

## Performance of laying hens on diets containing Eurolysine bacterial protein or Pekilo protein

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**Abstract.** The investigation was made to determine to what extent soybean meal and fish meal protein in the diet of laying hens can be replaced by Eurolysine bacterial protein, a by-product of lysine fermentation, or with Pekilo protein. In a 24-week laying trial the inclusion levels of Eurolysine in the diet were 0-4.0-7.9-11.9 % and those of Pekilo 0-6.3-12.7-18.0 %, or 0-33-66-100 % of the protein supplement. Eurolysine contained 68.5 % crude protein 53.5 % true protein and 6.9 % ether extract in DM and the corresponding values of Pekilo were 43.3 %, 38.8 % and 1.4 %. The average laying rates decreased with increasing inclusion of Eurolysine, and replacement of the protein supplement by Pekilo also lowered egg production, but the differences between the treatments were not significant ( $P > 0.05$ ). Feed intakes were increased ( $P < 0.05$ ) by inclusion of SCP. Feed conversion efficiency did not differ significantly among the treatments ( $P > 0.05$ ). Mortality increased with inclusion of both types of SCP in the diets, but the principal cause of death was cannibalism.

### Introduction

In recent years there has been a great deal of interest in the development of single cell proteins (SCP) from yeasts, fungi and bacteria grown on various substrates. In many experiments equally good laying results have been achieved when SCP products have replaced conventional protein sources (WALDROUP and HAZEN 1975, VOGT et al. 1975, SHANNON et al. 1976, VOGT et al. 1978, WHITTEMOORE et al. 1978).

In Finland investigations have been made with diets for broilers and layers including protein from the microfungi (*Paecilomyces varioti*) known as Pekilo and from *Torula* yeast (Silva) cultivated in sulphite spent liquor from the pulping industry (POUTIAINEN 1973, SALO 1977, KIISKINEN 1979). In these experiments at least half of the protein supplement could be of microbial origin without adverse effect on production or feed conversion.

Eurolysine is a by-product of *Bacterium lactofermentum*, which is used during the aerobic fermentation of beet molasses for manufacturing L-lysine. The crude protein content is 76 % and the lysine content is also high.

This report concerns the performance of layers in a feeding trial in which soybean and fishmeal protein were gradually replaced by Eurolysine or Pekilo protein.

## Material and Methods

The feeding experiment was carried out with 504 White Leghorns, strain SK-51 (Siipikarjanhoitajain Liitto). The hens were housed in stair-model cages containing three birds. The temperature, relative humidity, light and ventilation rate in the henhouse were controlled. The trial started when the hens were 30 weeks old. After a two-week standardization period, when the hens were fed a commercial layers' ration, the birds were distributed at random among seven different treatments, 4 or 5 subgroups of 12–18 hens being allocated to each treatment. During a two-week transition period the hens were gradually changed to the experimental diets. The experimental mashes were prepared by Vaasa Mills Co. and had the form of granules 2.5 mm in diameter. The ingredients of the experimental feeds are shown in Table 1.

The treatments and codes were as follows

1. EPO, Fish meal (FM) and soybean meal (SMB), (50 % + 50 %) protein supplement
2. E33, Eurolysine replacing 33 % of FM + SBM supplement
3. E66, Eurolysine replacing 66 % of FM + SBM supplement
4. E100, Eurolysine replacing 100 % of FM + SBM supplement
5. P33, Pekilo replacing 33 % of FM + SBM supplement
6. P66, Pekilo replacing 66 % of FM + SBM supplement
7. P100, Pekilo replacing 100 % of FM + SBM supplement

Table 1. Composition of the mixtures.

Ingredients, %	Treatments						
	1. EPO	2. E33	3. E66	4. E100	5. P33	6. P66	7. P100
Barley	55.7	56.2	56.8	57.2	53.9	52.0	50.3
Oats	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Fish meal	6.8	4.4	2.0	—	4.4	2.0	—
Soybean meal	6.8	4.4	2.0	—	4.4	2.0	—
Eurolysine	—	4.0	7.9	11.1	—	—	—
Pekilo	—	—	—	—	6.3	12.7	18.0
Rapeseed oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0
CaCO <sub>3</sub>	6.6	6.7	6.8	7.0	6.7	6.8	7.0
CaHPO <sub>4</sub> ·2H <sub>2</sub> O	1.5	1.7	1.9	2.1	1.7	1.9	2.1
Trace elements mix. <sup>1</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin supplement <sup>2</sup>	0.115	0.115	0.115	0.115	0.115	0.115	0.115

<sup>1</sup> Provided per kg feed: magnesium 130 mg, sodium 992 mg, iron 30 mg, chloride 900 mg, zinc 60 mg, iodine 0.70 mg, copper 3.5 mg, manganese 79 mg, cobalt 0.798 mg, molybdenum 0.020 mg and selenium 0.066 mg.

<sup>2</sup> Provided per kg feed: vitamin A 24000 IU, vitamin D 2000 IU, vitamin E 18.20 mg, vitamin K<sub>3</sub> 2.22 mg, vitamin B<sub>2</sub> 4.8 mg, B<sub>6</sub> 3.42 mg, B<sub>12</sub> 0.01 mg, biotin 0.02 mg, niacin 20 mg, folic acid 0.58 mg, pantothenic acid 9.39 mg, choline 100 mg, carophyllred 0.65 mg.

The period during which the treatments were compared lasted 24 weeks, from week 34 to week 58 in the life of the hens. The diets were fed *ad libitum* and the feed consumption was recorded for each subgroup for a 28-day period. Egg production was recorded daily by weighing and counting the eggs. The feed ingredients and experimental mixtures were analysed according to the standard methods. The mineral contents of the feeds were analysed with an atomic absorption spectrophotometer (Varian Techron AA 1000) and phosphorus by the method of TAUSSKY and SHORR (1953). The amino acid contents of Eurolysine and Pekilo were determined with a gas chromatograph, Hewlett Packard 5710 A, as described by NÄSI and HUIDA (1982). Pepsin-HCl-soluble protein was determined after 20-h digestion of a 0.5 g sample in 50 ml 0.1 N HCl containing 50 mg pepsin. The HYLPS programme (ANON. 1981) was used for calculating the production results and for the statistical analysis.

## Results and Discussion

The chemical composition of the feed ingredients and the experimental mixtures is presented in Tables 2 and 4. The crude protein (CP) content of Eurolysine, 68.5 %, was lower than that given by the manufacturer, 76 % of DM. The protein content of Pekilo, 43.4 %, was also lower than the values reported earlier. This caused a lower protein content in the diets containing single cell protein than in the others. The crude protein content of the treatments varied from 16.1 to 17.9 % of DM. However, the metabolizable energy (ME) content of the mixtures containing SCP was also lower because of inclusion of larger calcium and phosphorus supplements, and thus the CP:ME ratios were almost the same, 12.8–13.9 g CP/MJ ME.

The ME content of Eurolysine has been reported to be 8.4 MJ/kg for poultry (LARBIER 1979). When Eurolysine was used as protein in a digestibility trial with pigs the ME value obtained was 11.1 MJ/kg DM (NÄSI 1982). The present digestibilities for organic matter and crude protein were 55.0 % and 64.3 %, respectively. The pepsin-HCl-soluble protein in Eurolysine constituted only 52.9 % of crude protein, which is rather low compared with

Table 2. Chemical composition of the ingredients of the mixtures (% of DM).

Composition %	Barley	Oats	Fish meal	Soybean meal	Eurolysine	Pekilo
Dry matter %	87.6	87.5	91.4	87.7	92.6	94.1
Ash	3.0	3.0	14.2	6.4	4.3	5.4
Crude protein	10.9	13.6	72.3	49.2	68.5	43.3
True protein	10.2	12.5	57.9	47.4	53.5	38.8
Ether extract	2.4	5.6	9.0	2.2	6.9	1.4
Crude fibre	6.8	8.4	0.3	7.5	0.6	7.7
N-free extract	76.9	69.4	4.2	34.7	19.7	42.2

Table 3. Comparison of the amino acid composition of Eurolysine and Pekilo protein.

Amino acids g/16 g N	Eurolysine	Pekilo
Alanine	7.0	5.6
Arginine	3.4	4.6
Aspartic acid	7.1	7.1
Glutamic acid	9.7	11.5
Glycine	3.7	4.2
Histidine	0.9	1.2
Isoleucine	3.6	3.5
Leucine	5.8	6.1
Lysine	8.3	5.5
Methionine	0.6	0.5
Phenylalanine	3.0	3.3
Proline	2.6	3.5
Serine	3.0	3.7
Threonine	3.7	3.7
Tyrosine	2.1	2.7
Valine	4.5	4.3

Table 4. Chemical composition (% of DM) and feed values of the mixtures.

	Treatments						
	1. EPO	2. E33	3. E66	4. E100	5. P33	6. P66	7. P100
<i>Chemical composition</i>							
Dry matter %	88.4	88.1	88.4	88.2	88.9	88.9	88.6
Ash	9.8	9.9	10.2	10.0	10.0	9.8	9.4
Crude protein	17.9	17.1	16.9	16.1	17.6	16.8	16.2
True protein	16.1	15.1	14.8	13.6	15.7	15.0	14.6
Ether extract	5.5	5.6	5.5	5.4	5.1	5.2	5.0
Crude fibre	8.1	6.6	6.7	6.5	6.5	6.7	7.7
NFE	58.7	60.8	60.7	62.0	60.8	61.5	61.7
Phosphorus g/kg	7.03	7.51	7.61	7.84	7.34	7.78	7.80
Calcium g/kg	21.08	23.38	25.38	25.34	24.62	24.24	23.35
Magnesium g/kg	2.03	1.86	1.82	1.68	1.83	1.95	1.90
Potassium g/kg	5.84	5.57	4.88	4.56	4.91	4.40	4.40
Sodium g/kg	0.25	0.20	0.19	0.18	0.18	0.18	0.15
Iron mg/kg	267	248	325	241	234	233	226
Copper mg/kg	15	15	15	15	13	15	17
Zinc mg/kg	180	162	183	184	183	198	206
Manganese mg/kg	135	124	141	141	149	163	161
<i>Feed values</i>							
ME MJ/kg feed	11.43	11.20	10.97	10.77	11.37	11.29	11.22
Crude protein %	15.9	15.1	14.9	14.2	15.6	14.9	14.4

93.4 % for soybean meal, 88.7 % for fish meal and 71.0 % for Pekilo protein. The low values for protein digestibility suggest careless treatment during preparation presumably in drying.

In bacterial protein the nucleic acid nitrogen constitutes 15–27 % of total



nitrogen and in yeast 13–27 % (SCHULZ and PETERSEN 1979). LEHTOMÄKI (1979) reports that the nucleic acid content in Pekilo is 9.6–10.8 % when the crude protein content is 51–63 %. Absorption of nucleic acids is fairly effective, 66–87 % (SHANNON and McNAB 1973, GREIFE et al. 1981), but they are of minor value as a protein source for monogastric animals (ROTH and KIRCHGESSNER 1980).

The amino acid composition of Eurolysine and Pekilo is shown in Table 3. The lysine content is high 8.3 g/16 g N, exceeding the value for fish meal. Like other microbial products Eurolysine is poor in sulphur-containing amino acids (SCHULZ and OSLAGE 1976). Gas chromatography gave a methionine content of only 0.6 g/16 g N. The manufacturer gives a value of 1.37 g. Methionine is the primary limiting amino acid in poultry diets and improved performance has been achieved by LD-methionine supplementation in microbial protein feeding (WALDROUP and HAZEN 1975, SCHULZ and OSLAGE 1976, WHITTEMORE et al. 1978, KIISKINEN 1979). In diets for pigs the digestibilities of most amino acids were lower with Eurolysine as protein supplement than with soybean meal (NÄSI and HUIDA 1982).

The production results with the different treatments are shown in Table 4. During the standardization period, when the hens were aged 31 weeks, the laying rate averaged 88 %.

During the experimental period of 24 weeks the average laying rates fell and the trends of each treatment are shown in Figure 1. As soybean meal and fish meal were replaced by Eurolysine, the laying rates gradually decreased, the difference between groups EPO and E100 being 6.1 %. Replacement with Pekilo also gave lower laying rates at the levels of 33 and 100 %, but at 66 % replacement the laying rate was 0.9 %-units higher than that of the control. The differences between the treatments were not statistically significant ( $P > 0.05$ ). The average egg weight also decreased gradually with inclusion of single cell protein in the diets, but not significantly ( $P > 0.05$ ).

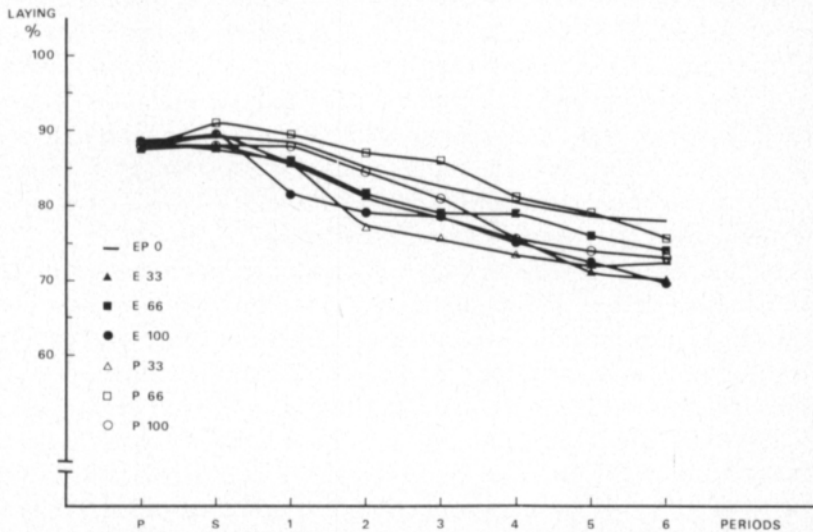


Figure 1. Average laying rates for the treatments during the standardization period and the 24-week test period.

Table 5. Production results from experiment with Eurolysine and Pekilo as protein supplement for laying hens.

	Treatments						
	1. EPO	2. E33	3. E66	4. E100	5. P33	6. P66	7. P100
<i>Number of hens</i>							
Beginning of expt.	75	76	79	76	59	62	59
End of expt.	73	72	72	68	52	59	49
Mortality %	2.7	5.3	8.9	10.5	11.9	4.8	16.9
<i>Laying</i>							
Laying rate, initial period (14d)	88.3 <sup>a</sup>	87.6 <sup>a</sup>	88.2 <sup>a</sup>	88.4 <sup>a</sup>	87.7 <sup>a</sup>	87.7 <sup>a</sup>	87.9 <sup>a</sup>
Laying rate, test period (168d)	82.1 <sup>a</sup>	77.0 <sup>a</sup>	79.2 <sup>a</sup>	76.0 <sup>a</sup>	76.0 <sup>a</sup>	83.0 <sup>a</sup>	79.4 <sup>a</sup>
Eggs g/hen/d	49.2 <sup>a</sup>	45.5 <sup>a</sup>	46.4 <sup>a</sup>	43.8 <sup>a</sup>	45.0 <sup>a</sup>	49.1 <sup>a</sup>	46.7 <sup>a</sup>
Egg weight, g	60.0 <sup>a</sup>	59.3 <sup>a</sup>	58.7 <sup>a</sup>	57.7 <sup>a</sup>	59.2 <sup>a</sup>	59.3 <sup>a</sup>	58.9 <sup>a</sup>
<i>Feed intake</i>							
Feed g/hen/d	126 <sup>b</sup>	125 <sup>bc</sup>	128 <sup>ab</sup>	134 <sup>ad</sup>	130 <sup>ab</sup>	128 <sup>ab</sup>	133 <sup>a</sup>
Feed DM g/hen/d	111 <sup>b</sup>	110 <sup>bc</sup>	111 <sup>b</sup>	118 <sup>ad</sup>	115 <sup>ab</sup>	114 <sup>ab</sup>	118 <sup>ad</sup>
Feed CP g/hen/d	19.9 <sup>ab</sup>	18.8 <sup>bc</sup>	18.8 <sup>ac</sup>	19.0 <sup>ab</sup>	20.2 <sup>ad</sup>	19.1 <sup>ab</sup>	19.1 <sup>ab</sup>
Feed ME MJ/hen/d	1.43 <sup>ab</sup>	1.40 <sup>b</sup>	1.38 <sup>bc</sup>	1.44 <sup>ad</sup>	1.47 <sup>a</sup>	1.45 <sup>ab</sup>	1.49 <sup>ad</sup>
<i>Feed conversion efficiency</i>							
Feed kg/kg eggs	2.56 <sup>a</sup>	2.76 <sup>a</sup>	2.72 <sup>a</sup>	3.07 <sup>a</sup>	2.97 <sup>a</sup>	2.62 <sup>a</sup>	2.88 <sup>a</sup>
Feed DM kg/kg eggs	2.26 <sup>a</sup>	2.43 <sup>a</sup>	2.40 <sup>a</sup>	2.71 <sup>a</sup>	2.62 <sup>a</sup>	2.32 <sup>a</sup>	2.55 <sup>a</sup>
Feed CP g/kg eggs	406 <sup>a</sup>	416 <sup>a</sup>	406 <sup>a</sup>	436 <sup>a</sup>	451 <sup>a</sup>	389 <sup>a</sup>	412 <sup>a</sup>
Feed ME MJ/kg eggs	29.2 <sup>a</sup>	30.9 <sup>a</sup>	29.8 <sup>a</sup>	33.1 <sup>a</sup>	33.6 <sup>a</sup>	29.5 <sup>a</sup>	32.2 <sup>a</sup>

Differences between means with different letters were statistically significant. (a, b, (P < 0.05), d, e, P < 0.01)

The feed intakes increased when the diets were supplemented with Eurolysine or Pekilo (P < 0.05, 0.01). The ME values of those diets were lower than the value of the control diet due to higher mineral supplementation. The crude protein and ME intakes differed significantly between treatments (P < 0.05, 0.01). The feed conversion deteriorated as an increasing percentage of conventional protein supplement was replaced by Eurolysine or Pekilo. The conversion ratios of crude protein and ME also deteriorated slightly, though the differences were not statistically significant (P > 0.05).

Mortality increased gradually with increasing supplementation with Eurolysine or Pekilo, but in almost every case the cause of death was cannibalism. Post-mortem and histological examinations made on hens from every group did not reveal any abnormality due to SCP feeding.

Single cell protein has been used successfully in poultry feeding, and the maximum level of safe replacement has been 50 % of dietary protein (POUTIAINEN 1973, SHANNON et al. 1975, VOGT et al. 1975, 1978, SALO 1977, KIISKINEN 1979). When the proportion of the SCP protein has been 50 % or more, production and feed utilization in layers has usually deteriorated. In this experiment the diets supplemented with SCP gave a poorer perform-

ance than the the control diet containing 6.8 % fish meal. The diets containing Eurolysine of Pekilo were not supplemented with methionine. The digestibility of methionine was also rather low in the pig digestibility trial (NÄSI and HUIDA 1982). The reason for the lower egg production could be the deficiency of sulphur-containing amino acids.

Eurolysine bacterial protein has been used as a protein supplement for broilers and 5 % Eurolysine inclusion did not affect weight gain or feed conversion. Higher inclusion rates, 10 % or 15 %, reduced weight gains (van WEERDEN and SCHUTTE 1977).

The results of this study suggest that Eurolysine bacterial protein and Pekilo protein can be used as protein sources for laying hens, and can compose up to 50 % of the protein supplement in the diet, provided account is taken of the requirements for sulphur-containing amino acids.

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## SELOSTUS

# Eurolysine bakteeriproteiini ja pekilo proteiini munivien kanojen valkuaisen lähteenä

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Tutkimuksessa selvitettiin kahden mikrobivalkuaisuutteen, eurolysine bakteeriproteiinin ja pekilo proteiinin käyttökelpoisuutta munivien kanojen valkuaisrehuna. Eurolysinen valkuaispitoisuus oli 68.5 % ja lysiinipitoisuus 8.3 g/16 g N. Pekilon valkuaispitoisuus oli 43.3 % ja lysiinipitoisuus 5.5 g/16 g N. Molemmissa mikrobiproteiinituotteissa metioniinipitoisuus oli alhainen. Kokeessa oli 504 kanaa jaettuna seitsemään ryhmään ja koe kesti 24 viikkoa. Eri rehuseoksissa oli eurolysineä 0-4.0-7.9 ja 11.9 % ja pekiloa 0-6.3-12.7-18.0 % eli 33, 66 tai 100 % valkuaislisästä, joka oli puoliksi soijaa ja kalajauhoa. Mikrobivalkuaista sisältävillä rehuseoksilla munintatulokset jäivät alemmiksi kuin soijalla ja kalajauholla. Samoin rehunkulutus lisääntyi mikrobiproteiinia sisältävillä ruokinnoilla. Erot rehuhyötysuhteessa eivät olleet merkitseviä. Kuolleisuus lisääntyi ryhmissä, jotka saivat mikrobiproteiinia, mutta kannibalismi oli pääasiallinen syy kuolemiin.