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The effect of processing treatments on the rumen microbial digestion in vitro of skimmilk powder protein

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Abstract. The microbial degradation of the protein of skimmilk powders manufactured and treated in different ways (high-, medium-, and low-heat spray, instant, roller, formaldehyde-treated spray) and milk powder products (skimmilk powder-wheat flour granules, skimmilk powder-barley flour pellets) was determined in anaerobic, rumen-simulating conditions *in vitro*. The tests showed that milk protein was decomposed rapidly and extensively in rumen fluid buffer. The heat treatment had a slight N solubility-reducing effect. Formaldehyde treatment decreased the degradation of the protein at the beginning of the incubation, but this effect became smaller as incubation proceeded. After 48 h the N solubility of the treated skimmilk powder was about the same as that of untreated spray powder. The variation in the *in vitro* N solubility of formaldehyde-treated skimmilk powder was greater than with the other powders and products.

The skimmilk powder-wheat flour granules were hard products which remained indigestible for several hours. At the end of the microbial digestion test the addition of skimmilk powder increased the N solubility of wheat flour protein.

On the basis of the results obtained from the digestion test on a pelleted skimmilk powder-barley flour product it can be assumed also that the addition of skimmilk powder caused an increase in the N solubility of barley flour protein.

Introduction

The significance of protein to the ruminant is decisively determined by its decomposition in the rumen. Those feed proteins which are soluble in the rumen fluid are fermented very rapidly (McDONALD 1952).

Casein is known to decompose extensively in the rumen fluid (PEARSON and SMITH 1943, CHALMERS et al. 1954. FERGUSON et al. 1967), and so its utilisation is less efficient than that of many other proteins generally used in the feed of ruminants. We have been unable to find any reports on the utilisation of milk powder as such by adult ruminants in the literature. It is of course true that the feeding of milk protein to a mature ruminant is both ureasonable and uneconomical. Milk powder is, as regards its solubility, like casein, but it contains easily soluble carbohydrate, and this may promote the utilisation of the protein in the rumen.

The decomposition of protein in the rumen can be prevented by different processes (heating, grinding, pelleting, rolling) or chemical treatments. The idea here is to shorten the time spent by the protein in the rumen or to inhibit the proteolytic activity of the rumen microbes. Several studies, particularly on the protection of casein with formaldehyde, have been performed (FERGUSON et al. 1967, HAGEMEISTER and PFEFFER 1973, HEMSLEY et al. 1973, FAICHNEY 1974, KELLAWAY et al. 1974), according to which the protection increased the amount of protein passing into the lower digestive tract, and decreased the nitrogen losses.

In the present study the degradation of the protein of skimmilk powders prepared in different ways (spray, roller, instant), formaldehyde-protected skimmilk powder, wheat flour granules containing various amounts of skimmilk powder, and skimmilk powder-barley flour pellets was investigated in *in vitro* experiments which simulated rumen conditions. These experiments formed a preliminary investigation for the following studies which elucidate the value of untreated and formaldehyde-treated milk protein as a protein source for dairy cows with high milk production (SYRJÄLÄ et al. 1978 a), and the utilization of untreated and formaldehyde-treated skimmilk powder and skimmilk powder-barley pellets by ruminants (SYRJÄLÄ et al. 1978 b).

Materials and Methods

In the *in vitro* experiments the microbial digestion of the protein of skimmilk powders[x] dried in different ways and at different temperatures was studied. The prepasteurisation times for the high-heat spray, medium-heat spray, low-heat spray, roller and instant powders prepared for this test were 93° C/9 min, 85° C/15 s, 68° C/15 s, 80° C/15 s and 80° C/15 s, respectively. The solubility index (determined by the ADMI method) of the roller powder was 9.90 ml and the other samples below 0.05 ml. The content of undenatured whey protein nitrogen (determined by the ADMI method) was 9.8, 5.3, 0.4, 6.3 and 0.9 mg per g skimmilk powder prepared by low-, medium-, high-heat, instant and roller processing. Formaldehyde-treated medium-heat spray powder contained 0.4 g formaldehyde per 100 g protein.[xx] The granulated milk powder preparations[xo] E-0, E-25, E-50 and E-75 contained 0, 25, 50 and 75 % skimmilk powder and 100, 75, 50 and 25 % wheat flour, respectively. They were prepared by an extruding process, in which the heating time ranged from a few seconds to some tens of seconds, the maximum temperatures for E-0, E-25, E-50 and E-75 being 162, 135, 128 and 120° C, respectively. The pelleted milk powder product[xo] contained 30 % skimmilk powder and 70 % barley flour. The heat treatment during pelleting was 60—70° C for a few seconds.

Fresh rumen fluid was obtained, from a ram on standard feed and fitted with a rumen fistula, less than 30 minutes before the start of each test series.

The determinations of the extent of decomposition of protein were performed using the first part of the two-stage method of TILLEY and TERRY (1963), according to which the samples were digested in a rumen fluid buffer under strictly controlled and standardised anaerobic conditions for two days. The second stage, that is pepsin-HCl digestion, was not performed, with the

x Manufactured by Kuivamaito Oy, Lapinlahti, Finland
xx » » Farmos Yhtymä, Turku, »
xo » » Vaasan Höyrymylly, Helsinki, »

exception of a few samples, so that the total digestibility of the samples on the basis of dry matter or organic substance was not determined. Sub-samples were taken after 2, 24 and 48 hours' incubation. Total nitrogen (TN), water-soluble nitrogen (WSN) and soluble protein nitrogen (SPN) in the samples were determined by the classical Kjeldahl method, using selenium as catalyst. Protein was precipitated with 10 % trichloracetic acid. Non-protein nitrogen (NPN) was obtained as the difference between WSN and SPN. Ammonia determinations were performed mainly with an ammonia electrode (Orion), and also by the Conway method (Conway 1962).

Results

The course of the digestion of the skimmilk powders and milk-powder products in the *in vitro* incubation with rumen fluid is seen in Figures 1 and 2. The results are means of 4—10 separate determinations. Fig. 1 gives the proportion of WSN and Fig. 2 that of NPN as a per cent of the TN.

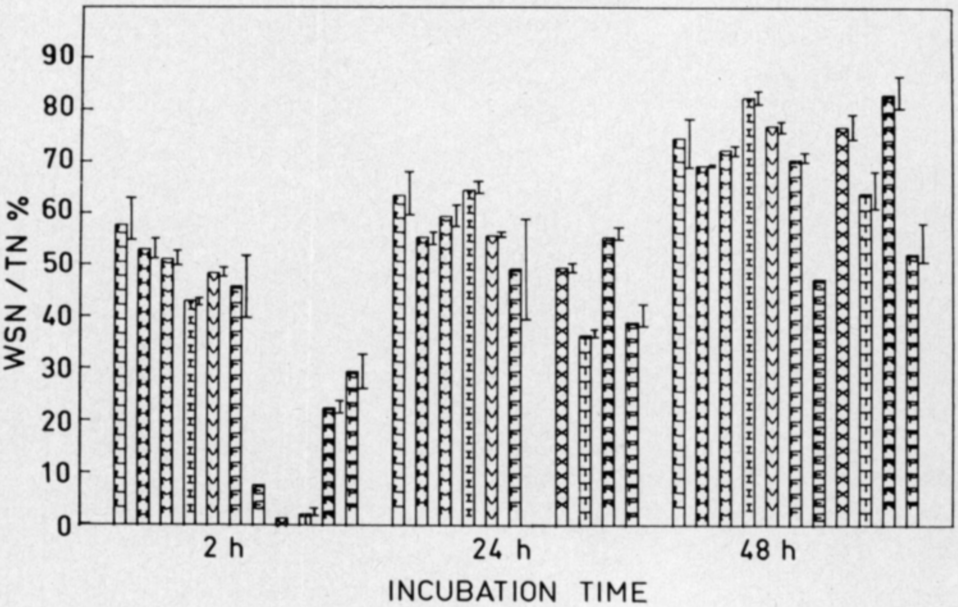

Fig. 1. The proportion of water-soluble nitrogen (WSN) as a percent of the total nitrogen (TN) in *in vitro* microbial digestion tests with different skimmilk powders and skimmilk powder products. The variation range of the results is given as a line beside each column. The symbols inside the columns mean:

L = low-heat spray
H = high-heat spray
M = medium-heat spray
I = instant
V = roller
F = formaldehyde-treated spray
E = granulated wheat flour
X = granulated milk powder (25 %) — wheat flour (75 %)
T = granulated milk powder (50 %) — wheat flour (50 %)
R = granulated milk powder (75 %) — wheat flour (25 %)
P = pelleted milk powder (30 %) — barley flour (70 %)

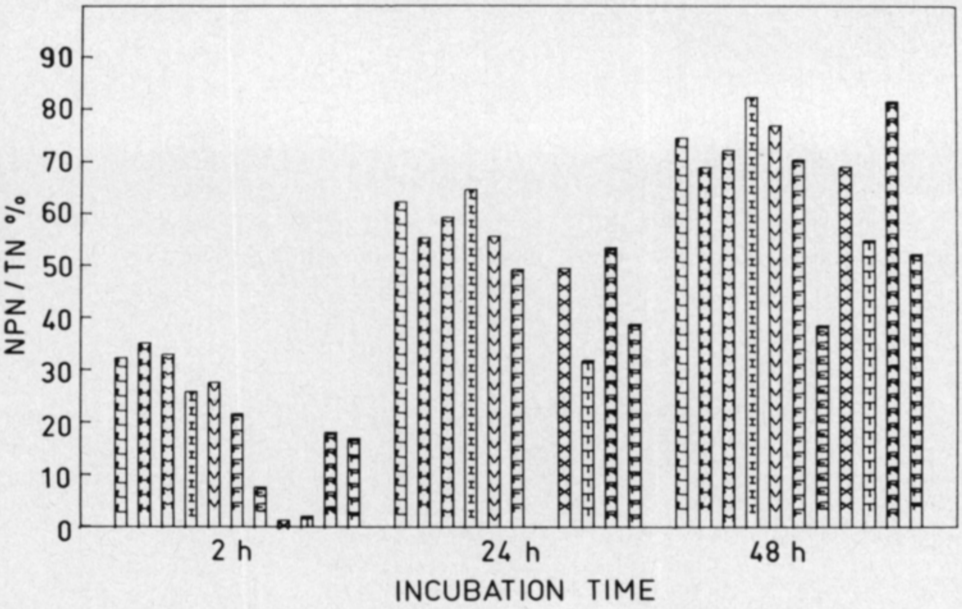

Fig. 2. The proportion of non-protein nitrogen (NPN) as a percent of the total nitrogen (TN) in *in vitro* microbial digestion tests with skimmilk powder samples. The symbols inside the columns are the same as in Fig. 1.

Of the TN of untreated skimmilk powders, 43—58 % was found in water-soluble form after an incubation of 2 h. Of the WSN, 20—25 % was still, however, SPN. Heat treatment had a slight protecting effect. The nitrogen (N) solubility of the high-, medium- and low-heat spray powders was 53, 51 and 58 % respectively. At this incubation stage the formaldehyde treatment decreased the N solubility by an average of 4 % units, that is to 47 %.

Variations in the *in vitro* N solubility of formaldehyde-treated milk powder proved greater than in that of high-, medium- and low-heat spray powders. Therefore the range of the N solubility results with powders is also given in Fig. 1.

After 24 h the N solubility of the skimmilk powder samples was on the average 55—65 %. Heating and formaldehyde treatment further decreased the degradation of protein. The N solubility of the roller powder (56 %), which had become partly denatured by the heat treatment, and the high-heat spray powder (55 %) was lower than that of the low-heat (64 %), medium-heat (59 %) and instant (65 %) powders. Formaldehyde treatment decreased the N solubility by 10 % units. The solubilised N was then almost entirely NPN, of which 90—100 % was ammonia; after two days' incubation the former figure had risen to about 70—80 % (Table 1). The protective effect of formaldehyde decreased during the incubation: after two days the N solubility of the treated spray-milk powder was about the same as that of the untreated powder.

The digestion of the granulated products containing 0, 25, 50 and 75 % skimmilk powder was slow at first. After one day, 0, 50, 36 and 56 % respectively

Table 1. The proportion of ammonium nitrogen (NH_3-N) as a percent of the non-protein nitrogen (NPN) after various incubation times in *in vitro* microbial digestion tests in rumen fluid buffer. E-0, E-25, E-50 and E-75 are extruded milk powder-wheat flour granules containing 0, 25, 50 and 75 % skimmilk powder respectively. The pelleted sample contained 30 % skimmilk powder, the rest being barley flour.

Milk powder sample	NH_3-N/NPN % 2 h	24 h	48 h
Low-heat spray	34.3	100	99.0
High-heat spray	30.4	94.7	100
Medium-heat spray	33.0	90.0	100
Instant	26.4	97.3	100
Roller	26.8	100	100
E-0	26.7	0	61.3
E-25	0	0	82.2
E-50	0	0	86.0
E-75	18.8	72.7	90.3
Pelleted	29.1	91.5	100
Formaldehyde-treated	29.1	93.7	100

of the TN or these samples was in soluble form, and after two days 47, 77, 64 and 83 %. The extruded product containing no milk powder was not as resistant as those containing milk powder. After two hours' incubation almost 8 % of the TN was in water-soluble form, which was subsequently used in microbial cell synthesis, since after 24 hours no soluble N was found. Ammonia was formed in only the 0 and 75 % milk powder samples after two hours' incubation and after 24 h in only the 75 % sample. After 48 h the WSN was not, however, solely ammonium nitrogen as was the case with skimmilk powders, as part of it was still SPN.

The barley flour pellets, which contained 30 % skimmilk powder, were not as resistant as the extruded products, and were decomposed in the rumen fluid buffer much more quickly. After the incubation times used the proportion of the WSN of the TN was 30, 39 and 52 %, of which 91 % was ammonium nitrogen after 24 h and 100 % after 48 h.

Discussion

The results showed that readily-soluble skimmilk powder protein was decomposed very rapidly in the rumen fluid *in vitro* due to microbial activity. The end product was ammonia. El Shazly (1952 a, b) observed that casein was decomposed in rumen fluid *in vitro* and *in vivo* in the same way. In the decomposition process the first stage was the proteolytic decomposition into amino acids, which in turn were deaminated. The amount of ammonia in the rumen fluid showed the degree of protein decomposition. When casein was given *via* a fistula straight into the abomasum or duodenum its digestibility was good and the animal was able to use the nitrogen better than that of casein entering *via* the rumen (Egan and Moir 1965, Reis 1969, Papas et al. 1971, Macrae et al. 1972).

Owing to its high solubility, skimmilk powder probably does not remain in the rumen as long as 48 h. In the *in vitro* experiments the two-hour incubation was also obviously too short for the development of microbial activity under the new conditions. When examining the results after the 24 h incubation it is found that the N solubility of the high-heat spray powder was 3 % units lower than that of medium-heat powder and 5 % units lower than that of low-heat powder. The high-heat treatment (93° C/9 min) had a slight degradation-reducing effect compared with the medium-heat (85° C/15 s) or low-heat (68° C/15 s) treatments. A brief heat treatment, which did not greatly denature proteins, had no effect on the fermentation extent of the product. After the 24 h incubation the N solubility of the roller powder was the same as that of the high-heat spray powder. When using the roller method, protein is partly denatured.

Skimmilk powder however was not degraded to the same extent as casein in rumen fluid. Hume (1974) observed that the extent of degradation of casein in the rumen fluid was as high as 91 %.

Hemsley et al. (1973) found that 86 % of untreated casein was decomposed in rumen fluid, but when the casein contained 1 % or more formaldehyde it protected the product from microbial decomposition. In the present study, treatment with formaldehyde (0.4 g/100 g protein) decreased the N solubility of medium-heat spray milk powder by an average of 10 % units after 24 hours' incubation. It is true, however, that the variation in the solubility results from the formaldehyde-treated milk powder samples was larger than with the other samples studied. This did not appear to be due to uneven distribution of formaldehyde in the sample, since according to the manufacturer the product was of uniform composition. The N solubility-reducing effect of formaldehyde decreased, however, in longer incubations. After 48 h no real difference was found in the N solubility of treated and untreated milk powder. The proportion of ammonium nitrogen of the TN or WSN of the treated powder after 2 hours' incubation was smaller than that of the untreated milk powder, but after 24 and 48 h no difference was found. Even very small amounts of free formaldehyde in the sample interfered with the determination of ammonia by various methods. According to the manufacturer the treated milk powder did not contain free formaldehyde.

Chalmers et al. (1954) observed that casein could be processed by heating or browning so that smaller amounts of ammonia were formed in the rumen and the utilisation of protein became more efficient. The authors' opinion was that although the heat treatment decreased the biological value of the protein, the decrease did not have the same importance in the feed of ruminants as in that of non-ruminants. The extruded milk powder-wheat flour granules (E-0, E-25, E-50 and E-75) also underwent a brief heat treatment. Wheat flour was used to convert the skimmilk powder to a granulated, rather hard product which does not dust during handling, with a degradation in rumen fluid lower than that of untreated milk powder. The higher the milk powder to wheat flour ratio, the smaller the process variables were to be. In spite of the milder processing the colour of samples E-50 and E-75 in particular was brown, and they had a distinct caramel flavour. The biological value of the protein may have been reduced as a result of the Browning reaction. The products were especially hard, too. At the beginning of the microbial digestion test they remained almost whole for several hours. After 24 hours' incubation ammonia was formed only in sample E-75. However, 47—83 % of the total TN of the extruded samples was in water-soluble form (Fig. 1), of which 0—1.7 % was SPN (Fig. 2). The decomposition of the total proteins had started, but the amino acids had not yet been deaminated. It should be noted that after 24 and 48 hours' incubation the amount of NPN of samples E-25 and E-75 was larger than when corresponding amounts of skimmilk powder and granulated wheat flour were incubated separately. Thus it is evident that the addition of skimmilk powder caused the increase in the N solubility of wheat flour protein in the rumen fluid buffer. In contrast, the decomposition of the 50 % skimmilk powder-containing granules (E-50) after 24 and 48 hours' incubation was the same as the decomposition of the corresponding amounts of skimmilk powder and granulated wheat flour.

Pelleting did not reduce the rate of digestion of the sample in the rumen fluid. No pelleted barley flour was available for digestion determination, but on the basis of these results it can be expected that the addition of milk powder to barley flour would also increase the N solubility of barley flour protein in rumen fluid buffer.

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SELOSTUS

Erilaisten käsittelyjen vaikutus rasvattoman maitojauheen typen liukoisuuteen *in vitro* pötsinestepuskurissa

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Eri tavoin valmistettujen ja käsiteltyjen rasvattomien maitojauheiden (high-, medium-, low-heat spray, instant, valssi, formaldehydikäsitelty spray) sekä maitojauhevalmisteiden (maitojauhe-vehnäjauhogranulaatit, maitojauhe-ohrajauhopelletti) typen liukoisuus määritettiin anaerobisissa pötsiä mukailevissa olosuhteissa *in vitro*.

Tulokset osoittivat, että maitojauheproteiini hajaantui nopeasti ja runsaasti pötsinestepuskurissa. Kuumennuskäsittely, joka denaturoi osittain maitojauheproteiinia valmistusvaiheen aikana, alensi typen liukoisuutta lievästi.

Formaliinikäsittely aikaansai liukoisuuden alenemisen inkuboinnin alkuvaiheessa, mutta liukoisuutta estävä vaikutus pieneni inkubaation kuluessa. Kahden vuorokauden kuluttua käsitellyn maitojauheen liukoisen typen osuus kokonaistypestä oli jokseenkin sama kuin käsittelemättömän spray-kuivatun maitojauheen. Vaihtelut formaliinikäsitellyn maitojauheen liukoisuuksissa osoittautuivat suuremmiksi kuin muiden näytteiden.

Maitojauhe-vehnäjauhogranulaatit olivat kovia, useita tunteja sulamattomina pysyviä tuotteita. Kahden vuorokauden sulatuksen jälkeen maitojauhelisäys oli aikaansaanut vehnäjauhoproteiinin liukoisuuden lisääntymisen.

Maitojauhe-ohrajauhopellettiä pötsinestepuskurissa sulatettaessa todettiin, että pelletoiminen ei hidastanut maitojauheen typen sulavuutta. Tulosten perusteella voidaan myös olettaa, että maitojauhelisäys aikaansai ohrajauhoproteiinin liukoisuuden lisääntymisen.