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THE EFFECT OF THE PROTEIN SOLUBILITY OF FISH MEAL AND THE ROUGHAGE CONTENT OF THE SUPPLEMENTED DIET ON ITS DIGESTION BY SHEEP

G.N. Louw, E.L. Steenkamp & P.K. van der Merwe

National Chemical Products Limited, P.O. Box 344, Germiston

OPSOMMING: DIE INVLOED VAN FORMALDEHIEDBEHANDELING EN PROTEÏENOPLOSBAARHEID OP DIE BENUTTING VAN VISMEEL DEUR SKAPE

Die invloed van formaldehiedbehandeling op die oplosbaarheid van vismeel in M.NaCl oplossing is ondersoek. Daarna is bepaal in hoe 'n mate die oplosbaarheid van verskillende vismele, afkomstig van verskillende bronne, varieer. 'n Reeks metaboliese proewe is vervolgens met Merino skape uitgevoer ten einde te bepaal in hoe 'n mate vismeelproteïene met verskillende oplosbaarhede deur die diere benut word wanneer hul rantsoene uit slegs laegraadse ruvoer bestaan of wanneer verskillende hoeveelhede mieliemeel en mieliestysel aangevul word. Wanneer growwe mieliemeel by die rantsoene gevoeg was, het skynbare proteïenverteerbaarheid en stikstofretensie toegeneem namate die mieliemeelvlak gestyg het. In die geval van mieliestysel egter, was spoedig 'n vlak bereik waar die waardes vir hierdie maatstawwe begin daal het. Die punt van daling was op 'n veel vroeër stadium by die meer onoplosbare proteïene teëgekom. Dit is moontlik dat die benuttiging van vismeelproteïene bepaal was deur 'n verband tussen hul oplosbaarheid of skynbare verteerbaarheid en die vloeispoed van kos deur die spysverteringskanaal. Die behandeling van vismeel met formaldehied het die verteerbaarheid van die produkte onderdruk. Onder sekere omstandighede mag die mikrobes in die rumen selfs aan 'n stikstofgebrek gely het, wat aanleiding kon gegee het tot onderdrukking van organiese materiaalverteerbaarheid en daaglikse inname van voer.

SUMMARY:

The effect of formaldehyde treatment on the solubility of fish meal in M.NaCl solution was investigated. Subsequently the variation in solubility of fish meals obtained from various sources was examined. A series of metabolic trials was then conducted with Merino sheep in order to determine to what extent fish meal proteins with different solubilities are utilized by animals on low quality roughage rations and the same rations supplemented with various amounts of maize meal and maize starch. The addition of coarse maize meal to the rations resulted in an increase in apparent protein digestibility and nitrogen retention with increasing levels of supplementation. In the case of maize starch, however, increasing levels of supplementation soon reached a stage when the values for these parameters started to decrease. The point of decrease was reached at a much lower level of supplementation in the case of the more insoluble proteins. It is possible that the utilization of fish meal proteins was determined by a relationship between their solubility or apparent digestibility and the rate of passage of digesta through the digestive tract. The treatment of fish meal with formaldehyde decreased its digestibility. The micro-organisms in the rumen may, under certain conditions even have suffered from a nitrogen deficiency, which could have lead to a suppression of organic matter digestibility and feed intake.

Introduction

It has been shown that the feeding of less soluble proteins to ruminants increases productivity and in the case of sheep, higher wool yields were obtained (Ferguson, Hemsley & Reis, 1967). These workers decreased the solubility of casein in the rumen by treatment with formaldehyde. Decreased solubility of proteins in the rumen was also obtained by application of heat (Whitelaw, Preston & Dawson, 1961; Tagari, Ascarelli & Bondi, 1962; Chalmers, Jayasinghe & Marshall, 1964) or by treatment with vegetable tannins (Zelter, Delort-Laval & Leroy, 1969).

Where success was obtained the proportion of readily digestible carbohydrate supplying energy to the animal, was high. However, where the dietary energy is derived mainly from roughage, as in the case of grazing ruminants under extensive conditions in South Africa, the proposition is quite different. Here the roughage is low in protein and in readily digestible carbohydrates, for the greater part of the year.

Thus a highly cellulolytic and hemi-cellulolytic ruminal flora is required to digest the roughage. To obtain this, the ruminal flora must be adequately supplied with nitrogen for growth, and this puts a limit to the proportion of treated protein which can be fed as a supplement. Variation in the solubility of certain products may limit indiscriminate treatment for protection against ruminal breakdown. Whitelaw & Preston (1963) procured two fish meals from different sources. The solubility of these products in M.NaCl solution was 6,2 and 77,2 %. When fed to early weaned calves the insoluble products resulted in a significantly higher nitrogen retention while the 'soluble' one gave rise to higher ammonia levels in the rumen (Preston, Whitelaw & McLeod, 1963).

In South Africa the supplementation of low quality grass veld with fish meal in winter is commonly propagated and practised. Fish meal is obtained from different sources and its nutritional value may vary considerably. The present work was undertaken to demonstrate the effect of the solubility of fish meal protein on the utilisation of high roughage diets by Merino sheep. It would be impossible to subject all fish meals to be tested to metabolic trials. For this reason the principle demonstrated by Henderickx & Martin (1963) was employed. These workers believe that the solubility of proteins in salt solutions may serve as a criterion of their digestibility by ruminants.

In order to establish this relationship the reaction between fish meal protein and formaldehyde was first studied. The variation in solubility of fish meals, as produced by the fishing industry, was investigated and finally a series of digestibility trials was carried out with sheep. Data obtained from these trials should indicate what reaction might be expected if fish meal, either treated or untreated with formaldehyde, was supplemented to grazings poor in nutritive value.

Procedure

Fish meal solubility

Sources: Commercial fish meals, produced at different factories at different times, were obtained from feed merchants.

Formaldehyde treatment: One fish meal sample was sub-divided into five samples of 600 g each. To each sample was added 700 ml water plus zero; 9,25; 18,50; 27,75 and 37,00 ml 40 % formaldehyde solution, respectively. The treated samples were kept in closed jars at 65°C for 24 hours and then dried at 65°C. The formaldehyde content of the different samples was determined by the method of Nitschmann & Hadorn (1943).

Method of determination: Solubility of fish meal protein in M.NaCl was determined with the method used by Kay *et al.* (1966).

Digestibility trials

Animals: Mature Merino wethers, divided into groups of six animals each.

Diets: A series of three metabolic trials was carried out. The first trial consisted of Rations A and B, the second of Rations C, D, E and F and the third of Rations G, H, I, J, K, L, M and N. The compositions of these rations are given in Table 1.

Formaldehyde treatment: One batch of fish meal each for every metabolic trial was divided into two portions and one portion treated with formaldehyde. Treated fish meal was wetted with an excess of 2,5% formaldehyde solution. It was left at room temperature for 24 hours and dried in a slight draught with infra red light after being spread evenly in shallow pans. The maximum temperature was 75°C and drying lasted 72 hours.

Chemical composition of the rations: The chemical compositions of the rations are given in Table 2.

Experimental design: Randomized block.

Table 1

Compositions of rations (air dry basis)

Constituent		Ration													
		Experiment 1		Experiment 2				Experiment 3							
		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Wheat straw	%	90	90	87,5	77,5	67,5	57,5	97,5	83,5	68,5	53,5	83,9	68,9	53,9	38,9
Formaldehyde treated fish meal	%	–	7,5	10,0	10,0	10,0	10,0	–	14	14	14	–	–	–	–
Untreated fish meal	%	7,5	–	–	–	–	–	–	–	–	–	13,6	13,6	13,6	13,6
Maize meal	%	–	–	0	10	20	30	–	–	–	–	–	–	–	–
Maize starch	%	–	–	–	–	–	–	–	–	15	30	–	15	30	45
Mineral-vitamin mix*	%	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5

* Composition: 49% salt + 40% Di-calcium phosphate + (145000 I.U. vitamin A + 10000 I.U. vitamin D_3) per lb of phosphate-salt mixture.

Table 2

Chemical composition of the rations (on dry basis)

		Ration												
		Experiment 1		Experiment 2				Experiment 3						
		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Crude protein	%	8.5	8.5	10,5	11,0	10,1	11,2	2,1	10,2	11,3	10,2	10,6	11,1	11,1	10,7
Crude fibre	%	38,0	36,6	35,1	31,6	29,1	25,9	44,8	36,6	33,6	28,1	34,1	31,8	27,9	20,3
Ether extract	%	1,8	1,5	2,0	2,0	2,5	2,6	1,9	1,9	1,8	1,5	1,9	2,4	1,6	1,6
Ash	%	6,5	6,5	6,8	6,8	6,3	6,0	6,2	7,2	8,3	7,6	7,1	7,1	6,9	6,2
N.F.E.	%	45,2	46,9	45,5	48,6	52,0	54,3	44,0	44,1	45,0	52,6	46,3	47,6	52,5	61,2
Bruto energy K. cal per gm		4,51	4,27	4,43	4,41	4,41	4,10	4,23	4,41	4,35	4,32	4,28	4,35	4,30	4,23
Solubility of protein in M.NaCl	%	12,0	5,9		8,2			–		17,8			23,4		
Bound formaldehyde	%	–	0,33		0,40			–		0,26		–	–	–	–

Digestion trials: Standard procedure for digestion trials were followed. Adaptation periods lasted 21 days and collection periods 7 days.

Results

Solubility of formaldehyde treated fish meal protein: The effect of treatment with various concentrations of formaldehyde on the solubility of fish meal protein in M.NaCl solution is presented in Table 3.

Table 3

Solubility of fish meal protein in M.NaCl solution when treated with different quantities of formaldehyde

N content of sample (% on dry basis)	Formaldehyde bound to protein (% on dry basis)	Protein solubility %
11,03	0	28,2
11,01	0,084	27,5
10,95	0,184	26,3
10,86	0,407	23,8
10,73	0,665	19,9

Solubility in M.NaCl solution of proteins in fish meals obtained from different sources: The results are given in Table 4.

Digestibility trials: The results of the different digestibility trials are given in Tables 5, 6 and 7.

Table 4

Solubility in M.Nacl solution of fish meal proteins obtained from various sources

Sample	N content (% on dry basis)	Solubility of protein (% on dry basis)
1	10,84	7,2
2	11,32	11,8
3	11,88	14,1
4	11,21	14,9
5	11,61	15,1
6	11,16	15,9
7	11,79	19,4
8	11,26	22,4
9	10,97	25,3
10	11,21	28,8
11	11,94	32,1
12	10,91	33,4

Table 5

Metabolic data from Rations A and B

		Ration		* F calculated	L.S.D.	
		A	B	treatment	P < 0,05	P < 0,01
Daily intake (on a dry basis)	g	822	762	3,57	175	–
Digestible organic matter	%	47,0	38,5	25,63	4,0	4,6
Apparent protein digestibility	%	34,8	5,3	13,46	13,1	18,1
N Retention	g / day	1,13	– 0,97	9,24	1,05	1,45
Apparent digestible energy	Mcal/kg	2,06	1,45	47,33	0,18	0,24

* F required : P < 0,05 = 3,29
P < 0,01 = 5,42

Table 6

Metabolic data from Rations C, D, E and F

		Ration				* F calculated treatments	L.S.D.	
		C	D	E	F		P < 0,05	P < 0,01
Daily intake (on a dry basis)	g	760	868	1,324	1,284	20,2	294	407
Digestible organic matter	%	43,9	45,5	51,9	52,3	16,79	3,19	4,42
Apparent protein digestibility	%	25,1	23,5	28,5	31,2	1,86	–	–
N. retention	g / day	1,26	1,52	2,55	3,18	2,73	–	–
Apparent digestible energy	Mcal/kg	1,83	1,88	2,18	2,22	18,31	0,14	0,20

* F required: P <0,05 = 3,29
P <0,01 = 5,42

Table 7

Metabolic data from Rations G, H, I, J, K, L, M and N

		Ration								* F Calculated treatments	L.S.D.	
		G	H	I	J	K	L	M	N		P< 0,05	P< 0,01
Daily intake (on a dry basis)	g	340	725	853	967	804	884	991	620	17,48	127	171
Digestible organic matter	%	38,3	45,9	51,0	53,5	47,0	54,0	59,9	67,5	34,25	4,3	5,6
Apparent protein digestibility	%	–	47,8	45,1	38,0	67,2	71,1	69,5	66,7	76,36	14,7	19,8
N retention	g/day	–2,51	1,40	2,23	1,47	1,00	2,65	4,82	4,02	18,49	1,42	1,91
Apparent digestible energy	Mcal/kg	1,38	1,96	2,13	2,21	2,08	2,30	2,50	2,77	35,03	0,19	0,26

* F required: P < 0,05 = 2,42
P <0,01 = 3,47

From the data in Table 3 it is evident that the solubility of fish meal protein is linearly related to the percentage of formaldehyde bound to it (Y = 28,51 −12,51 X, r = 0,99). It would therefore be possible to change the solubility of a particular fish meal protein in order to investigate the relationship between this factor and the digestibility of the protein, as well as the relationship between protein solubility and the utilisation of roughages by sheep.

According to Table 4 the solubility of different fish meals in M.NaCl solution varies extensively. Should there be a relationship between this factor and the digestibility of fish meals by ruminants, it would jeopardise the suitability of these products as supplements to low quality veld grasses grazed by ruminants.

In order to determine the relationship between the solubility of the protein from different fish meals in M.NaCl solution and their digestibilities by sheep fed a poor quality roughage, it is necessary to compare the results obtained in the three different digestibility trials. Apart from nitrogen balance data this is permissible since all experimental conditions were standard.

Rations A, B, C, H and K consisted basically of wheat straw and fish meal only, the only differences being the variation in solubility of their protein content in M.NaCl solution. The relationship between protein solubility and digestibility illustrated in Figure 1.

The data in Figure 1 agree highly significantly with the regression line (r = 0,82) despite the fact that Rations A and B (dotted marks) contained less crude protein and that the pertinent data were more scattered than those of the protein of the other rations.

This relationship supports the finding of Preston, Whitelaw & MacLeod (1964) that the protein solubility of fish meal products provide a reliable guide to their nutritive value by ruminants. It also supports the results obtained by Henderickx & Martin (1963) that the solubility of different proteins in salt solutions is correlated with their rate of digestion by rumen bacteria. However, it would be expected that the regression line should flatten off very sharply at solubility values greater than 25 to 30% . The fact that the fish meal proteins in Rations A and K were not treated with formaldehyde, but fitted significantly into the regression, indicates that formaldehyde only had a precipitating effect on the proteins.

Consequently it is evident that the solubility of fish meal proteins in M.Nacl solutions may, within certain ranges, be used as a method to indicate the approximate digestibility of these nutrients when they are used to supplement rations for ruminants consisting predominantly of low quality roughages. By substituting the data in Table 4 into Figure 1 a very dark picture is obtained of the results which may be obtained if fish meal is supplemented to low quality roughages.

Since the investigation concerned the resistance of proteins to bacterial breakdown in the rumen, it would be advisable to determine the ammonia level in the rumen fluid. However, this could not be done in the present experiment and some indirect method of evaluating this process had to be employed. Chalmers & Synge (1954) found that the extent of ammonia production in the rumen could be correlated with differences in utilisation of protein. These differences were mainly due to an increased urinary nitrogen excretion which was associated with higher values of ruminal ammonia concentrations.

The relationship between protein solubility in M.NaCl solution and the daily urinary nitrogen excretion by the sheep is depicted in Fugure 2 (r = 0,91) while the relationship between apparent protein digestibility and daily urinary nitrogen excretion is given in Figure 3 (r = 0,91). The data for Rations A and B were not included in these regressions since they contained two per cent less crude protein than the other rations.

From Figures 2 and 3 it is evident that apparent protein digestibility, nitrogen excretion in the urine and the solubility of protein in M.NaCl solution are interrelated. A given value for the latter factor may thus be indicative, not only of the approximate digestibility of the protein, but also of the extent to which it will be broken down in the rumen. Again, if the data in Table 4 are substituted into Figures 2 and 3, it is evident that the resistance to ruminal break-down of fish meals de-

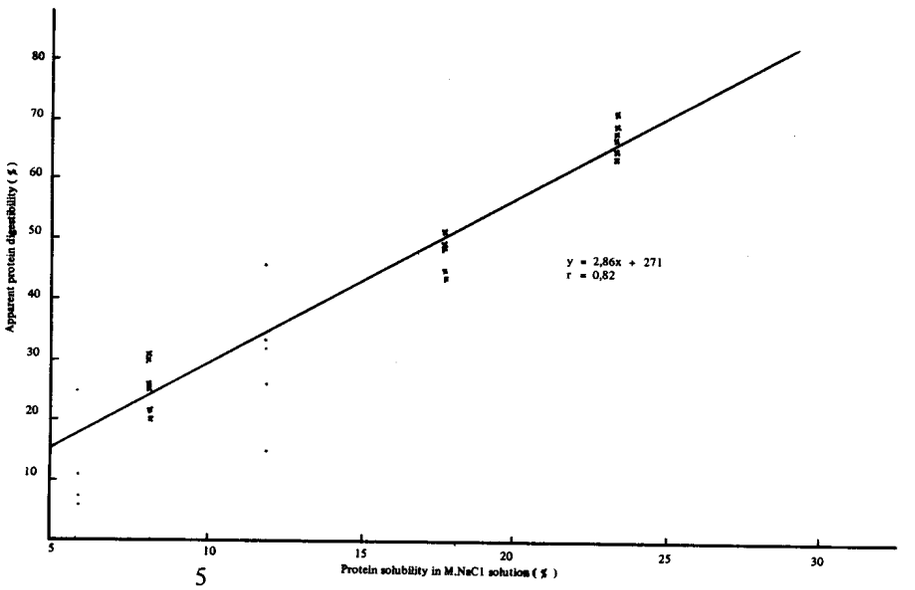

Fig. 1. – *Relationship between protein solubility in M.NaCl and apparent protein digestibility*

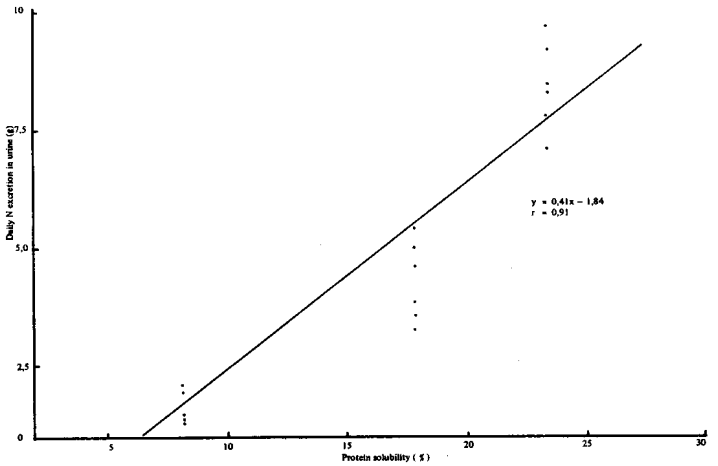

Fig. 2. – *Relationship between protein solubility in M.NaCl and daily nitrogen excretion in urine.*

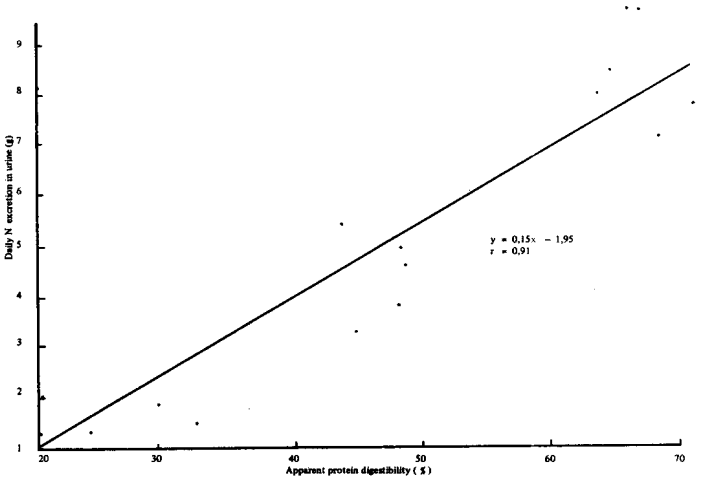

Fig. 3. – *Relationship between apparent protein digestibility and nitrogen excretion in urine*

rived from different sources may vary greatly.

The effect of decreased solubility of fish meal protein on its utilisation by sheep, as well as that of low quality roughage diets as a whole, may be illustrated by comparing Rations A and B and Rations H and K, respectively (See Tables 5 and 7). In terms of daily intake, organic matter digestibility and apparent digestible energy content, the less soluble proteins depressed feed utilisation, although statistically not significantly. This may be ascribed to the rumen microbes probably being comparatively starved for nitrogen when less degradation of the fish meal occurred.

In the case of nitrogen retention, however, there was a switch-back. In the first metabolic trial with Rations A and B (Table 5) the nitrogen balance was statistically significantly ($P < 0,01$) in favour of untreated fish meal. On the contrary, with Rations H and K (Table 7) the formaldehyde treated fish meal (H) resulted in more nitrogen retained, although statistically no t significantly.

It therefore seems that when fish meal is supplemented to low quality roughage diets, the more soluble proteins result in better roughage utilisation. However, maximum protein utilisation apparently obtained when the protein is approximately 50 % digestible or 17 % soluble in M.NaCl. The question arises, how many fish meals comply with this apparent ideal and how much protein is wasted when these products are used to supplement low quality grass veld.

The addition of a readily fermentable carbohydrate like starch to rations, contributes towards better utilisation of proteins since it decreases ammonia concentrations in the rumen fluid (McDonald, 1952; Phillipson, Dobson, Blackburn & Brown, 1962; Annison, Chalmers, Marshall & Synge, 1954). The rations in the second metabolic trial (C,D, E and F) and those in the third metabolic trial (G, H, I, J, K, L, M and N) were tested to determine the influence of various carbohydrate levels on the utilisation of the fish meals under discussion. Although Ration N had no counterpart containing formaldehyde treated fish meal, it was included in the experiment to determine the influence of excessive starch on the utilisation of the rations.

From Table 6 it is evident that, although increasing quantities of maize meal in the ration resulted in significantly increased daily feed intake, organic matter digestibility and apparent digestible energy, the apparent protein digestibility and nitrogen balance were not affected significantly. However, the values of the latter two factors increased with increase in maize contents of the rations.

Rations C, D, E and F contained formaldehyde treated fish meal only and the maize meal contributed towards their total crude protein contents. For this reason Rations G to N were formulated to effect a comparison between treated and untreated proteins in the absence of maize protein.

The data in Table 7 indicate that fish meal, treated or untreated, increased the daily intake, digestibility and other nutritional characteristics of wheat straw, statistically significantly. The addition of 15 % maize starch to Rations I and L and 30 % to Rations J and M increased daily feed intake, organic matter digestibility and apparent digestible energy, progressively. However, as in the case of Rations H and K the formaldehyde treatment depressed results in all instances.

This phenomenom may be ascribed to the fact that the formaldehyde treated protein liberated too little ammonia, resulting in the microbes being nitrogen starved. The wheat straw and starch were absolutely protein deficient.

Formaldehyde treatment decreased digestibility of proteins significantly. In the case of Rations H, I and J (formaldehyde treated) apparent protein digestibility de-

creased as the starch content of the rations increased. In Rations K, L, M and N (untreated) protein digestion increased till the 15 % starch level and then it decreased with increase in starch content. Although the effects were not statistically significant, the trends were consistent.

The picture with regard to the nitrogen balances was slightly different. The maximum values for both treated and untreated values were obtained at one higher level of starch content. Among the treated rations the nitrogen retention for I was higher than that for H while that for J was less. With the untreated fish meal the retention of nitrogen increased from Rations K to M and it only started to decrease in Ration N.

The data indicate that the relationship between protein digestibility and solubility, as well as nitrogen retention, was apparently influenced by the time spent in the digestive tract. The more soluble proteins were utilised more effectively at the higher carbohydrate levels than the insoluble proteins. The movement of digesta was apparently too fast for proper ruminal fermentation, with the result that the depressive effect was more pronounced with a decline in protein digestibility.

The fact that maize starch gave such different results compared with the maize meal, may possibly be ascribed to the particle size of these carbohydrate sources. The starch was in a state of fine division, while the maize was coarse. The latter could have stayed in the rumen much longer (cf. Campling & Freer, 1962) producing better fermentation conditions to break down the fish meal protein.

According to the regression lines in Figures 1, 2 and 3, there are apparently no other obvious differences between the nutritional characteristics of treated and untreated fish meals apart from those determined by their respective solubilities. For this reason the various reactions obtained with Rations C to N may be ascribed to differences in protein solubility in the rumen. With the data in Table 4 in mind it is thus obvious that the big variation which occurs in the solubility of commercial fish meals may have a pronounced influence on the success of ruminant feeding. When these products are treated with formaldehyde the situation is aggrevated.

It is quite possible that rations containing levels of up to 16 % crude protein, derived from fish meal, will give more favourable results. However, it should be borne in mind that wool is mostly produced off low quality grazing, usually containing less that 6 % crude protein. It would be unrealistic to supplement these grazings with fish meal to such an extent that the total crude protein exceeds 10% of the daily diet.

Conclusions

The apparent digestibility of different commercial fish meal proteins by sheep differ widely when these products are used to supplement low quality roughage diets. By using protein solubility in M.NaCl solution as a criterion of its digestibility by sheep, it appears that the coefficient of digestibility may vary between 20 and 70%. The wastage of protein when fish meals with low solubilities are supplemented to low quality grass veld, is probably high.

The apparent digestibility of fish meal protein, as well as nitrogen retention by sheep, increases with an increase in maize meal content of the rations. However, when maize starch was included as the source of carbohydrate, nitrogen digestibility and retention values started to decrease at different starch levels with an increase in starch content. The decrease started with the more insoluble protein first. It seems that in high energy rations the utilisation of fish meal proteins by sheep is determined by a relationship between their solubility or apparent digestibility and the rate of passage of the feed through the digestive tract.

The treatment of fish meal with formaldehyde results in an overall depression of protein digestibility by sheep. If supplemented to low quality roughage this may lead to nitrogen starvation of the rumen microbes with subsequent depression of organic matter digestibility and daily feed intake.

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