*Ptycholoma lecheanum*	51	*Noctuidae*, spp.	5
Archips rosanus (A)	*A. rosanus*	102	*Argyresthia arcella* ♀♂	25
	Phalonidia manniana	12		
	Yponomeuta spp.	15		
Adoxophyes orana (H)	*A. orana*	119	*Argyresthia arcella* ♀	1
	Mamestra pisi	37	*Cucullia umbratica*	9
	Amphipoea fucosa	48	*Epigena polygona*	6
	Eulia ministrana	13	*Euhyponomeutoides rufella*	1
			Gelechia rhombella ♀	1
Hedya nubiferana (H)	*H. nubiferana*	388	*Apotomis infida*	4
	Cydia tenebrosana	62	*Argyresthia arcella* ♀♂	4
	Eucosma cana	16	*Argyresthia conjugella*	1
	Cnephasia stephensiana	6	*Coleophora* spp.	3
	Epiblema foenella	8		
Spilonota ocellana (H)	*S. ocellana*	111	*Apotomis turbidana*	3
			Archips rosanus	1
			Argyresthia arcella	5
			Coleophora spp.	1
			Cydia pomonella	1
			Gelechia rhombella	3
			Olethreutes mygindianus	4
			Pandemis spp.	1
			Xanthorhoe montanata	1
Pammene rhediella (H)	*P. rhediella*	8	*Argyresthia arcella* ♀♂	3
	Pammene populana	24	*Callisto denticulella*	1
	Celypha striana	22	*Cnephasia stephensiana*	3
	Yponomeuta spp.	12	*Cydia pactolana*	2
			Diarsia brunnea ♀	1
			Elachista apicipunctella	1
			Gelechia rhombella	6
			Ptycholoma lecheanum	1
Cydia funebrana (H)	*Cydia tenebrosana*	190	*Pammene populana*	3
	Cnephasia stephensiana	86		
	Apotomis infida	42		

DISCUSSION

Many studies have dealt with the specificity of the codling moth pheromones (e.g. CHAMBON and d'AGUILAR 1974, STENMARK 1978). In Finland, Codlemone (Zoecon Corp.) also attracted the tortricids *Pammene rhediella* (Clerck), *Pammene populana* (F.), *Hedya nubiferana* (Haw.), *Celypha* spp. and *Eucosma campoliliana* (Den and Shiff.) (HEIKINHEIMO 1978). The traps are considered to be specific enough for use by farmers because the target species is easily recognizable (HEIKINHEIMO 1981).

The traps for *P. blancardellus* attracted an

abundance of the target species and only one irrelevant species which is easily distinguished from *P. blancardellus*. *P. blancardellus* is very common in Finnish orchards although no severe damages have been noted (VAPPULA 1962). The other trapped species, *Cydia tenebrosana* (Dup.), was also caught by traps for *H. nubiferana* and *C. funebrana*.

The diamondback moth *Plutella xylostella* (L.) was quite rare in 1984 and only a few specimens were caught. *P. xylostella* is easily separated from other species so that the traps are suitable for use by farmers. The other main species caught by the traps, the antler moth *Cerapteryx graminis* L., is a common species which caused severe damage to hay fields in the early 1900's (VAPPULA 1962). Nowadays, the species is of no importance in Finland.

S. exitiosa preparate attracted no males of *S. tipuliformis*, but did attract one female of the species as well as one male of *S. culiciformis* (L.). The traps were situated in blackcurrant fields where *S. tipuliformis* was known to cause damage. 3,13-ODDA acts as a sex attractant of several *Synanthedon* species but attractivity is strictly dependent on the ratios of different isomers of the compound (BARRY et al. 1978, VOERMAN et al. 1978, REED et al. 1981). It is possible that certain isomers of the compound also attract *S. tipuliformis*, but only in strictly determinated ratios.

The pheromone preparate for *Archips podanus* (Scop.) attracted the target species and additionally the currant bud moth *Euhyponomeutoides rufella* (Tengström) which has caused severe damage in Finnish blackcurrant cultivations during last few years. In one locality, blackcurrant cultivations were situated 100 m apart from the trap and in another quite near the trap. 16 and 9 males of *E. rufella* were trapped, respectively. HEIKINHEIMO (1978) found that *A. podanus* pheromone (Zoecon Corp.) attracted the species *Aspilapteryx tringipinnella* (Zell.), *Colocasia coryli* (L.), *Hysterosia sodaliana* (Haw.) and *Acompsia cinerella* (Clerck). Of these species, 5 males of *A. cinerella* and one male of *C. coryli* were trapped in 1984. The target species is quite easily distinguishable from the other species. In Finland *A. podanus* is quite rare and there is no common need of a monitoring system for this species.

Archips xylosteanus (L.) traps caught no specimens of the target species, but did catch in abundance the plain yellow twist *Aphelia paleana* (Hübn.). However, there is no need for control or monitoring of *A. paleana* in Finland, although the species has sometimes injured timothy grass (VAPPULA 1962).

ARN et al. (1974) found that cis-11-TDA attracted *Yponomeuta padellus/malinellus* complex and the same compound has been determined as the main component of *Archips* spp. pheromone (PERSOONS et al. 1974). All *Archips* pheromones, with the exception of *Archips rosanus* (L.) supplied by Hoechst AG also attracted *Yponomeuta* species in 1984. According to the information from Hoechst AG the *A. rosanus* traps were baited with cis-11-TDA but the species trapped by the preparate were quite different from those of *A. rosanus* pheromone supplied by Albany International. Instead, *A. rosanus* (H) pheromone attracted many *Ptycholoma lecheanum* (L.) in all localities. Other species were not trapped at all. MINKS et al. (1976) found that pheromones of *P. lecheanum* are cis-11-TDol and cis-11-TDA in the ratio of 3/1. The pheromone components of *A. rosanus* have been determined to be the same compounds but in the ratio of 1/9 (DESCOINS and FREROT 1979). *A. rosanus* (A) traps also attracted some males and females of *Argyresthia arcella* (F.) which is quite common in many orchards and damages the buds of apple trees in the spring.

Adoxophyes orana (F. v R.) pheromone attracted the target species well. Of the other species caught, *Mamestra pisi* (L.) is easily separated from *A. orana* as well as *Amphipoea fucosa* (Fr.), which flies later than *A. orana*. *Eulia ministrana* (L.) may be confused with *A. orana*

but the flying periods of *A. orana* and *E. ministrana* are different. The traps are quite suitable for the monitoring of *A. orana.*

The traps for *Hedya nubiferana* (Haw.) operated well. Of the other species the most common one, *C. tenebrosana,* is easily distinguishable from *H. nubiferana.* The traps are considered to be well suited for monitoring of *H. nubiferana.*

The pheromone preparate for the bud moth *S. ocellana* was very specific. The bud moth is quite easy to recognize and separate from the other, more or less randomly trapped species.

The traps for *P. rhediella* caught only a few specimens of the target species. Of the other species, *P. populana* may be confused with *P. rhediella* but the flying period of *P. populana* is much later. HEIKINHEIMO (1978) found that pheromone traps baited with Codlemone attracted *P. rhediella* and *P. populana* in abundance. According to ARN et al. (1982) the unpurified pheromone for *C. pomonella,* trans-8, trans-10-DDol, attracted *P. rhediella.* Purified pheromone was an effective attractant only after having been in field conditions for several weeks. In 1984 some traps for *C. pomonella* (H) were situated in the same localities as *P. rhediella* traps but no specimens of *P. rhediella,* and only a few specimens of *P. populana* were trapped by them. It seems obvious that the earlier pheromone preparates were not purified as well as the newer ones. According to GUERIN et al. (1983) cis-8, trans-10-DDol acts as the sex pheromone of *P. rhediella* but the codling moth pheromone, instead, acts as an inhibitor.

No specimens of the target species were trapped by the plum moth *Cydia funebrana* pheromone, but large numbers of *C. tenebrosana, Cnephasia stephensiana* (Doup.) and *Apotomis infida* (Heinr.), which all are minor pests of fruit trees in Finland, were trapped. It is possible that the quite rare and fluctuating species *C. funebrana* was not present at the trapping localities in 1984. *C. tenebrosana* and *C. funebrana* are not easily distinguishable, and their flying periods do not differ much, although *C. tenebrosana* is a somewhat later species. HEIKINHEIMO (1978) lists many species caught by traps baited with Funemone (Zoecon Corp.) among others *C. tenebrosana,* but neither *C. stephensiana* nor *A. infida.* Also STENMARK (1978) found that Funemone attracts *C. tenebrosana* and *Pammene* spp. Differences in the catch from Funemone traps and the traps supplied by Hoechst AG may be due to the relative amounts of the trans-isomer of 8-DDA in the pheromone preparate (ARN et al. 1976, CHARMILLOT et al. 1982).

According to the specificity of the preparates the pheromone traps for *P. blancardellus* (H), *P. xylostella* (H), *A. rosanus* (A), *A. orana* (H), *H. nubiferana* (H) and *S. ocellana* (H) proved to be suitable for use by farmers. Additionally, the pheromone trap for *A. podanus* (H) attracted *E. rufella* in such an amount that the traps should be studied in blackcurrant cultivations to determine if they could be used to monitor the currant bud moth, too. Earlier, the preparates for *C. pomonella* and *C. nigricana* have been proved to be adequately specific.

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SELOSTUS

Feromonivalmisteiden lajispesifisyys ja käyttökelpoisuus perhostuholaisten esiintymisen tarkkailussa

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Maatalouden tutkimuskeskus

Hyönteisten sukupuoliferomoneja voidaan käyttää houkutteena pyydyksissä, joiden avulla tietyn hyönteislajin runsautta ja esiintymisaikoja voidaan tarkkailla. MTTK:n tuhoeläinosastolla on aikaisemmin tutkittu mm. omenakääriäisen ja hernekääriäisen feromonien käyttöä torjunnan tarpeellisuuden ja oikean ajankohdan määrittämiseen ja menetelmät ovat viljelijöiden käytettävissä.

Tutkimuksessa selvitettiin 15 perhoslajin esiintymisen seurantaan tarkoitetun kaupallisen feromonivalmisteen houkuttelemaa perhoslajistoa ja arvioitiin pyydysten käyttökelpoisuutta. Feromonivalmisteet hankittiin Hoechst AG:sta Saksan Liittotasavallasta ja Albany International'ista USA:sta.

Feromonivalmisteiden houkutusvaikutusta ja pyydystettyä lajistoa tutkittiin kolmella paikkakunnalla Etelä-Suomessa. Koepaikat valittiin pyydystettävän lajin ravintokasvien ja levinneisyyden perusteella. *Synanthedon exitiosa*-pyydykset sijoitettiin lisäksi neljälle muulle alueelle, missä tiedettiin esiintyvän samaan lasisiipisukuun kuuluvaa herukanlasisiipeä. Pyydyksiin tulleet perhoset määritettiin tarvittaessa genitaalipreparaateista.

Seuraaville perhoslajeille tarkoitetut feromonivalmisteet osoittautuivat käyttökelpoisiksi ja riittävän lajispesifisiksi: omenanpoimukalvaja, kaalikoi, omenankuorikääriäinen, versokääriäinen ja silmukääriäinen. Näiden lajien esiintymisen seuranta torjunnan tarpeellisuuden arvioimiseksi on monessa tapauksessa tarpeen.

Ruostekääriäiselle tarkoitettua feromonivalmistetta kannattaa kokeilla herukansilmukoin esiintymisen seurantaan. Herukansilmukoi soveltuisi lisäksi erittäin hyvin feromonin avulla tapahtuvan seurannan kohteeksi merkityksensä ja toisaalta vaikean havaittavuutensa vuoksi. *Archips xylosteanus*-lajille tarkoitettu feromonivalmiste houkutteli runsaasti timoteikääriäistä, pensaskääriäisen tarkkailuun tarkoitettu feromoni jonkin verran *Ptycholoma lecheanum*-lajia ja *Archips*-feromonivalmisteet omenankehrääjäkoita. Omenankehrääjäkoita varten on olemassa feromonivalmiste, jota ei kuitenkaan tässä tutkimuksessa ollut mahdollista kokeilla. Muiden mainittujen lajien osalta ei meillä ole tarvetta käyttää feromonipyydyksiä. *Synanthedon exitiosa*-feromonivalmiste ei sovellu sellaisenaan herukanlasisiiven esiintymisen seurantaan.

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Review

THE POSSIBILITIES TO INCREASE BIOLOGICAL NITROGEN FIXATION BY BREEDING

MARKETTA SAASTAMOINEN

SAASTAMOINEN, M. 1986. **The possibilities to increase biological nitrogen fixation by breeding.** Ann. Agric. Fenn. 25: 147—155. (Agric. Res. Centre, Dept. Pl. Breed., SF-31600 Jokioinen, Finland.)

The ability of living organisms to use the atmospheric source of nitrogen is limited. Legumes in symbiosis with *Rhizobium* bacteria have the highest significance in biological nitrogen fixation when studying agricultural ecosystems. Nitrogen fixing bacteria that live on the roots (root-associated nitrogen fixation) are also important. This article reviews some previous results concerning the possibilities to increase the biological nitrogen fixation by breeding.

Genetic variation in the root-associated nitrogen fixation has been found in rice, and when breeding maize, root-associated nitrogen fixation has been increased.

Symbiotic nitrogen fixation can be increased by breeding techniques. These techniques can be directed towards selection and breeding of *Rhizobium*, breeding of the host plant, and breeding of the interaction between the *Rhizobium* and the host. There are many differences between the *Rhizobium* strains in their ability to fix nitrogen. This ability depends greatly on the genotype of the host plant, for it is clear that there are interactions between the *Rhizobium* strains and host genotypes. A great genetic variation in the ability to fix nitrogen has been found in the host plant, in the pea, alfalfa, and in the soybean. The estimated heritability to fix nitrogen in the pea has been high.

The possibilities to transfer the nitrogen fixation genes (nif genes) from the *Rhizobium* to higher plants by genetic engineering has been greatly explored. Some questions that must be solved by the techniques of genetic engineering are nif gene transfer and expression, energy supply, and oxygen protection.

Index words: biological nitrogen fixation, legumes, root-associated nitrogen fixation, nitrogenase, symbiotic nitrogen fixation, plant breeding, selection.

Atmospheric nitrogen is the greatest source of nitrogen in the world. The ability of living organisms to use the atmospheric nitrogen is although limited. The atmospheric nitrogen is being fixed by free living bacteria, cyanobacteria (called bluegreen algae), lichens, leguminous plants in symbiosis with *Rhizobium* bacteria, by other root nodules forming higher plants in symbiosis with micro-organisms, and by the leaf nodules forming higher plant (*Ardisia crispa*), in

Table 1. Atmospheric nitrogen fixation and nitrogen fixing organisms (STANIER et al. 1971, LETHBRIDGE and DAVIDSON 1983, MURTY 1983).

Type of N_2 fixation	Host plant/Associated plant	N_2 fixing micro-organism
Nonsymbiotic nitrogen fixation		heterocyst forming cyanobacteria (blue green algae): f.ex. *Anabaena, Nostoc* aerobic bacteria: f.ex. *Azotobacter, Beijerinckia* other bacteria: fotosynthetic bacteria, *Clostridium* sp., *Bacillus polymyxa*
Symbiotic nitrogen fixation	Lichens	*Nostoc, Anabaena,* Polypothrix
	Root nodules forming, leguminous plants	*Rhizobium* sp.
	Root nodules forming, nonleguminous plants: *Coriaria, Myrica, Alnus, Casuarina, Hippophae, Shepherdia, Ceanothus, Discaria*	
	Leaf nodules forming, nonleguminous plants: *Ardisia crispa*	
Root-associated nitrogen fixation	Several plants: wheat	*Azotobacter beijerinchii* *Azospirillum brasilense*
	maize	*Azospirillum brasilense*
	sugarcane	
	rice	
	Paspalum	
	Digitaria	
Nitrogen fixation on leaf surfaces	Several plants: sugarcane	*Beijerinckia*
	sorghum	,,
	Eleusine coracana	,,
	Bamboosa spp.	,,
	Morus indica	,,

symbiosis with micro-organisms (Table 1).

In the agricultural production the most important nitrogen fixers are the legumes. The annual rates of N_2 fixation in well-managed alfalfa (*Medigaco sativa*) vary from 300 to 600 kg N/ha (LYON and BIZZEL 1934, MISHUSTING and SHIL'NIKOVA 1971, SCHERTZ and MILLER 1972). Free living nitrogen fixing bacteria may give some support in the cultivation. Root-associated nitrogen fixing micro-organisms may fix one part of the nitrogen needed by the plants. Accumulation of nitrogen in the range of 38—165 kg per ha per year in standing vegetation of plant fallows has been measured according to the review article of BERKUM and BOHLOOL (1980). Nitrogen-fixing micro-organisms have been found on the leaf surfaces of sugarcane, sorghum, and other plants (MURTY 1983).

During the fixation process the atmospheric dinitrogen, N_2, is reduced to ammonia, NH_3. The nitrogen fixing enzyme, nitrogenase, ATP, and one of the reducing components, ferredoxin or flavodoxin, are needed for nitrogen fixation. 12—24 ATP molecules are needed in the fixation of each N_2 molecule. Nitrogenase is always synthetized by the prokaryotic micro-organisms (BRILL 1980).

Some breeding techniques have been used to

increase N_2 fixation. When these techniques were applied to maize there was an increase in root-associated N_2 fixation (ELA et al. 1982). Symbiotic nitrogen fixation can be increased by breeding the *Rhizobium*, by breeding the host plant for higher N_2 fixation, and by breeding for a better interaction between the host plant and the *Rhizobium*.

Breeding techniques used to possibly increase root-associated N_2 fixation

Root-associated nitrogen fixation has been found in many plants (BERKUM and BOHLOOL 1980, ELA et al. 1982, IYAMA et al. 1983). Inoculation with *Azospirillum brasilense* increased the grain and straw yields of barley and maize as much as 40 kg/ha N fertilizer (SUBBA RAO et al. 1979).

Some chemical components that are secreted by the roots of grasses, support the adhesion of bacteria to the root epidermis (UMALI-GARZIA et al. 1980). All nitrogen fixing bacteria have fimbriae by which they adhere to the roots. Type-3 fimbriae found in *Klebsiella* are more effective in adhesion ability than the mannose-binding type-1 fimbriae found in *Enterobacter* strains (HAAHTELA and KORHONEN 1985). Plant lectins seem to be inactive in adhesion (HAAHTELA and KORHONEN 1985), even if they play an important role in the initiation of the nodulation in symbiotic nitrogen fixation. By selecting the bacteria species with type-3 fimbriae for inoculation, it is maybe possible to increase the root-associated nitrogen fixation.

In root-associated nitrogen fixation the type of bacteria, and the genotype of the plant, play a significant role in the effectiveness of nitrogen fixation. A genetic variation in root-associated nitrogen fixation has been found in rice (LEE et al. 1977, HIROTA et al. 1978, SANO et al. 1981, IYAMA et al. 1983) and in maize (ELA et al. 1982). ELA et al. (1982) have been able to increase the root-associated nitrogen fixation in maize from 0,0—5,0 nmol C_2H_4/plant/h to 39 nmol C_2H_4/plant/h by breeding techniques. The best of the selected maize plants have however been only ca 0,5 % as active as soybeans inoculated with *Rhizobium japonicum*, and grown and assayed in the same manner as the maize plants. The results suggest that plants can be bred for higher root-associated nitrogen fixation.

By using a diallel analysis IYAMA et al. (1983) have estimated the heritability of $0{,}266 \pm 0{,}245$ for the root-associated nitrogen fixation in rice. Some other researchers have found that substitutions of special chromosomes have increased the root-associated nitrogen fixation in wheat (NEAL and LARSON 1976, RENNIE and LARSON 1979). Results have shown that by applying plant breeding techniques it is possible to increase the root-associated nitrogen fixation in cereals.

Some results show that the root-associated nitrogen fixation may be significant in leguminous plants, when associated with the symbiotic nitrogen fixation. Lentil (*Lens culinaris*) inoculated with *Azospirillum brasilense* or its mutant strains, and with *Rhizobium* strains simultaneously, has increased nodule dry weight. It has also increased the nitrogenase activity of nodules and roots and grain yield compared to the uninoculated control and the plants inoculated only with *Rhizobium* strains, or with *A. brasilense* strains (RAI 1985).

Symbiotic N_2 fixation in legumes

The symbiotic nitrogen fixation is the most effective nitrogen fixation in the agricultural ecosystems, but it is restricted to the *Leguminosae* plants. However, all *Leguminosae* species are not able to form nodules and fix N_2. About 90 % of the subfamilies *Mimosioideae*

and *Papilionatae* and 30 % of the *Caesalpinioideae* are able to nodulate (BURNS and HARDY 1975). The ability of *Rhizobium* species to nodulate legumes is very host specific; e.g. *Trifolium* spp. are nodulated by *R. trifolii*, *Phaseolus* spp. by *R. phaeseoli* and *Pisum*, *Vicia*, *Lathyrus*, and *Lens* by *R. leguminosarum*. *Rhizobium* usually fixes nitrogen only in symbiosis, but it has also been able to produce nitrogenase on culture media in microaerophilic conditions.

The nodulation and the activity of nitrogenase are influenced by many factors. In the symbiotic nitrogen fixation, the *Rhizobium* needs some carbohydrates and energy from the host plant in order to give the ammonia to the plant. It has been suggested that there is a positive correlation between the rate of nitrogen fixation and photosynthesis (BERGERSEN 1977).

Breeding for higher N_2 fixation in legumes

It is possible to breed for a higher nitrogen fixation in legumes by selection and breeding of *Rhizobium*, by selection and breeding of the host plant, and by breeding for a better interaction between the host and *Rhizobium* genotypes. Most of the work to achieve a higher nitrogen fixation has been made in selecting better *Rhizobium* strains which can be bred artificially. Transduction and conjugation are possible gene-transfer systems in *Rhizobium* (ROBERTS and BRILL 1981).

Selection and breeding of *Rhizobium*

It has been known that the *Rhizobium* strains vary in their nitrogen fixation ability. Inoculation using the effective strains belongs to the practical cultivation of the leguminous plants.

Some *Rhizobium* strains have a hydrogenase enzyme which makes them more effective in nitrogen fixation. Nitrogenase has a side reaction that hydrolyzes adenosine triphosphate (ATP) and forms H_2. This reaction uses ATP, but it has no special function (HARDY et al. 1965). Some *Rhizobium japonicum* strains, Hup^+, have a hydrogenase that regenerates ATP by oxidation of H_2, thus the ATP is available for use in further nitrogen fixation. Soybeans inoculated with Hup^+ strains of *Rhizobium japonicum* have produced higher yields and the protein content of the soybeans has increased (ALBRECHT et al. 1979, HANUS et al. 1981). LA FAVRE and FOCHT (1983) have found that the pigeon peas (*Cajanus cajan*) inoculated with the hydrogenase-positive *Rhizobium* strain, P132, have yielded significantly more total shoot N than other inoculated or uninoculated treatments. However two other hydrogenase-positive strains have not increased the total N of the pigeon peas. By using transduction or conjugation it is possible to transfer the hydrogenase gene(s) to other *Rhizobium* strains.

Nitrogen fertilization and availability of NO_3^- may decrease nodulation and the activity of nitrogenase (FRANCO et al. 1979). The research to find the relationship between nitrate and nitrite reduction, and nitrogenase activity has become a current topic. STEPHENS and NEYRA (1983) have found in nitrate reductase-negative

mutant strains of *R. japonicum* that the addition of 20 mmol KNO_3 to bacteroids of wild-type strains has caused a decrease in nitrogenase activity by more than 50 %, but the nitrate reductase-negative strains have been insensitive to nitrate.

Most of the legumes do not grow well in acid or alkaline conditions, although *Medicago sativa, Glycine wightii* and some *Trifolium* species can grow quite well at pH 4,0 in a solution culture (MUNNS 1965, MULDER et al. 1966, HELYAR and ANDERSON 1971). The *Rhizobium* strains vary in their ability to grow in acid soils (KEYSER and MUNNS 1979, KEYSER et al. 1979). In the strains of *R. japonicum* and *R. trifolii* there is a genetic variation in their ability to grow and fix nitrogen in acid conditions (KEYSER and MUNNS 1979, THORNTON and DAVEY 1983). By artificial selection it is possible to find acid tolerant *Rhizobium* strains.

Selection and breeding of the host plant for higher N_2 fixation

Genetically very little is known about the effects of the host plant on the intensity of nitrogen fixation. However a rather great genetic variation has been found in the ability to nodulate and fix nitrogen in alfalfa (SEETIN and BARNES 1977, DUHIGG et al. 1978, HOFFMAN and MELTON 1981), in soybean (PATTERSON and LARUE 1983), in peas (HOBBS and MAHON 1982 a), in southern pea (*Vigna unquiculata*) (ZARY et al. 1978) and in Spanish clover (*Desmodium sandwicence*) (PINCHBECK et al. 1980). HOBBS and MAHON (1982 a) have found a heritability value of 0,76 for $N_2(C_2H_2)$ fixation in peas. They assume that simple selection could be used in improving nitrogen fixation in peas. SEETIN and BARNES (1977) have found that crosses between alfalfa clones with high acetylene reduction values have produced progenies with doubled acetylene reduction rates compared to the progenies from low × low crosses. High nitrogen fixation has been found to be associated with late maturity in soybeans (PATTERSON and LARUE 1983) and in peas (RYDBERG 1983). In peas the short stalk and the low nitrogen fixation are associated (RYDBERG 1983).

Very little practical breeding has been done to increase nitrogen fixation in the host. PULVER et al. (1985) suggested that the breeding of the host plant for a higher nitrogen fixation is a more effective and economical way for the developing countries than the production and distribution of high quality rhizobia. This is because these countries usually lack facilities to take care of the production and distribution of the inoculants. They have also tested 400 different soybeans at five locations in Nigeria for the ability to nodulate with indigenous rhizobia. They found only 10 promiscuous soybeans capable of forming an effective symbiosis at all locations. The best of the soybeans was a local Nigerian cultivar.

It has been found that the host genotype has great effect on the hydrogenase activity of *Rhizobium* strains. DIXON (1972) found that *R. lequminosarum* strain, ONA 311, expressed strongly Hup^+, slightly Hup^+, or Hup^- phenotypes on *Pisum sativum, Vicia bengelensis,* and *Vicia faba,* respectively. Two *R. japonicum* strains have been Hup^+ on three cowpea (*Vigna unguiculata*) cultivars and Hup^- on three soybean cultivars (KEYSER et al. 1982). The *R. leguminosarum* strain, 128C53, has been described in this literature as Hup^+ (RUIZ-ARGÜESO et al. 1978, BETHLENFALVAY and PHILLIPS 1977, NELSON and CHILD 1981, NELSON and SALMINEN 1982), or as Hup^- (DIXON et al. 1981) on *Pisum sativum.* BEDMAR et al. (1983) found that the *R. leguminosarum* strains, 128C53 and 3960, showed only 10 % of the hydrogenase activity in symbiosis with pea cultivar Feltham First when compared to that in symbiosis with pea cultivars Alaska and JI1205.

Breeding for better inreraction between host plant and *Rhizobium*

Nodulation and the regulation of the symbiotic nitrogen fixation is a complex process in which the host plant and the *Rhizobium* are taking part. The *Rhizobium* genes involved in host specificity, nod genes for nodulation, and nif genes for the synthesis of nitrogenase enzyme are located in a large plasmid, sym, of the *Rhizobium* (AUSUBEL 1982, GOVERS et al. 1985). Leghemoglobin which binds O_2 in the nodule, thus preventing the inactivation of the nitrogenase is coded partly by the host plant (CUTTING and SCHULMAN 1971) and partly by the *Rhizobium* (NADLER and AVISSAR 1977). Nodule-specific proteins, nodulins, are coded by the host plant (LEGOCKI and VERMA 1980, GOVERS et al. 1985). While the genes responsible for the nodulation and the symbiotic nitrogen fixation are separated in the genome of the host plant and the genome of the *Rhizobium,* it is clear that there are great interactions between the host genotype and the *Rhizobium* strain.

It is well known that the intensity of the nitrogen fixation is dependent on the host plant and the *Rhizobium* strain, simultaneously, causing an interaction between the genotypes (e.g. HOBBS and MAHON 1982 b, HOBBS and MAHON 1982 c). In *Pisum* a resistance to *Rhizobium* has also been found. OHLENDORF (1983 a, 1983 b) has found from a pea collection of 120 cultivars, 5 cultivars, 3 from Afghanistan, 1 from China and 1 from Iran, which are resistant to the effective *Rhizobium* strain 311d. Two effective *Rhizobium* strains from Israel and Turkey, however, have been able to nodulate these resistant pea lines (OHLENDORF 1983 a). LIE et al. (1982) found that the European *Rhizobium leguminosarum* strains, that are effective on the cultivated pea, induce ineffective nodules on pea ecotypes, elatius and abyssinicum. The elatius and abyssinicum plants have been nodulated by *Rhizobium* strains from the Middle East. LIE et al. (1982) conclude that there is a co-evolution between the legumes and the local *Rhizobium* strains.

Gene technology and biological N_2 fixation

The possibility that the nif genes of bacteria could be transferred to cereal plants is being explored in many laboratories. BARTON and BRILL (1983) have discussed the difficulties in this work. These difficulties included the expression and regulation of the prokaryotic nif genes in an eukaryotic cell. BRILL (1980) has mentioned the O_2 lability of the nitrogenase.

In the engineering work the following questions must be answered: nif gene transfer and expression, energy supply, and oxygen protection (KRIEGER 1984). It is possible to incorporate bacterial DNA into plant cells, but we still do not know if the nif genes will activate in a plant, and thus supply the plant with the N_2 needed. Because CO_2 is the limiting factor in photosynthesis and the chloroplast is a prokaryotic organelle an attempt to solve the above problems is to incorporate the nif genes into the chloroplast of the plant cell (KRIEGER 1984, MERRICK and DIXON 1984). Chloroplasts evolve large quantities of oxygen which inactivates nitrogenase. There are however oxygen sensitive cytochromes within the chloroplast inner membrane, which must somehow be protected from contact with oxygen (KRIEGER 1984).

MERRICK and DIXON (1984) have listed some suggestions to the problem of the oxygen sensitivity of the nitrogenase: in *Klebsiella pneumoniae,* the nifL gene product acts to repress nif gene transcription in the presence of oxygen; in cyanobacteria the nitrogen fixation takes place in heterocysts which lack photosystem II of photosynthesis; in certain tissues of higher plants

(the bundle sheath cells of C4 plants, such as maize) oxygen evolution by photosystem II does not occur; a possibility to protect the nitrogenase from oxygen is to regulate nif genes so that they are expressed only in non-photosynthetic roots.

Plant breeders see many of the expectations for applying gene technology to crop improvement unrealistic. It must be remembered that even if a nitrogen fixing cereal would be created, it would only be raw material for a practical breeder. We do not know what problems there would be after transferring nif genes to the higher plants.

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SELOSTUS

Kasvinjalostuksen mahdollisuudet parantaa biologista typensidontaa

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Maatalouden tutkimuskeskus

Maapallon suurin typpivaranto sijaitsee ilmakehässä. Kuitenkin ainoastaan harvat eliöryhmät pystyvät käyttämään hyväkseen tätä ilmakehän vapaata typpeä. Sitä pystyvät sitomaan siniviherlevät, eräät vapaana elävät bakteerit, jäkälät, palkokasvit ja eräät muut juurinystyröitä muodostavat bakteerien kanssa yhteiselämässä eli symbioosissa elävät kasvit sekä lehtinystyröitä muodostavat kasvit. Myös juurien ja lehtien pinnoilla elävillä typpeä sitovilla bakteereilla on merkitystä typen sidonnassa.

Typensidonnan aikaansaava entsyymi, nitrogenaasi, on aina prokaryoottisen mikrobin, bakteerin tai siniviherlevän, syntetisoima. Symbioottiseen typensidontaan osallistuu myös isäntäkasvin perimä eli genomi. Esim. nystyrässä tavattava leghemoglobiini on osittain isäntäkasvin, osittain symbioottisen bakteerin aikaansaama, syntetisoima.

Assosiatiivista kasvien juurien pinnalla tapahtuvaa typensidontaa pystytään lisäämään kasvinjalostuksen avulla. Ainakin maissilla on saatu tämänsuuntaisia tutkimustuloksia.

Symbioottista palkokasveilla tapahtuvaa typensidonnan tehokkuutta voidaan lisätä kasvinjalostuksen avulla. Jalostustyö voi suuntautua kolmeen eri kohteeseen: 1) *Rhizobium*-bakteerin valinta ja jalostus, 2) isäntäkasvin jalostus, 3) *Rhizobium*-in ja isäntäkasvin välisen yhteisvaikutuksen eli interaktion jalostus. Hyvin suurelta osalta tutkimus- ja jalostustyö on painottunut *Rhizobium*-kantojen tutkimiseen. Kuitenkin myös isäntäkasvilla näyttää olevan suuri merkitys typensidonnan tehokkuudessa. melko suuri perinnöllinen eli geneettinen vaihtelu tässä suhteessa on mm. sinimailasessa, soijapavussa ja herneessä. Herneen typensidontakyvyn periytymisaste eli heritabiliteetti on korkea, joten sen pitäisi olla myös helposti jalostettavissa.

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