



Methods and procedures for the processing of feather from poultry slaughterhouses and the application of feather meal as antioxidant

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Abstract. The research subject is the elaboration of a method and procedure for processing feather from poultry slaughterhouses and using it as antioxidant as well as for satisfying the sulphurous amino acid needs of ruminants. We investigated the level of digestion of the meal feather obtained with our technology, its antioxidant effect and role in the rumen fermentation of the ruminants. Making use of the digested feather meal's antioxidant effect and amino acid composition, we make a suggestion for the preparation to be used as antioxidant and for the satisfaction of the sulphurous amino acid needs of ruminants. By adopting this procedure, the valueless feather can be transformed into a useful feed supplement (natural antioxidant, sulphur source) that can bring about significant economic growth. Pre-trials have been performed successfully, and in what follows we'll need to prove through field trials and pilot-scale experiments that feather meal can be produced and utilized economically as antioxidant in monogastric animals and as a sulphur source in the studying of ruminants.

Keywords and phrases: feather, feather meal, poultry, antioxidant, cysteine, sulphurous amino acids

1 Introduction

Feather meal is a feed ingredient derived from the feather of healthy poultry, intended for human consumption, heat treated at a minimum of 145 °C, at a minimum of 0.4–0.5 MPa pressure, and for a minimum of 40 minutes, which is then followed by drying, grinding, sorting, and packaging (Kovács, 2017). According to the regulations, it may be used exclusively for the feeding of pets. It has a maximum of 10% water content and 80–90% crude protein content, of which about 55–65% is digestible crude protein (Csapó & Sarudi, 1985). (Protein digestibility is around 80% on the average.) Its fat content is between 5 and 7% and its ash content between 1 and 6%, depending on ingredient type and technology. Neither *Clostridium perfringens* nor *Salmonella* can be detected, while enterobacteria is less than 10 in 25 g. Nocek (1988) measured its energy content at 22–23 MJ/kg, while Dale (1992) investigated in detail its true metabolizable energy content.

The feather having 30–50% dry matter content on the average is produced as the by-product of poultry slaughtering and is suitable for producing feather keratin meal. This product was previously used as animal feed, but today is mostly utilized as a fertilizer of high nitrogen content on plough-lands and in horticulture.

Its use as animal feed is also hampered by its very unique amino acid composition that contains such a little amount of essential amino acids, especially lysine, that it can only reduce the biological value of any protein it is admixed with. Many have investigated the nutritional value, first of all, the amino acid composition of feather meal. In the 1950s, Binley & Vasak (1951) were the first to study the possibilities of feather meal production, followed by Naber *et al.* (1961), who investigated feather protein composition and the utilization of amino acids in broiler chickens. Morris & Balloun (1973a,b) studied the effects of feather meal production technology on nitrogen retention, on net protein value, and on the activity of xanthine dehydrogenase in broiler chickens, while Baker *et al.* (1981) examined the protein content and amino acid composition of feather treated for various periods of time and at different pressures. According to our own investigations, due to its high cystine content, it could be considered as a cystine/cysteine supplement in lamb feed only or perhaps in the feed of angora rabbits (Csapó & Csapóné, 1985; Csapó *et al.*, 1986).

Besides the extremely low biological value of feather meal, its utilization as feed is also rendered difficult by the fact that keratin, in its original state and due to its high number of disulphide bridges, is fully resistant to digestive en-

zymes, that is, it passes through the digestive tract without any change. Protein digestibility can be improved by splitting the disulphide bridges, digesting the hydrogen bridge bond stabilizing the protein molecule, and the partial hydrolysis of protein (*Blasi et al.*, 1991; *Latshaw et al.*, 1994; *Cotanch et al.*, 2006, 2007; *Garcia et al.*, 2007); however, the biological value will continue to decrease owing to further essential amino acid decomposition consequent upon technological interventions (*Papadopolous et al.*, 1985, 1986; *Wang*, 1997). Treatment carried out at high pressure in an autoclave dissolves disulphide bridges, protein is denatured, but this treatment does not yield significant protein hydrolysis. Hydrolysis can only be achieved with chemical (alkaline or acidic) methods or the use of enzymes.

Among acidic methods, both hydrochloric and sulphuric acid treatment can be applied to the hydrolysis of keratin. Following hydrochloric hydrolysis, the salt content may increase due to acid neutralization, while during sulphuric acid neutralization the gypsum precipitate is relatively easy to remove from the system. In the course of alkaline hydrolysis, lanthionine is produced from cysteine via dehydroalanine (*Robbins et al.*, 1980; *Moritz*, 2000), which reduces cystine content; but the most relevant problem is the racemization of amino acids, which may even result the racemic mixture of amino acids during the entire procedure leading to hydrolysis (*Pohn et al.*, 1999).

In addition to chemical methods, enzymatic methods have also been developed for keratin hydrolysis, which is a much more gentle procedure considering amino acids. The last twenty years have seen many adopting enzymatic treatment for keratin digestion and to improve its digestibility (*Williams et al.*, 1991; *Hood & Healy*, 1994; *Onifade et al.*, 1998; *Tiwary & Gupta*, 2002; *Yamamura et al.*, 2002; *Bertsch & Coello*, 2005; *Grazziotin et al.*, 2006; *Gupta & Ramnani*, 2006; *Brandelli et al.*, 2010; *Zaghloul*, 2011). Besides the aforementioned, *Bockle & Muller* (1997) successfully used particular enzymes for splitting disulphide bridges to obtain an improved digestibility. Each of the processes is based on the selection of such microorganisms that are able to break the disulphide bonds of the keratin mostly via reduction, whereby they can eliminate protein compactness, thus the protein exerting smaller resistance to digestive enzymes. Changing the technology and optimizing the kinetics of enzymatic reactions make the methods suitable to completely or partially break down the protein and produce free amino acids as well as oligo- and polypeptides.

These procedures, however, do not affect amino acid composition (*Eggum*, 1970). Compared to chemical methods, their only advantage is that the decomposition and transformation of amino acids take place to a lesser degree

under optimal conditions; this is quite insignificant in the case of certain technologies.

In Hungary, 40–50,000 tonne of wet feathers are produced on an annual basis as by-products of poultry slaughterhouses, for which no reassuring placement or utilization has been found yet. The perishable organic material of high protein content causes environmental issues, which is why the destruction or utilization thereof is an economic task. Its destruction is costly, it has high land-use requirements (burying), and the expenses are not compensated by any product.

Summing up the previously mentioned research results, in the utilization of feathers, e.g. in animal feeding, we must consider that the protein, the keratin it contains is insoluble in water and digestive juices, and it resists all substances that do not attack it chemically and digest it. We may conclude from the above that without digestion the meal produced from feathers has a very low feeding value and digestibility, wherefore feather meal, despite its high protein content, cannot be efficiently used for feeding our farm animals. Subsequent to the digestion of feather with chemical or microbiological methods, digestibility is essentially improved, but the biological value of the protein remains unchanged; therefore, the digested feather, the feather protein hydrolysate is difficult to include in the feeding process.

Looking at literature data, we cannot find a single reference – apart from our conference lecture (*Csapó & Albert, 2015*) – to anyone ever using hydrolysed feather meal as antioxidant in feeding. The foregoing prompted us to develop a procedure for the digestion of feather in such a way that the digested and processed material can be suitable for feeding purposes. We examined the feather meal obtained with the application of our technology in terms of its level of digestion, antioxidant effect, and its role played in the rumen fermentation of the ruminants. Making use of the digested feather meal's antioxidant effect, we make a suggestion for the preparation to be used as antioxidant and, due to its high cystine content, for the satisfaction of the sulphurous amino acid needs of ruminants.

2 Materials and methods

Overview of the experiments for feather digestion

We placed 150 kg of air-dried feather into a 500-l alkali-resistant steel container and poured on it 350 l of 1M sodium hydroxide. (14 kg of solid sodium

hydroxide or a corresponding quantity of liquid lye was dissolved in 150 l of water, after which the solution was supplemented to 350 l.) The obtained substance was carefully heated to boiling point, but not too rapidly as that would have led to intensive foaming. After boiling commenced, we boiled it for one hour and then let it cool down. The cooled substance, the protein hydrolysate with a pH around 12.5 was set to pH = 6. The neutralization of the 500 l of hydrolysate requires about 35 l of hydrochloric acid – 37% technical grade. Neutralization must be performed with due care and diligence as foam materials produced during protein precipitation may leave the vessel. A pH meter must be used to carry out and check the setting of the hydrolysate to pH = 6. The hydrolysate produced as described above was desiccated at 60 °C in an exsiccator and then ground.

Assessment of the digested feather meal's antioxidant effect

We set up two experiments for the demonstration of antioxidant properties. In the first experiment, we analysed the correlations between feather meal and the acid, peroxide index variations, while the second experiment looked into the relation between feather meal and the changes occurring in the vitamin A and E concentration of the experimental compound feed.

Over a period of five months, we studied as a function of time the changes in the peroxide and acid number of the following feather meal mixtures: 900 g of complete soybean meal + 100 g of butter, 800 g of complete soybean meal + 100 g of butter + 100 g of feather meal, and 500 g of complete soybean meal + 100 g of butter + 400 g of feather meal. From the samples presented above, we also carried out the determination of vitamins A and E, analysing the changes in the concentration of the two vitamins as a function of time.

Assessing the suitability of hydrolysed feather meal in ruminants

We investigated the effects of feeding the obtained digested feather meal to cows with a rumen fistula. During the experiment, the feed ration consisted of 20 kg of maize silage, 4 kg of pasture hay, and 4 kg of dairy feed. The dairy feed was made up of 21.5% maize, 21.5% wheat, 21.5% wheat bran, 30.1% extracted sunflower meal, 3.2% monocalcium phosphate, and 2.2% feed

lime. When ten days of feeding with the above ration had elapsed, after 2, 4, and 8 hours following the morning feeding on the tenth day, we sampled the rumen fluid, from which we determined the ammonia content, pH value, and acetic acid, propionic acid, and butyric acid content. The obtained results were considered the control data in our experiment.

In the first pilot phase, 1 kg of extracted sunflower meal was replaced by 0.5 kg of feather meal and 0.3 kg of maize meal, and so the experimental feed included 11.2% feather meal. The pilot phase lasted for seven days – after 2, 4, and 8 hours following the morning feeding on the third and seventh day, we sampled the rumen fluid, from which we determined the previously mentioned parameters.

In the second pilot phase, 1.4 kg of extracted sunflower meal was replaced by 0.7 kg of feather meal and 0.4 kg of maize meal, and so the experimental feed included 15.8% digested feather meal. The pilot phase lasted for 14 days – after 2, 4, and 8 hours following the morning feeding on the 3rd, 7th, 10th, and 14th day, we sampled the rumen fluid, from which we measured the previously mentioned parameters.

3 Results

The composition of feather meal obtained following digestion, neutralization, and grinding is included in tables 1 and 2.

Investigation of the digested feather meal's antioxidant effect

Feather as well as the feather meal that has undergone various technological processes contain about 5–7% cystine. From this cystine content, the following are generated: 0.05–0.20% free cysteine due to digestion procedures, the same amount of free cystine, and about 0.2–0.5% cysteic acid due to contact with air and high temperature. Based on theoretical considerations, we may conclude that the hydrolysed feather meal had antioxidant effects due to the cystine-cysteine-cysteic acid system it contains.

Table 1: Effects of digestion time on protein extraction and on the free amino acid content of the hydrolysate

Component	Digestion time (minutes)				
	15	30	45	60	120
Feather mass (g)	1,500	1,500	1,500	1,500	1,500
Volume of the hydrolysate (cm ³)	4,850	4,830	4,820	4,800	4,610
Protein content of the hydrolysate (%)	24.1	24.0	24.0	23.9	22.7
Protein mass (g)	1,169	1,159	1,157	1,148	1,047
Protein extraction (%)	94.0	93.2	93.0	92.3	84.1
Protein loss (%)	6.0	6.8	7.0	7.7	15.8
Free amino acid content (%)					
Aspartic acid	0.089	0.130	0.283	0.417	0.823
Threonine	0.027	0.039	0.089	0.102	0.184
Serine	0.154	0.380	0.642	0.129	2.342
Glutamic acid	0.014	0.022	0.044	0.072	0.138
Proline	0.112	0.187	0.415	0.639	1.124
Glycine	0.153	0.301	0.681	0.681	0.143
Alanine	0.172	0.342	0.712	0.960	1.821
Cystine	0.024	0.038	0.064	0.078	0.141
Valine	0.033	0.063	0.099	0.177	0.321
Methionine	0.010	0.015	0.027	0.045	0.083
Isoleucine	0.010	0.019	0.043	0.051	0.092
Leucine	0.041	0.083	0.181	0.222	0.431
Tyrosine	0.038	0.061	0.136	0.180	0.359
Phenylalanine	0.088	0.167	0.302	0.498	0.887
Lysine	0.021	0.023	0.054	0.066	0.119
Histidine	0.011	0.018	0.020	0.027	0.052
Arginine	0.010	0.014	0.030	0.036	0.073
Total	1.007	1.902	3.822	5.721	10.142

Table 2: The amino acid composition of feather meals produced with different digestion times – expressed in units of g amino acid/100 g feather meal (A) and g amino acid/100 g protein (B)

Amino acid	Digestion time (minutes)											
	15		30		45		60		120			
	A	B	A	B	A	B	A	B	A	B	A	B
Aspartic acid	5.19	6.1	5.20	6.1	5.04	6.0	5.53	6.4	5.12	6.4	5.12	6.4
Threonine	3.63	4.2	3.59	4.2	3.43	4.1	3.28	4.0	3.14	3.9	3.14	3.9
Serine	8.54	10.0	8.50	10.0	8.48	10.1	8.32	10.1	8.20	10.2	8.20	10.2
Glutamic acid	8.89	10.4	8.91	10.5	8.72	10.4	8.54	10.3	8.02	10.0	8.02	10.0
Proline	7.97	9.3	8.01	9.4	7.79	9.3	7.78	9.5	7.43	9.3	7.43	9.3
Glycine	6.17	7.2	6.12	7.2	6.05	7.2	6.04	7.3	6.05	7.6	6.05	7.6
Alanine	5.08	5.9	4.90	5.8	4.87	5.8	4.80	5.8	4.72	5.9	4.72	5.9
Cystine	6.14	7.2	5.89	6.9	5.72	6.8	5.64	6.8	4.98	6.2	4.98	6.2
Valine	7.32	8.5	7.30	8.6	7.28	8.7	7.21	8.7	7.02	8.8	7.02	8.8
Methionine	0.69	0.8	0.67	0.8	0.66	0.8	0.60	0.7	0.59	0.7	0.59	0.7
Isoleucine	3.54	4.1	3.50	4.1	3.48	4.2	3.26	3.9	3.25	4.1	3.25	4.1
Leucine	7.92	9.2	7.90	9.3	7.94	9.5	7.91	9.6	7.69	9.6	7.69	9.6
Tyrosine	2.51	2.9	2.48	2.9	2.33	2.8	2.30	2.8	2.14	2.7	2.14	2.7
Phenylalanine	4.13	4.8	4.10	4.8	4.21	5.0	4.03	4.9	3.99	5.0	3.99	5.0
Lysine	1.50	1.8	1.50	1.8	1.47	1.8	1.42	1.7	1.42	1.8	1.42	1.8
Histidine	0.57	0.7	0.58	0.7	0.52	0.6	0.52	0.6	0.51	0.6	0.51	0.6
Arginine	5.89	6.9	5.91	6.9	5.82	6.9	5.73	6.9	5.75	7.2	5.75	7.2
Total	85.68	100.0	85.06	100.0	83.81	100.0	82.71	100.0	80.02	100.0	80.02	100.0

Effects of feather meal on the changes in peroxide and acid number

According to our investigations (*Table 3*), the control sample underwent the following changes: its peroxide number increased from 2 to 6 in 60 days, to 15 in 120 days, and to 22 in 150 days, while its acid number increased from 4 to 14, 22, and 28 respectively. Samples containing feather meal underwent significantly less changes. The peroxide number doubled, while the acid number barely changed, which is a clear demonstration of the feather meal's antioxidant effect and of that it is able to have the same effect in compound feeds as well.

Table 3: Effects of feather meal content on the changes in peroxide and acid number

The studied sample	Peroxide number (P) Acid number (S)	Time elapsed from the beginning of the experiment (days)					
		0	30	60	90	120	150
900 g soy + 100 g butter (control)	P S	2 4	4 8	6 14	8 20	15 22	22 28
900 g soy + 100 g butter + 100 g feather meal	P S	2 4	2 4	2 4	2 4	4 5	5 6
900 g soy + 100 g butter + 400 g feather meal	P S	2 4	2 4	2 4	2 4	4 4	4 5

Effects of feather meal on the changes in vitamin A and E content

According to our investigations, in the control feed, not including feather meal: 15% of vitamin A and 20% of vitamin E decomposed in 90 days, while 25% of vitamin A and 30% of vitamin E decomposed in 150 days. Upon adding feather meal, the decomposition in 90 days remained under 10% in both vitamins, and neither of them reached a 15% decomposition in 150 days.

In the light of all the foregoing, we can say that feather meal obtained with the procedure described above has antioxidant properties, wherefore it can partially or fully replace the artificial antioxidants annually imported to Hungary, worth several million dollars. Beyond its economic benefits, it allows us to have antioxidants with not fully explored effects on the animal organism replaced by a natural substance completely harmless to animals and their environment.

Examination of the hydrolysed feather meal's suitability in ruminant feed to replace sulphurous amino acids

Effects of the digested feather meal feeding on the relevant rumen parameters

The free ammonia content of the rumen fluid had a moderate decrease in the first experimental phase (*Table 4*) and a more substantial decrease in the second experimental phase (*Table 5*). This is presumably the consequence of the fact that we have removed from the feed a relatively rapidly and well-degradable nitrogen source, the extracted sunflower meal, and replaced it with digested feather meal. Based on our assumptions, the rumen's microorganisms directly utilize the free amino acids and part of the small-size peptides without the occurrence of a significant protein decomposition in the rumen. The pH of the rumen fluid is the same as that of the control in the first experimental phase, and it shows a certain increase in the second experimental phase only. By increasing the feather meal portion, the acetic acid content of the rumen fluid also increases while exhibiting high variability, and the propionic acid content shows a slight increase in the first experimental phase, whereas in the second experimental phase it has similar values to that of the control. Butyric acid content decreased upon increasing feather meal quantity.

Analysing the parameters pointing at rumen fermentation, we may conclude that regarding ammonia and propionic acid content a greater proportion of feather meal may act depressively. Based on our results, the maximum ration of the digested feather meal in the case of lactating cows should be 10% of the dairy feed or not exceeding 0.5 kg per day.

Effects of feeding the various feather meal rations on rumen fermentation and on the break-down of the feeds used

The break-down of the silage-maize silage's and the dairy feed's dry matter content slightly decreases, whereas their crude protein content decreases minimally upon the increasing feather meal feeding. The cellulolytic activity of the rumen is slightly reduced due to feather meal feeding despite that the rumen pH fell within the range of the cellulose-decomposing bacteria's activity optimum. There is a likelihood that for lack of a satisfactory nitrogen source, the number of bacteria decomposing structural carbohydrates slightly decreased. Based on the foregoing, we suggest feeding 0.5 kg of digested feather meal and the use of a well- and rapidly degradable nitrogen source in the feed ration.

Table 4: Changes in the ammonia, pH, and volatile fatty acid content of the rumen in the first experimental phase

Examined parameters	Control phase			First experimental phase					
	Sampling on the 10 th day of the preliminary feeding			Sampling on the 3 rd day of the experiment			Sampling on the 7 th day of the experiment		
	Time elapsed after feeding (hours)								
	2	4	8	2	4	8	2	4	8
Ammonia	295	218	198	231	73	104	188	68	113
pH	6.36	6.38	6.35	6.39	6.37	6.53	6.27	6.31	6.15
Acetic acid	0.65	0.64	0.65	0.62	0.65	0.65	0.63	0.66	0.67
Propionic acid	0.18	0.18	0.17	0.21	0.19	0.20	0.20	0.19	0.19
Butyric acid	0.15	0.12	0.13	0.13	0.12	0.11	0.12	0.11	0.10

* Ammonia, acetic acid, propionic acid, butyric acid: (mg/100g)

Effects of the digested feather meal feeding on the dry matter and protein content of milk as well as on its protein fractions

Upon hydrolysed feather meal feeding, the dry matter and crude ash content of milk as well as its protein fractions (total protein, true protein, whey protein, true whey protein, casein, non-protein nitrogen) remain unchanged compared to control specimens not consuming feather meal.

Discussion of the results

Unfortunately, we cannot evaluate our results in the light of the literature as we have not found any studies on hydrolysed feather meal used as antioxidant or investigating the changes of the concentration of volatile fatty acids produced in the rumen as a function of feather meal feeding rate. Our description of the procedure and the suggested utilization open up new avenues for using feather meal as feeding stuff. Our aim was the processing and utilization of a polluting by-product high in protein content, that is, the several tens of thousands of tonnes of wet feather produced annually, in such a way that over and above the elimination of adverse effects (protein degradation, soil contamination, air pollution) a useful end-product is produced in livestock-farming, whose price can partially or entirely cover the processing costs.

Table 5: Changes in the ammonia, pH, and volatile fatty acid content of the rumen in the main experiment

Examined parameters	Main experiment – Phase 1				Main experiment – Phase 2							
	Sampling on the 3 rd day		Sampling on the 6 th day		Sampling on the 9 th day		Sampling on the 12 th day					
	2	4	8	2	4	8	2	4	8			
	Time elapsed after feeding (hours)											
Ammonia	203	57	48	127	46	73	203	69	44	97	40	73
pH	6.23	6.30	6.39	6.51	6.47	6.82	6.39	6.44	6.29	6.41	6.37	6.43
Acetic acid	0.67	0.69	0.71	0.66	0.67	0.69	0.65	0.67	0.68	0.70	0.71	0.71
Propionic acid	0.19	0.18	0.17	0.20	0.19	0.18	0.19	0.19	0.18	0.17	0.17	0.17
Butyric acid	0.09	0.08	0.08	0.10	0.10	0.10	0.11	0.10	0.10	0.09	0.009	0.09

* Ammonia, acetic acid, propionic acid, butyric acid: (mg/100g)

The high cystine content of the digested feather meal creates a low-priced solution to meet the sulphurous amino acid needs of the ruminants, whereas the cystine-cysteine-cysteic acid system is able to protect the oxidation-sensitive components of premixtures and feeds due to its antioxidant properties. The antioxidant properties of the obtained digested feather meal can be clearly demonstrated, which is why its application can replace the use of artificial antioxidants. Its natural quality speaks for the utilization of feather meal since we cannot yet fully and accurately assess the physiological effects of artificial antioxidants.

Our cattle feeding experiments showed that applying a sufficient dose of digested feather meal: has beneficial effects on rumen fermentation; decreases the break-down of protein feed in the rumen; in rations of 0.5 kg per day, it increases propionic acid production; does not make significant changes to the utilization of dry matter, cellulose, and hemicellulose content of the feeding stuffs.

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