

Poultry feather hydrolysate as a protein source for growing rats

Adriane GRAZZIOTIN^{1,2}
 Fernanda Araújo PIMENTEL¹
 Erna Vogt DE JONG¹
 Adriano BRANDELLI^{1,2}

1 - Instituto de Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul, Porto Alegre-RS
 2 - Programa de Pós-Graduação em Ciências Veterinárias da Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul, Porto Alegre-RS

Corresponding author:

Dr. A. Brandelli, ICTA-UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, Brasil; fax: +5551 3316 7048; e-mail: abrand@ufrgs.br

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Abstract

Feather protein has been considered as a protein complement for animal diets, since it is largely available as a by-product of poultry processing. In this work, a feather protein hydrolysate produced by the keratinolytic microorganism *Vibrio* sp. kr2 was evaluated as a feed additive. Wistar rats were fed seven experimental diets (n = 6 rats per diet) containing different protein sources: casein (CAS), soybean protein, feather hydrolysate, feather meal, and soybean protein supplemented with 10 or 20% (w/w) feather hydrolysate, and 20% feather hydrolysate supplemented methionine. Values for weight gain, feed ingest, true digestibility, feed:gain ratio, Protein Efficiency Ratio and Net Protein Ratio were similar for diets containing soybean protein and 20% feather hydrolysate supplemented methionine. Lowest values for all nutritional parameters were observed for diets containing soybean protein supplemented with 10 or 20% (w/w) feather hydrolysate, feather hydrolysate and feather meal. Protein source had a considerable influence in the final weight of liver, kidney and hearth, although no significant differences were observed for brains. These results indicate that feather hydrolysate may be useful as supplementary protein in feed formulations.

Key words:

Diet.
 Digestibility.
 Feed.
 Nutritional value.
 Protein.

Introduction

Feathers represent 5 to 7% of the total weight of mature chickens and are generated in huge amounts as a waste by-product at commercial poultry processing plants. Currently, feathers are converted to feather meal, produced by steam pressure cooking, which require high-energy input. Because of the large growth of the poultry industry, a great quantity of feather meal is available for use in animal feeds.¹

Feather meal have long served as alternative animal feed supplement, but variability in protein quality is one of the most important concerns regarding its use in livestock rations. Feather meal presents variable nutrient composition and nutrient bioavailability.^{2,3,4} Questionable amino acid balance and availability have limited their use in feeds. Feather protein is poorly digested

by birds and mammals, which has been attributed to the high degree of cross-linking and compacted structure within the keratin molecule together with the lack in animals of proteolytic enzymes capable of hydrolyzing this protein.⁵

Considering that feathers are composed by at least 90% keratin, the utilization of this protein source should be investigated. Production of feather hydrolysates by microbial proteases has been considered as a viable alternative. A feather-lysate produced by *Bacillus licheniformis* PWD-1 supplemented with amino acids produced a growth curve of chickens identical to that of soybean meal.⁵ This enzyme could increase the digestibility of commercial feather meal and replace up to 7% of the dietary protein for growing chicks.⁶ Although an increased number of feather-degrading microorganisms have been described,^{1,7} few

reports on utilization of microbial feather hydrolysates are available. In addition, the concerns about bovine spongiform encephalopathy (mad cow disease) have gaining importance, restraining some traditional protein sources for diets.⁸ In this context, feather protein hydrolysates may constitute an interesting alternative protein source for animal feed.

The feather-degrading microorganism *Vibrio* sp. kr2 produces a protein hydrolysate with similar amino acid composition to that described for the feather-lysate of *B. licheniformis* PWD-1.⁹ In a recent report, we showed that this hydrolysate has higher predicted nutritional parameters than feather meal and composition analysis indicated that methionine is the limiting amino acid.¹⁰ These previous results suggest that feather hydrolysate might be used as a protein source in diet formulations. The purpose of this study was to evaluate the feather hydrolysate produced by the strain kr2 as protein source in diets for growing rats.

Materials and Methods

Raw feathers were obtained from a local poultry-processing plant (Avipal, Porto Alegre, Brazil). Feathers were washed with warm tap water and distilled water, and dried at 45°C for 48 h in a circulating air-drying oven. After drying, feathers were autoclaved for 15 min prior to microbial treatment.

The bacterium used for production of feather hydrolysate was a *Vibrio* sp. kr2 strain, previously isolated from decomposing chicken feathers.⁹ The bacterium was grown aerobically at 30°C on raw feathers as unique source of carbon, nitrogen, sulfur and energy. Raw feather broth contained: 10 g/L raw feathers, 0.5 g/L NaCl, 0.3 g/L K₂HPO₄, 0.4 g/L KH₂PO₄; the pH was adjusted to 6.0.

The strain kr2 was grown in raw feather broth to reach 10⁹ cfu/mL and 10 mL of this culture were inoculated to 290 mL of medium containing 60 g/L feathers.¹¹ The organism was cultivated for up to 7 days at 30°C under agitation in an orbital shaker

at 180 rpm. After incubation the culture was autoclaved for 15 min, concentrated at 50°C under vacuum and dried in an air-circulating stove at 45°C for 48 h. The product was hammer milled to reach 1 mm mesh screen and stored at room temperature until used.

The experimental diets were formulated according the guidelines of the American Institute of Nutrition (AIN-93G) for growing rodents,¹² with reduction of protein to 10% to accomplish the calculation of Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) indices. The composition is detailed in table 1. Seven protein formulations were tested: casein (diet CAS), soybean protein (diet SP), feather hydrolysate (diet FH), feather meal (diet FM), and SP supplemented with 10 or 20% (w/w) FH, and 20% FH supplemented with 3 mg of methionine per g of protein (diets FH10, FH20 and FH20M, respectively). Soybean protein, soybean oil and feather meal were from Bunge (Esteio, Brazil), casein and cellulose were from Farmaquímica (São Paulo, Brazil), vitamin mix was from Roche (São Paulo, Brazil) and mineral mix was prepared according to AIN-93G.¹²

Experimental design consisted of seven complete random blocks. Forty two Wistar albino rats (recently weaned, 3-week-old animals) with an initial mean live weight of 50 g were used. The animals were divided into seven groups of six animals per group and kept in individual cages designed for separate collection of feces. The cages were placed in a well-ventilated, thermostatically controlled 22 ± 2°C room with 12 h light/dark periods. Each group consumed *ad libitum* one of the experimental diets made up of different protein sources for a period of 10 days, which consisted of a 3 day adaptation period to the diets followed by an experimental period of 7 days during which feces were collected on alternated days and stored at -21°C until analyzed. Daily food intake was determined by weighing the amounts of diet given, refused and spilled. Live weight was recorded daily. Throughout the trial, all rats had free access to water. Animals were killed under ether anesthesia.

Table 1 - Ingredients and chemical composition of experimental diets¹

Ingredient ²	Diets (g/kg)						
	CAS ³	SP	FH10	FH20	FH20M	FH	FM
Casein	123.30	-	-	-	-	-	-
Soybean protein	-	125.95	113.35	100.75	100.75	-	-
Feather hydrolysate	-	-	11.07	22.15	22.15	110.74	-
Feather meal	-	-	-	-	-	-	123.92
Sucrose	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Soybean oil	70.00	70.00	70.00	70.00	70.00	70.00	70.00
Cellulose	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Mineral mix (AIN-93G-MX)	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Vitamin mix (AIN-93-VX)	10.00	10.00	10.00	10.00	10.00	10.00	10.00
L-methionine	-	-	-	-	3.00	-	-
L-cystine	3.00	-	-	-	-	-	-
Choline bitartrate	2.50	-	-	-	-	-	-
Terbutyl hydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014
Corn starch	q.s.p.	q.s.p.	q.s.p.	q.s.p.	q.s.p.	q.s.p.	q.s.p.

¹ Feed formulation according AIN-93G for growing rodents,¹² with reduction of protein amount to 100g/kg

² Protein content of ground samples were (g/kg): casein (811), soybean protein (794), feather hydrolysate (903), feather meal (807)

³ CAS, casein; SP, soybean protein; FH10, soybean protein supplemented with 10% feather hydrolysate; FH20, soybean protein supplemented with 20% feather hydrolysate; FH20M, soybean protein supplemented with 20% feather hydrolysate and methionine; FH, feather hydrolysate; FM, feather meal; q.s.p. quantity sufficient to reach 1 kg

Adequacy of anesthesia was tested by the absence of withdrawal response to toe pinching. The abdominal surface was shaved and the skin cleansed, and the organs were taken out through a cavity opened along *striae alba*. This work followed the animal welfare guidelines of American Veterinary Medical Association¹³ and was approved by the Ethics Research Committee of the University.

Total nitrogen content was determined in food and feces according to the micro Kjeldahl's method,¹⁴ using mineralization (Block Digestor Kjeldatherm,

Gerhardt, Bonn, Germany), distillation units (Marconi, Piracicaba, Brazil) and titration units (Schott-Geräte GmbH, Mainz, Germany). Crude protein was calculated as N x 6.25.

True digestibility values were obtained by subtracting the endogenous excretion corrected for the amount of diet consumed from the apparent fecal losses.¹⁵ The Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) were calculated as follows: PER = gain in body weight (g) / protein consumed (g); NPR = (weight gain of TPG – weight loss of NPG) / protein consumed (g), where TPG = test protein group, and NPG

= nonprotein group.¹⁶

Experimental data were analyzed by one-way ANOVA and Tukey's highly significant difference test.¹⁷ Differences were considered significant at a confidence level of 95% ($P < 0.05$).

Results

Dietary intake of keratin-based diets by growing rats was low compared with a standard casein-based diet containing similar protein concentration (Table 2). The protein source in diet formulation had a great influence in the weight gain of the animals ($P < 0.05$). A decrease of weight gain was observed as the percentage of feather hydrolysate increased in the diets. When methionine was added to diet FH the rats showed a higher weight gain than those fed soybean protein (Table 2). The growth curves are shown in the figure 1. The weight gain ratio was calculated from the slopes of growth curves as 6.22, 3.41, 2.07, 1.78 and 4.03 g/day for diets CAS, SP, FH10, FH20 and FH20M, respectively.

The addition of feather hydrolysate to the diets resulted in decreased values of PER and NPR, except for the methionine supplemented diet (Table 2). Diets FH10 and FH20 were not significantly different ($P > 0.05$) to each other despite the later had twice the amount of feather protein.

True digestibility was not affected by the substitution of the protein source up to 20% feather hydrolysate, even when compared with casein. Significant differences were only observed when feather protein was used as sole protein source, as the case of the diets FH and FM (Table 2).

The weight of some individual organs was measured (Table 3). As observed for weight gain, the protein source had a considerable influence ($P < 0.05$) in the final weight of liver, kidney and heart, although no significant differences were observed for the

brain ($P > 0.05$).

Discussion

A feather hydrolysate was used as protein source in experimental diets for rats, showing a performance comparable to soy protein when added at 20% plus methionine supplementation. Dietary intake of keratin-based diets by growing rats was low compared with a standard casein-based diet of similar protein concentration. Low feed intake of legume-based diets has been related to the presence of antipalatable components (alfa-galactosides or tannins) and deficiencies in certain essential amino acids, minerals and vitamins, leading to

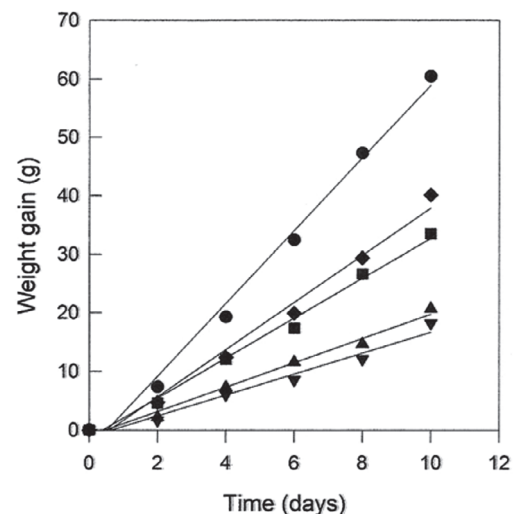


Figure 1 - Growth curves of Wistar rats feeding the standard diet (casein, ●), or diets containing soybean protein (■), soybean protein supplemented with 10% feather hydrolysate (▲), soybean protein supplemented with 20% feather hydrolysate (▼), or soybean protein supplemented with 20% feather hydrolysate and methionine (◆). Each data point represents the mean of five animals

nutrient imbalance.¹⁸ Elmayergi and Smith¹⁹ tested feather meal and soy protein as standard in diets for chickens. They observed a decrease in dietary intake from 166.3 g to 76.3 g when comparing the standard diet with feather meal and a consequent decrease in weight gain from 59 g to 7 g. The deficiency in essential amino acids had harmful consequences for growth

Table 2 - Food intake, growth performance and digestive utilization of nitrogen in rats fed experimental diets

	Diets						
	CAS ¹	SP	FH10	FH20	FH20M	FH	FM
Dietary intake (g)	131.9 ^a	115.2 ^{ab}	92.1 ^{bc}	81.4 ^c	112.3 ^{ab}	48.3 ^d	70.2 ^c
Weight gain (g)	60.5 ^a	33.4 ^{bc}	20.7 ^{cd}	18.2 ^d	40.1 ^b	-5.3 ^e	2.7 ^e
AEC	0.46 ^a	0.34 ^b	0.27 ^c	0.22 ^c	0.36 ^b	-0.11 ^e	0.04 ^d
PER	4.2 ^a	3.2 ^b	2.6 ^c	2.0 ^c	3.5 ^b	-0.9 ^e	0.4 ^d
NPR	4.9 ^a	4.0 ^{bc}	3.7 ^{cd}	3.2 ^d	4.4 ^{ab}	1.1 ^f	1.9 ^e
True N digestibility (%)	96.1 ^a	94.9 ^a	92.9 ^a	92.9 ^a	90.4 ^a	82.3 ^b	83.8 ^b

¹ CAS, casein; SP, soybean protein; FH10, soybean protein supplemented with 10% feather hydrolysate; FH20, soybean protein supplemented with 20% feather hydrolysate; FH20M, soybean protein supplemented with 20% feather hydrolysate and methionine; FH, feather hydrolysate; FM, feather meal; AEC, alimentary efficacy coefficient; PER, Protein Efficiency Ratio; NPR, Net Protein Ratio. Different superscript letters indicate significant differences within the same row at $P < 0.05$ by Tukey's test

Table 3 - Weight of organs of rats fed experimental diets

	Diets						
	CAS ¹	SP	FH10	FH20	FH20M	FH	FM
Liver	4.7 ^a	3.4 ^b	3.0 ^b	2.9 ^{bc}	3.8 ^b	1.6 ^d	2.2 ^c
Brain	1.5 ^a	1.4 ^a	1.4 ^a	1.4 ^a	1.5 ^a	1.3 ^a	1.4 ^a
Kidney	1.1 ^a	0.8 ^b	0.8 ^b	0.7 ^{bc}	0.8 ^b	0.5 ^d	0.6 ^{cd}
Heart	0.5 ^a	0.5 ^{ab}	0.4 ^b	0.4 ^{bc}	0.5 ^a	0.2 ^d	0.3 ^c

¹ CAS, casein; SP, soybean protein; FH10, soybean protein supplemented with 10% feather hydrolysate; FH20, soybean protein supplemented with 20% feather hydrolysate; FH20M, soybean protein supplemented with 20% feather hydrolysate and methionine; FH, feather hydrolysate; FM, feather meal. Different superscript letters indicate significant differences within the same row at $P < 0.05$ by Tukey's test

and lowered feed intake. Soybean, and particularly feather hydrolysates, have shown limiting amounts of sulfur amino acids,^{10,20} and in both cases the weight gain was lower than the casein-based diet.

The evaluation of digestive utilization of feather protein revealed its reduced biological value. Proteins with PER values below 1.5 are considered as low quality, between 1.5 and 2.0 as intermediate and good quality for those of PER higher than 2.0.¹⁶ The PER values found in this work suggest that feather keratin, either as feather meal or feather hydrolysate, is a low quality protein. This result is in agreement to other reports describing the poor nutritional value of feather meal.^{4,21} In chick bioassays, no significant differences were found in PER and NPR among FM, enzyme-treated FM

and NaOH-treated FM, although metabolizable energy of enzyme-treated FM was significantly higher.²² Instead, feather protein structure and low solubility derived from aggregation caused by disulphide bonds and hydrophobic interactions may be determinant for protein digestibility, like suggested for some legume proteins.²³

However, feather protein is considered an excellent source of metabolizable protein²⁴ and microbial feather-lysate has similar nutritional features to soybean meal,⁵ indicating the potential use of feather keratin as feed protein. The amounts of some limiting amino acids as methionine, lysine and arginine are often higher in microbially treated feathers than in feather meal.^{1,9,25} Feeding trials indicate that fermented feather meal allows better

performance than feather meal, despite sub-deficiency of methionine, resulting in improved digestibility and lower lack of weight gain in rats.¹ In agreement with these results, the feather hydrolysate obtained with strain kr2 showed best *in vitro* nutritional features than feather meal¹⁰ and could substitute up to 20% of soybean protein when supplemented with methionine.

Protein hydrolysates obtained from submerged cultivation of keratinolytic bacteria on poultry feathers show upgraded nutritional value of feather keratin.^{10,25} Recycling of feathers is a subject of great interest for animal nutrition, because of its potential as an inexpensive and alternative protein source. Despite the limited nutritional value of keratin, both the digestibility and amino

acid balance of feather protein might be improved by microbial fermentation.^{10,25}

Conclusion

The microbial feather hydrolysate produced by the strain kr2 can replace up to 20% of soybean protein in feeds when supplemented with methionine. Additional experiments should be accomplished to test different levels of inclusion of this hydrolysate to allow its satisfactory utilization as supplementary protein in feed formulations.

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Hidrolisado de penas como fonte de proteína para ratos

Resumo

A proteína da pena é uma boa fonte proteica para dietas de animais, sendo um material de grande disponibilidade como subproduto da produção avícola. Neste trabalho, um hidrolisado protéico de penas produzido pelo microrganismo queratinolítico *Vibrio* sp. kr2 foi avaliado como suplemento em rações. Ratos da linhagem Wistar foram alimentados com sete dietas experimentais (n = 6 ratos por dieta) contendo diferentes fontes de proteína: caseína (CAS), proteína de soja, hidrolisado de pena, farinha de pena, e proteína de soja suplementada com 10 ou 20% (w/w) hidrolisado de pena, ou 20% hidrolisado de pena suplementado com metionina. Os valores de ganho de peso, consumo, digestibilidade verdadeira, coeficiente de eficiência alimentar, coeficiente de eficiência proteica (PER) e eficiência líquida proteica (NPR) foram similares para as dietas contendo proteína de soja e proteína de soja suplementada com 20% hidrolisado de pena e metionina. Valores inferiores para todos parâmetros nutricionais foram observados para as dietas contendo 10 ou 20% hidrolisado de pena, hidrolisado de pena e farinha de pena. A fonte protéica influenciou no peso final do fígado, rins e coração, porém as diferenças não foram significativas para cérebros. Estes resultados indicam que o hidrolisado de penas pode ser usado como fonte de proteína suplementar na formulação de rações desde que suplementados com metionina.

Palavras-chave:
Dieta.
Digestibilidade.
Ração.
Valor nutricional.
Proteína.

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