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Infulence of feathers hydrotermal processing on bioactive substances in obtained feather meal

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ABSTRACT

Raw feather is a favorable material for processing into feed rich in proteins convenient for nutrition of certain animals species. Since proteins of raw feathers are poorly digested, the improved digestibility of proteins may be achieved by hydrothermal processing of raw feathers. In this study the effect of water quantity at hydrothermal feathers treatment in industrial processing conditions on the nutritive value of hydrolysed feather meal was investigated. By these technological conditions in vitro digestibility of protein in hydrolysed feather meal was significantly increased in relation to digestibility of raw feathers. Hydrolysis contributes to the decrease of protein nitrogen content, whereas contents of non-protein nitrogen, ammonia and α-amino nitrogen were increased. Significant decrease of cystine content in the proteins of hydrolysates was noticed: 3.81, 4.07 and 4.21 g/100 g protein, depending on the technological treatments with the moisture content in raw feathers of 50%, 60% and 68%, respectively.

Keywords : Cystine, digestibility, feather, hydrolysis

INTRODUCTION

Nowadays, the main concern worldwide is growing interest for problems solving regarding to the waste materials. Currently waste materials present a great danger due to increasing environmental pollution, and thereby endangering the health of people and animals [1].

On the other hand, it is a required alternative to turn waste materials into raw materials which could lead to reduce amount of the waste and in the same time, it would be obtained new useful products [2, 3].

In industrial poultry slaughterhouses significant amounts of by-products are created (38.29% in relation to the live mass before slaughtering). The largest part of this waste (17.36%) represent moist feathers (non squeezed), followed by an intestines with the spleen and gizzard (7.77%), legs (5.29%), head (3.23%), blood (3.05%), trachea and crow with esophagus (0.92%), cloacae (0.49%) and cuticle with gizzard (12.28%) [4]. Inedible by-products from slaughtering represent a meaningful source of proteins, fats and minerals, which can be used for the production of high-protein feed for pigs, fish and pets [5, 6, 7]. However, proteins of raw feathers (keratins) are poorly digested, and even inert in the digestive tract of animals. Due to presence of strong disulfide bonds between polypeptide chains, keratin is resistant to the activity of digestive enzymes (trypsin, pepsin) and therefore very poorly digestible [8, 9]. This characteristic of keratin requires that the cystine-disulfide bonds in keratin complex be broken so that the proteins from feathers become digestible, and their amino acids biologically active. Cleavage of cystine-disulfide bonds in keratin complex is achieved by hydrolysis (alkaline, enzymatic, microbial and hydrothermal). Guillermo et al. [10] processed poultry feathers by combined method (thermo-chemical treatment) in order to obtain a porridge of feathers rich in amino acids and polypeptides, intended for animal nutrition. In this case, keratin of feathers was treated with calcium hydroxide. In the organism of ruminants, the production of ammonia from the soluble keratin was similar to that from soybean or cottonseed meal, which is much lower than from urea. This is an indication that the ammonia released from the body of cattle, which are fed with soluble keratin, is not toxic [10]. Grazziotin *et al*. [11] applied microbiological treatment to produce hydrolsates of feathers. Hydrolysate of feather proteins was obtained using keratolytic bacteria *Vibrio* sp. strain kr2. Bacterial hydrolysate of feathers can be used as feed for animals or as an organic fertilizer. Bertsch and Coello [12] examined the possibility of biotechnological processing of poultry feathers with the aim of recycling feathers into feed for animals. Hydrolysis of raw feathers was performed using aerobic keratolytic bacteria *Kocuria rosea* LPB 3. Bacterial cells produced carotenoids during fermentation. The meal made from feathers enriched with *Kocuria rosea* may be useful in animal feeding as a source of proteins and pigments. Enzymatic hydrolysis is achieved using an enzyme mixture consisting of proteases, lipases and amylases.

In practice, hydrothermal hydrolysis of keratin under pressure is widely used. The main problem with the hydrothermal treatment of feathers is a poor thermal conductivity of dry feathers. In order to achieve better heat transfer to the place where thermohydrolysis of keratin should be carried out, it is necessary to add water to the raw feathers. The amount of added water affects the profitability of processing, due to the fact that the final product, hydrolysed feather meal, can contain up to 11% of moisture according to the requirements of the Regulation on the quality of feed [13]. This means that the obtained hydrolysate must be dried. In order to obtain an adequate quality of the products, with the minimal power consumption, it is necessary to investigate the parameters that affect the thermohydrolysis of feathers and the recycling into useful products.

The aim of this study was to investigate the influence of water quantity, during industrial processing of feathers using thermohydrolysis, on nutritional value of hydrolysed feathers, as feed component.

MATERIALS AND METHODS

Feathers analysis

Moist feathers from slaughtered broilers were used as a material for the analysis. The characteristics of raw feathers are presented in Table 1. The analysis of nutritional values of raw feather and hydrolysed feather meals were conducted in 10 replicates (n=10).

Moisture content, crude proteins, ammonia nitrogen, α-amino nitrogen and protein digestibility *in vitro* were determined according to A.O.A.C. methods [14].

Determination of the level and the share of non-protein nitrogen in total nitrogen was used as the basis for the ability of the trichloroacetic acid (TCA) to deposit proteins only. From the filtrate, after treatment with TCA, amount of non-protein nitrogen was determined using Kjeldahl method.

Amino acids content was determined by ion-exchange method on aminoanalyzer BIOTRONIK LC 5001, and cystine content was determined by analyzing previously oxidized samples [15].

Table 1. Chemical composition of raw feathers (in % DM*)

Crude proteins (%)	Protein nitrogen (%)	Non-protein nitrogen (%)	α-amino nitrogen (mg/100g)	Ammonia nitrogen (%)	Protein digestibility (%)
89.35	14.15	0.13	0.00	0.00	18.72

**DM – dry matter.*

Hydrolysis treatment

For the hydrolysis of raw feathers, a semi-continuous production under strictly controlled conditions was used. During processing of feathers, hydrolysis of raw, moist feathers and partial drying of hydrolysed mass take place in a hydrolyser with indirect heating. For final drying, a continuous pipe dryer with recycled air was used.

Starting from the basic requirements for the processing of feathers into meal for animal nutrition by thermohydrolysis (the presence of water), influence of water quantity of 50, 60 and 68% in raw feathers on the quality of feather meal, at a constant pressure of 3.5 bars, during constant time of the hydrolysis of 30 min, were examined.

Statistical analyses
All results were expressed as an average mean of ten replicates with standard deviation (±SD). Comparison of obtained values was performed by analysis of variance, while the statistical significance (P<0.05) beetwen individual averages was determined by *Duncan*'s multiple interval test. For data analysis, the software package Statistica v.12.0 [16] was used.

RESULTS AND DISCUSSION

Nitrogen fractions of hydrolysed feather flours
The influence of the water quantity in the raw feathers on the production results of hydrothermal treatment of feathers was tested at constant pressure of 3.5 bars and constant duration of hydrolysis (30 min). The moisture contents in the raw feathers were 50%, 60% and 68%. Results of the influence of water quantity in raw feathers on nutritional value of hydrolysed feather meals are presented in Tables 2, 3 and 4.

The results showed that during thermohydrolysis the content of protein nitrogen in the hydrolysed feather flours produced by hydrolysis at a water content in raw feathers of aproximately 50 and 60% were not statistically different (P>0.05). On the other side, the content of protein nitrogen in the meal obtained with the content of moisture in raw feathers of 68% is significantly different (P<0.05) from the amount in meals produced with 50 and 60% of moisture in raw feathers (Table 2).

Table 2. Analysis of nitrogen fractions of hydrolysed feather flours (% in DM*)

Treatment	Protein nitrogen (%)	Non-protein nitrogen (%)	α-amino nitrogen (mg/100g)	Ammonia nitrogen (%)
Hydrolysis with 50% water	13.67±0.22^b	0.28±0.087^a	121.20±15.5^a	0.24±0.075^a
Hydrolysis with 60% water	13.77±0.26^b	0.39±0.099^b	150.24±17.3^b	0.28±0.109^a
Hydrolysis with 68% water	13.53±0.14^a	0.44±0.011^b	192.80±18.3^c	0.38±0.102^b

**DM – dry matter; Means with common superscript no differ significantly at P < 0.05.*

Increase of the crude protein, the total nitrogen and the protein nitrogen losses with increasing water content in treated feathers (Table 2) are a result of a higher degree of keratin hydrolysis. This fact indicates an increase *in vitro* digestibility of protein, increasing the moisture content in the treated feathers. A feather is poor conductor of heat. Water addition to raw feathers provided the loose structure of treated feathers in the device and better heat transfer to the protein keratin. From the obtained results it is obvious that a higher amount of water in the raw feathers improves the effect of thermohydrolysis. By reducing the protein nitrogen content, an increase in the non-protein nitrogen, ammonia and α-amino nitrogen was noticed. The differences in the content of non-protein nitrogen in the hydrolysates produced with moisture content in the raw feathers of 60 and 68% are not significant (P>0.05), whereas the non-protein nitrogen content in the meal obtained by the hydrolysis with the moisture content of approximately 50% is a significantly different (P<0.05) from the two above mentioned meals. The quantities of ammonia nitrogen in the feed obtained by hydrolysis with an amount of water of approximately 50 and 60% are not statistically different (P>0.05). The content of ammonia nitrogen in feed produced by treatment with water content of about 68% was significantly different (P<0.05) from the content in the meals produced with the amount of water in raw feathers of approximately 50 and 60%. The contents of α-amino nitrogen in the dry matter (DM) of produced meals from hydrolysed feathers were 121.20 mg/100 g (the moisture content was 50%); 150.24 mg/100 g (the moisture content was 60%) and 192.77 mg/100 g (the moisture content was 68%), respectively. Differences in the content of α-amino nitrogen in the produced feeds were significantly different (P<0.05). The raw feathers obtained from broilers slaughterhouse, used as an initial raw material for the hydrolysis did not contain ammonium nitrogen and α-amino nitrogen, which indicates that is processed in a short period from the moment of obtaining.

Crude protein and protein digestibility of hydrolysed feather meals
Applied technological mode of raw feathers processing (50, 60 and 68% moisture content in the raw feathers at a pressure of 3.5 bars for 30 min) significantly increased the *in vitro* digestibility of the proteins in the hydrolysed feather meals, compared to the digestibility in the raw feathers (Table 3). Analysis of the results related to the digestibility of proteins from the hydrolysed feather meals obtained with different water contents in raw feathers, showed that the digestibility of the proteins in the flour produced with 50% of the water is significantly different (P<0.05) from the digestibility of the proteins of meals produced with moisture content of 60 to 68%. The values of protein digestibility of the meals obtained with 60 and 68% of moisture were no significantly different (P>0.05).

Table 3. Crude protein and protein digestibility of hydrolysed feather flours

Treatment	Crude protein (% in DM*)	Protein digestibility (%)
Hydrolysis with 50% water	87.22±1.22[a]	77.33±2.72[a]
Hydrolysis with 60% water	88.53±1.75[b]	84.85±2.95[b]
Hydrolysis with 68% water	87.35±0.99[a]	85.80±3.04[b]

**DM – dry matter; Means with common superscript no differ significantly at P < 0.05.*

Results regarding to the loss of proteins in feather meals is closely related to the results of Kormanjoš et al. [17], who performed the thermohydrolysis at a pressure of 3.5 bars during a period of 40 min and with different water contents in the raw feathers. The contents of crude proteins in the dry matter of feather meals was decreased by 0.99%, 1.49% and 2.97%, with the moisture contents of 50.45%, 60.30% and 68.06%, respectively [17].

Sakač et al. [6] in their research with thermohydrolysis of raw feathers at a pressure of 3.5 bar during the 20 min and with a water content in the raw material of 62% achieved an increase in protein digestibility from 22.08% to 85.73% (protein digestibility increased 3.9 times compared to the digestibility of proteins of raw feathers). The increase in protein digestibility of feathers in ours study (protein digestibility increased 4.3 times in comparison to protein digestibility of raw feathers), which is presented in Table 3, is in agreement with the results of the above mentioned authors [6], due to the fact that they applied a shorter treatment period of raw feathers (20 min).

The present study reported the time extension of thermohydrolysis has a positive effect on protein digestibility of high-protein feed produced from feathers and intended for consumption by certain types and categories of animals, which is in accordance with statement reported by Kormanjoš et al. [17, 18] and Sakač et al. [6].

Increase of the protein keratin digestibility in feathers during the thermohydrolysis of raw feathers in the research of Kormanjoš et al. [17] is in accordance with the results of this study. Kormanjoš et al. [17], during the production of protein porridge by thermohydrolysis of feathers, at a pressure of 3.6 bars during the period of 30 min and with the moisture content in the raw material of 61.33%, reached acidic pepsin digestibility of 83.14%. The digestibility was 4.4 times higher than the protein digestibility in the raw feathers (digestibility increased from 18.85% to 83.14%). The similar results were obtained by Ristić et al. [19] who applied the treatment of the raw feathers at a pressure of 3.5 bars for a period of 40 min with the water content of the raw feathers of 60.82%. The protein digestibility of raw feathers from the slaughter house of broilers increased from 19.75% to 85.91% (digestibility increased 4.4 times compared to the digestibility of raw feathers).

Comparison of data on *in vitro* protein digestibility of hydrolysed feather meals produced with different amounts of water in raw feathers, showed that the water in thermohydrolysis of feathers positively affect the protein digestibility of obtained products.

Table 4. Amino acids in the proteins of raw and hydrolysed feathers

Amino acids	Treatment of feathers hydrolysis			
	Raw feathers	Hydrolysis with 50% water	Hydrolysis with 60% water	Hydrolysis with 68% water
Essential amino acids g/100 g protein				
Histidine	0.56±0.063[a]	0.59±0.042[ab]	0.60±0.069[ab]	0.64±0.079[b]
Lysine	1.84±0.184[a]	1.71±0.198[a]	1.76±0.176[a]	1.80±0.179[a]
Phenyl alanine *	3.82±0.225[a]	4.16±0.244[b]	4.22±0.253[b]	4.31±0.253[b]
Methionine	0.59±0.071[a]	0.57±0.067[a]	0.59±0.064[a]	0.58±0.076[a]
Threonine	3.94±0.492[a]	4.22±0.521[a]	4.16±0.520[a]	4.10±0.511[a]
Leucine	7.15±0.47[a]	7.70±0.515[a]	7.43±0.518[a]	7.50±0.505[a]
Isoleucine	4.69±0.385[a]	4.96±0.405[a]	4.89±0.400[a]	4.93±0.397[a]
Valine	7.18±0.265[a]	7.76±0.310[b]	7.76±0.335[b]	7.66±0.35[b]
Semi-essential amino acids g/100 g protein				
Arginine	6.18±0.344[a]	6.12±0.359[a]	6.02±0.339[a]	6.15±0.344[a]
Glycine	6.23±0.304[a]	6.69±0.342[b]	7.03±0.336[c]	7.07±0.347[c]
Cystine	7.00±0.482[b]	3.87±0.261[a]	4.07±0.294[a]	4.21±0.361[a]
Tyrosine	2.52±0.23[b]	2.18±0.209[a]	2.18±0.187[a]	2.26±0.258[a]
Non-essential amino acids g/100 g protein				
Asparaginic acid	6.76±0.432[a]	7.10±0.468[a]	7.27±0.477[a]	7.16±0.459[a]
Alanine	4.37±0.399[a]	4.44±0.405[a]	4.34±0.402[a]	4.39±0.399[a]
Glutaminic acid	8.25±0.366[a]	8.74±0.395[ab]	8.58±0.393[ab]	8.42±0.377[b]
Prolyne	8.43±0.479[a]	8.18±0.467[a]	8.17±0.452[a]	8.37±0.492[a]
Serine	11.07±0.569[b]	10.56±0.559[a]	10.39±0.543[a]	10.23±0.529[a]

Means with common superscript no differ significantly at P < 0.05.

Amino acid profile in the proteins of raw and hydrolysed feathers
The analysis of the amino acid composition of the protein of raw and hydrolysed feathers with different amounts of water indicate that there has been a significant reduction of cystine content in hydrolysed feathers compared to the content in proteins of raw feathers (Table 4). During hydrolysis with approximately 50% of the water in the raw feathers cystine in proteins decreased from 7% to 3.87% (the loss of cystine was around 44.71% compared to the content in the raw feathers). By applying water content in the raw feathers of 60% the loss of cystine was 41.86% (the content of cystine is reduced from 7% to 4.07 % in comparison to the content in the raw feathers). At the highest moisture content in raw feathers (68%) the lowest reduction of cystine in proteins of hydrolysed feather meal (39.86 %) were observed. Using this feather-processing technological procedure cystine content in proteins decreased from 7% to 4.21%. Decrease of the cystine content in the produced feed compared to the starting raw material was statistically significant ($P<0.05$) (Table 4). The difference in the contents of cystine in the proteins of hydrolysates obtained by hydrolysis of the raw feathers at a water content of approximately 50, 60 and 68 % were not statistically significant ($P>0.05$). The results of cystine contents in proteins of raw feathers and in proteins of hydrolysed feathers meal obtained by thermohydrolysis under pressure with different water contents in raw feathers showed that water generally have positive influence during thermohydrolysis on the content of cystine in proteins of derived products.

Kormanjoš et al. [17] examined the effect of water content in the raw feathers on the nutritional values of the meals produced from the thermohydrolysed feathers. They have reported the cystine reduction in proteins of hydrolysed feather meals similar to the results of current research. According to Kormanjoš et al. [17] hydrolysis of feathers led to changes in the content of cystine and some other amino acids in the proteins of feathers and to increases *in vitro* protein digestibility of feathers. Comparing the data on the amino acid composition in hydrolysed feather meals produced with adding different amounts of water prior to the hydrolysis, it is concluded that water has positive impact on the stability of the amino acids composition. Moreover, the protein digestibility in hydrolysed feather meals is improved during the thermohydrolysis processing of feathers. Therefore, obtained results indicated the importance of water content in raw feathers on the nutritional characteristics of hydrolysed feather meals.

The results of Sakač et al. [6] indicated that the thermohydrolysis of raw feathers at 3.6 bars for 30 minutes at a moisture content of treated feathers from 61.33%, causing a reduction of cystine content in proteins of hydrolysed feathers of 33.18%. This loss of cystine was lower (41.86%) compared to the loss in this study at approximately same conditions during thermohydrolysis of raw feathers (pressure of 3.5 bar at the time of 30 minutes and with a moisture content in raw feathers of approximately 60%) obtained from the broilers slaughter house. The observed difference is the result of the applied regime of hydrolysis. The above mentioned authors obtained hydrolysates in the moist state (moisture content 55.12%) used for further investigations.

CONCLUSION

Based on the obtained results and comparative analysis of the impact of the examined technological process of feathers thermohydrolysis on nutritional value of the final products, it is concluded that the amount of water in raw feathers of approximately 60% is the most favorable in the processing of this material (hydrolysis time 30 minutes and pressure of 3.5 bar).

Processing of raw feathers obtained from slaughtered broilers gave the high protein feed with a very high content of protein nitrogen (13.77% expressed in DM) and favorable *in vitro* protein digestibility (84.85 %).

Applied regimes of technological processing of raw feathers caused a significant reduction of cystine in proteins of feather meals as compared to the content in proteins of raw feathers.

Regarding to the regulations of the European Community on harmless removal of by-products of slaughtered poultry, using technological process of feathers hydrolysis it is achieved nutritionally valuable feed supplement intended for nutrition of certain types of animals, and at the same time is significantly reduced the problem of the environment protection.

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