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Effect of the crude protein level on the utilization of untreated and formaldehyde-treated urea *in vitro*

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Abstract. Utilization of untreated urea (F_0) and urea treated with 1.5 % formaldehyde ($F_{1.5}$) was tested *in vitro* on incubation substrates with different levels of crude protein: 9, 10, 11, 12, 13, 14, 15 and 16 % of the substrate dry matter. The content of crude protein was adjusted by addition of urea, the lowest level (9 %) being that of the substrate without urea.

The incubation time was five hours. When F_0 urea was used microbial protein synthesis, determined by tungstic acid-sulphuric acid precipitation, reached its maximum at the crude protein level of 11 %. When $F_{1.5}$ urea was added, the synthesis increased up to the level of 15 % crude protein. At the levels of 13–15 % crude protein, the synthesis was significantly ($P< 0.05$) higher with $F_{1.5}$ urea than with F_0 urea.

In the bacterial mass obtained by ultracentrifugation the content of methionine was significantly higher ($P< 0.01$) when treated urea was used. With untreated urea, the proportion of lysine was significantly higher ($P< 0.05$). Addition of urea did not affect the amino acid composition of the bacterial mass but increased the yield of microbial protein during incubation.

Introduction

In many of the papers reviewed by MØLLER (1979) rumen ammonia concentration has been shown to be a critical factor for ammonia utilization in the rumen, when the basal ration is supplemented with urea. Since the ammonia level in the rumen is chiefly dependent on the nitrogen intake and hence on the nitrogen content of the ration, it has been suggested that addition of urea is not justified in rations whose crude portein content exceeds 13–14 % of dry matter (SATTER and SLYTER 1974).

However, the critical crude protein level can vary considerably, depending on such factors as the solubility and degradability of the nitrogen in the basal ration (AITCHISON et al. 1976, ROY et al. 1977) and the content and quality of energy in the ration (MØLLER 1973, SATTER and ROFFLER 1976, KROPP et al. 1977). The object of this experiment was to study how urea utilization is affected by the rate of urea degradation on diets with different levels of crude protein and a constant energy content.

Materials and methods

The incubation technique was the same as used by SETÄLÄ and SYRJÄLÄ-QVIST (1982). The urea supplements consisted of untreated urea (F_0) and urea treated with 1.5 % formaldehyde ($F_{1.5}$). The formaldehyde treatment was as described earlier (SETÄLÄ and SYRJÄLÄ-QVIST 1982).

The crude protein levels were 9, 10, 11, 12, 13, 14, 15 and 16 % of the substrate dry matter. The crude protein content was adjusted by adding urea to the substrate to be fermented. The first level was the content of the NaOH-treated wheat straw and barley-molassed beet pulp mixture used as substrate (SETÄLÄ and SYRJÄLÄ-QVIST 1982).

The criterion of urea utilization was the formation of protein precipitable with tungstate – sulphuric acid during five hours' incubation. After incubation the fermentor contents were centrifuged at 2000 rpm for 10 minutes, the sediment was discarded and protein was determined on the supernatant according to SETÄLÄ and SYRJÄLÄ-QVIST (1982).

The bacterial mass was separated from the supernatant without precipitation by ultracentrifugation at 25 000 rpm for 20 minutes (ARNOULD et al. 1976). This was done at the levels of 9, 11, 14 and 15 % of crude protein. The mass was analyzed for amino acids, excluding cysteine and tryptophan, by hydrolysis with 6 N HCl at 110°C for 24 hours.

Statistical analyses were performed with a MONROE 1860 computor and its statistical programs. Both analysis of variance and the t-test were used. The differences between treatment means were tested with the Tukey test (STEEL and TORRIE 1960).

Results

The addition of urea to the substrate increased microbial protein synthesis (Fig. 1). When untreated urea (F_0) was used, the peak value for synthesis was reached at the level of 11 % crude protein. With the treated urea ($F_{1.5}$) protein synthesis was lower than with the untreated urea up to the level of 13 % crude protein. After this level microbial protein synthesis increased in the $F_{1.5}$ incubations and the peak of synthesis was not reached until the level of 15 % crude protein. At the levels of 13–15 % crude protein, synthesis was significantly ($P< 0.05$) higher with $F_{1.5}$ urea than with Fo urea.

The addition of urea to the substrate did not change the amino acid composition of the bacterial mass (Table 1). The proportions of isoleucine and leucine in the total amino acids tended to increase, but this change was not statistically significant.

When the incubations were performed with treated urea, the proportion of methionine in the amino acids was significantly ($P< 0.01$) higher than when untreated urea was used. On the other hand, the proportion of lysine was significantly ($P< 0.05$) lower than in incubations with untreated urea. There were also some differences in the proportions of threonine, proline and leucine but these were not statistically significant.

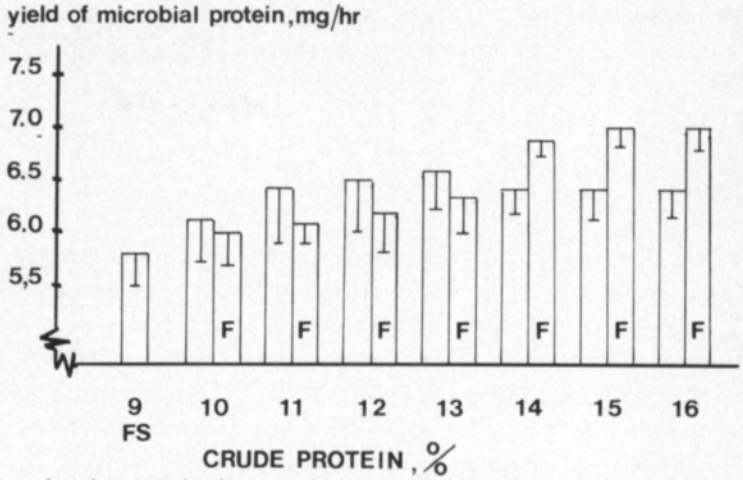

Figure 1. Effect of crude protein level on microbial protein yield (mg/hr/40 mg fermentor contents), when untreated or formaldehyde-treated urea was used.

FS = feed substrate, no urea, F = formaldehyde-treated (1.5 %) urea.

Table 1. Effect of crude protein level and added urea on the amino acid composition of the bacterial mass, when untreated (F_0) and treated ($F_{1.5}$) urea were used.

	Untreated urea			Treated urea			Untreated urea	Treated urea	No urea
	Level of crude protein, % of DM								
	11	14	15	11	14	15	$\bar{X}$	$\bar{X}$	$\bar{X}$
	% of total amino acids								
Essential	45.8	45.7	44.9	44.2	45,8	45,4	45.4	45.1	44.1
Arginine	2.9	2.5	2.7	2.6	3.3	2.7	2.7	2.8	2.7
Histidine	1.9	2.2	1.8	1.9	1.9	1.9	1.9	1.9	1.8
Isoleucine	5.9	6.3	5.6	5.9	6.0	6.3	5.9	6.0	5.6
Leucine	8.2	8.6	8.2	8.1	8.0	8.6	8.0	8.2	7.4
Lysine	9.1	9.1	8.3	8.1	7.4	8.1	8.8^{a}	7.8^{b}	8.6^{ab}
Methionine	0.9	0.6	0.7	1.0	1.3	1.3	0.7^{d}	1.2^{e}	0.9^{de}
Phenylalanine	5.3	5.1	5.4	5.3	5.5	5.2	5.2	5.3	5.4
Threonine	6.3	6.6	6.8	6.1	7.1	6.0	6.6	6.3	6.4
Valine	5.3	5.7	5.4	5.2	5.3	5.3	5.4	5.3	5.3
Non-essential	54.2	54.3	55.1	55.8	54.2	54.6	54.6	54.9	55.9
Alanine	6.6	6.9	6.8	7.0	6.9	6.9	6.7	6.9	7.2
Glutamic acid	16.0	15.6	16.5	16.4	16.3	15.8	16.0	16.2	16.3
Glycine	5.2	5.4	5.3	5.4	5.4	5.3	5.2	5.3	5.5
Proline	3.6	4.4	4.2	3.5	3.4	3.9	4.0	3.6	3.6
Serine	4.9	4.8	5.0	5.1	5.1	4.9	4.9	5.0	5.0
Tyrosine	4.7	4.7	4.7	4.6	4.6	4.6	4.7	4.6	4.7

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

The ratio of essential to non-essential amino acids was not significantly changed by the addition or the treatment of urea. There was a tendency, however, for the proportion of the essential amino acids to be higher when untreated or treated urea was added to the substrate. The proportion of the

non-essential amino acids tended to be higher when microbial protein synthesis did not increase with a rise in the amount of urea and the crude protein level.

Discussion

When untreated urea was used, the results for microbial protein synthesis agreed fairly well with the observations of SATTER and SLYTER (1974), KAUFMANN (1977) and SLYTER et al. (1979). Since the energy level was kept constant, lower degradation of urea was the main factor influencing the ratio between ammonia and energy to be fermented. The peak value for microbial protein synthesis was reached when untreated and treated urea constituted 18 and 35 % of the total nitrogen in the substrate, respectively. If the ammonia levels are calculated according to the results obtained earlier (SETÄLÄ and SYRJÄLÄ-QVIST 1982) for the feed substrate alone and for both kinds of urea, the optimum ammonia concentration was 10–11 mmol NH_3/1, which is higher than those suggested elsewhere, for instance in the review by MØLLER (1979).

Energy is very important when ammonia utilization is concerned (MØLLER 1973, KROPP et al. 1977). For instance, EDWARDS and BARTLEY (1979) showed *in vitro* that urea, given in the form of starea, was efficiently utilized for protein synthesis, when the crude protein level was elevated from 17 to 30 % of dry matter. The fermentation of energy was shown to reach its maximum at the ammonia level of 11–12 mmol NH_3/1 (MEHREZ et al. 1977). In the present sudy, where the energy level was kept constant, the maximum in fermentation was evidently reached when no increase in microbial protein synthesis was found. Further addition of urea to the substrate gave too much ammonia in relation to the energy fermented and thus did not improve protein synthesis (McMENIMAN et al. 1976, HAGEMEISTER et al. 1980).

The amino acid composition of the bacterial mass was similar to that reported by SALTER et al. (1979), SYVÄOJA and KREULA (1979) and HVELPLUND and MØLLER (1980) for animals receiving urea-containing diets, although the proportion of methionine was rather low. According to SYVÄOJA and KREULA (1979), addition of urea caused slight changes in the proportions of threonine, glutamic acid, alanine, tyrosine and arginine in microbial protein. SALTER et al (1979) found that elevated levels of urea in the diet decreased the proportion of methionine.

The differences in the amino acid composition of the microbial mass found in this experiment between the diets probably arose from differences in the biosynthesis of amino acids. CHALUPA et al. (1970) reported that amination and transamination are the major mechanisms of ammonia assimilation by rumen microbes. Lysine, together with threonine and tryptophan, was synthesized through routes other than transamination (BHATIA et al. 1979), but the transaminase activities of the rumen protozoal and bacterial fractions were suggested to be slightly higher with respect to methionine than lysine.

The dominant enzyme in the biosynthesis reaction chain could be altered by the NH_4^+ level in the rumen. Glutamate synthase and glutamine synthase decreased in activity at high NH_4^+ concentrations (ERFLE et al. 1977). The activity of alanine amino transferase increased correspondingly. These changes may be of importance from the point of view of our results, because SALTER et al. (1979) suggested that glutamic scid and alanine, together with aspartic acid, can act as initial recipients for subsequent transfer to other amino acids in the chain of biosynthesis.

It was also reported by ALLISON and BRYANT (1963) that *R. flavefaciensis* can use the carbon of formate for methionine biosynthesis. If formaldehyde is degraded to formic acid by rumen microbes, as suggested by KAEMMERER and KERBER (1977), this could also contribute to the higher concentration of methionine in microbial protein when treated urea was used.

However the possibility cannot be excluded that in spite of careful preparation of the samples, some unsequestrated protozoa may have influenced the amino acid composition of the microbial protein (PURSER and BUECHLER 1966). Protozoal protein contains more lysine and less methionine than bacterial protein. The same authors have also observed that the bacterium *R. albus* has a relatively high methionine content in its cell protein. Since formaldehyde seemed to have a negative effect on protozoa *in vitro* (SETÄLÄ and SYRJÄLÄ-QVIST 1982), it is possible that in incubations with unteated urea some connection existed between the lysine content of microbial protein and precence of protozoa.

Contamination by feed particles and their effect in analysis have been discussed in the earlier paper of SETÄLÄ and SYRJÄLÄ-QVIST (1982).

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Rehuannoksen raakavalkuaistason vaikutus käsittelemättömän ja formaldehydi-käsitellyn urean hyväksikäyttöön *in vitro*

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Tutkimuksessa verrattiin rehuannoksen raakavalkuaistason vaikutusta käsittelemättömän urean ja 1.5 prosentin formaldehyditasolla käsitellyn urean hyväksikäyttöön. Tutkitut raakavalkuaistasot olivat 9, 10, 11, 12, 13, 14, 15 ja 16 prosenttia raakavalkuaista substraatin kuiva-aineessa. Raakavalkuaistasoa muutettiin urealisäyksen avulla. Perusrehusubstraatti ilman ureaa muodosti ensimmäisen tason.

Wolframaatti-rikkihappo -saostuksella määritetty mikrobiproteiinisynteesi viiden tunnin inkubaation aikana lisääntyi käsittelemätöntä ureaa käytettäessä 13 prosentin raakavalkuaistasolle saakka. Käsitellyllä urealla maksimiproteiinisynteesi saavutettiin vasta 15 prosentin raakavalkuaistasolla. Ureoiden välinen ero oli merkitsevä ($P < 0.05$) yli 13 prosentin raakavalkuaistason jälkeen.

Ultrasentrifugoimalla saadun bakteerimassan metioniinipitoisuus oli merkitsevästi ($P < 0.01$) korkeampi inkubaatioissa, joissa käytettiin käsiteltyä ureaa käsittelemättömällä urealla saatuihin tuloksiin verrattuna. Käsittelemättömällä urealla tehdyissä inkubaatioissa oli vastaavasti bakteerimassassa merkitsevästi ($P < 0.05$) korkeampi lysiinipitoisuus. Urean lisäys rehusubstraattiin ei vaikuttanut mikrobivalkuaisen aminohappokoostumukseen, mutta lisäsi proteiinisynteesiä inkubaation aikana.