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Composition, Digestibility, and Microbiological Quality of the Animal-Origin Meal

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Abstract

There is currently a growing interest of animal nutritionists and farmers in the use of by-products and/or agro-industrial residues in feeds. The purpose is to reduce production costs, since feed represents nearly 70% of the cost of production, as well as to allocate waste properly, minimizing potential environmental impacts. The characteristics of the residues used and their physical and/ or chemical limitations should be known, providing nutritionist with the correct information for the best choice and use of these ingredients for animal nutrition. This study aims to characterize the centesimal and energetic composition,

digestibility, and microbiological quality of animal-origin meals in non-ruminant feeds. Samples of animal-origin meals – AOM (n=210), hydrolyzed feather meals (n=70), chicken offal meal (n=70), and pig offal meal (n=70) were evaluated. The following variables were determined: moisture, crude protein, amino acids, ethereal extract, ash content, FAO grain size analysis, and protein digestibility. Peroxide and acidity levels were determined to evaluate the oxidative process. The microbiological quality of AOM evaluated by the presence/absence of *Salmonella* spp.; the apparent metabolizable energy was verified by the indirect method using prediction equations. The amounts of proteins, minerals, amino acids, and energy differed from those reported in the literature. These results were possibly due to the different operational processes performed in each one of the experiments, as well as the proportions of constituents in the compared raw materials compared. Moreover, we observed that the AOM is within the Brazilian hygienic-sanitary standards.

Keywords: Grain size, Metabolizable energy, Rancidity, *Salmonella*, Viscera

1. Introduction

In agribusiness, chicken meat and pork production chains constitute important segments that generate employment and income for people. Besides the expressive amounts of animal protein produced and exported, there is the generation of the most varied co-products and/or by-products from the slaughter process. The by-products consist of offal, feathers, sebum, blood, meat, and bones (Reis *et al.*, 2013).

In Brazil, the animal recycling industry works to prevent significant volumes of animal origin co-products (AOC) from inappropriately discharged in the environment. In 2014, 12.4 million AOCs tons were processed, and not destined for human consumption, generating 5.3 million tons of meal and oils. There was a production of 3.41 million tons of animal meal, of which 15.6% came from feathers, and 18.3% from offal (Brazilian Animal Recycling Association – ABRA, 2016).

The production of animal by-products presents an average annual growth of 25.4%, which shows the potential of these products, especially meat and bone meal, which accounts for 86% of Brazilian foreign sales. The concentration of flour and fat manufacturing is proportional to the concentration of factories of animal products, as a symbiotic relationship of the production chain, benefiting the environment of those regions (Silva *et al*., 2018).

Os coprodutos de origem animal são definidos como corpos inteiros ou partes de animais mortos e outros produtos provenientes de animais que não se destinam ao consumo humano, incluindo oocistos, embriões e sêmen. As principais fontes dos coprodutos são o abate de animais destinados a consumo humano, a produção de alimentos de origem animal e o abate sanitário de animais (ABRA, 2016; Geraldes, 2014).

Animal-origin meals – AOM is an important source of calcium, phosphorus, amino acids and energy (Ruis *et al*., 2013). The use of animal by-products in the monogastric diet aims not only to reduce feed costs, but also to be an alternative source of protein (Carvalho *et al*., 2012). Thus, the effect of animal meal on animal performance can be modified by several factors, including processing, an origin of the co-product used, and use of additives and

antioxidants (Aguiar *et al*., 2014).

The inclusion of up to 8% of animal meal in the feed, in addition to meeting the protein needs of the bird, plays an important role in nutrient recycling and environmental preservation, when considering the pollutant content of slaughterhouse co-products. Thus, harnessing waste from the meat industry for the production of animal meal (FOA) is of paramount importance in the economic and environmental aspects (Rostagno et al., 2017).

However, the lack of standardization in the manufacturing process can be inconvenient for use, as it causes variations in nutrient and energy content (Geraldes, 2014). Thus, the use of FOA in feed is limited by knowledge of the origin of the material and its processing, factors that affect the digestibility of nutrients provided by them. Therefore, technologies that improve the digestibility of these ingredients in poultry feed are important.

Numerous factors involved in the acquisition process of raw materials that have a wide variation in chemical composition depending on the type and proportion of materials (meat, blood, bones and fat) used; the form of processing, in this case the most influential factor is the temperature used in the cooking of the raw material, which can modify the chemical composition and nutritional (digestibility and metabolizability in nutrient) and microbiological quality AOM. However, there are economic, sanitary, and nutritional reasons for the use of AOM, such as lowering the final feed price, and providing a suitable destination for the waste generated by slaughterhouses (Eyng *et al.*, 2012, Troni *et al.*, 2016).

The continuous study of the composition of ingredients used in feed, especially of by-products, is relevant because there are evident variations due to the lack of standardization in the productive processes. Poultry meal is a crushed, powdered, semi-defatted product, resulting from the cooking of raw material originated in the slaughter of birds, consisting of meat parts, viscera, heads, feet and other organs, except feathers and blood removed in the animal's bleeding. Pig meal is a crushed, powdered, semi-defatted product resulting from the cooking of raw material of swine origin, consisting mainly of meat, offal and bones of swine. Hydrolyzed feather meal is a ground, powdered product resulting from the hydrolysis of feathers originating from the slaughter of birds.

The objective of this study was to characterize the centesimal and energetic composition, digestibility, and microbiological quality of animal-origin meals used in non-ruminant feeds, to keep the database update (matrices) and to improve the nutrient estimation for AOM´s.

2. Material and Methods

The evaluations were performed on animal meal samples - OMA (n = 210) from a Meal and Fat Factory (QFP) connected to a refrigerator located in Rio Verde, Goiás, Brazil. A sample of each load received collected, totaling in 70 days collection for evaluation. The studied ingredients were hydrolyzed feather meal - HFM (n = 70), poultry meal - POM (n = 70) and pig meal - SOM (n = 70).

The AOM processed in a cylindrical digester with a heat jacket, and with a capacity of 6,750 liters. The cooking process occurred in 35 minutes at a pressure of 2.0 kgf cm^{-2}. Pre-drying

then carried out in the digester for 20 min., and the AOM discharged in a percolator, routed through a propeller thread to a primary rotary dryer, with a temperature of ≅ 120°C. The ingredients subsequently passed to a secondary rotary dryer, reaching a temperature of 100°C. Milling carried out in a hammer mill in the last stage.

Near Infrared Reflectance Spectroscopy (NIRS) determined the following variables: moisture (M), crude protein (CP), amino acids, ethereal extract (EE), and ash content (AC). Peroxide (PL) and acidity (AL) levels were determined to evaluate the oxidative process. Analyses followed the methodologies described in the Brazilian Compendium of Animal Feeding (Sindirações, 2013).

Nitro-perchloric digestion (wet digestion) used for calcium (Ca) and phosphorus (P) quantification in the samples. Visible spectrophotometry in the Exata Laboratory (Jataí-GO, Brazil); and Ca determined phosphorus content by atomic absorption spectrophotometry (GBC-932 AA, Scientific Equipment PTY LTD, Melbourne, Australia) readings performed in

spectrophotometer at 660 nm.

The microbiological quality of AOM evaluated by the presence/absence of *Salmonella* spp. The technique used was Enzyme Linked Fluorescent Assay (ELFA) in the VIDAS® automated system, according to AOAC recommendations (2005).

Protein digestibility was determined using a pepsin assay (0.002%), carried out in an oven while stirring. The in vitro pepsin digestibility test has a useful purpose and place in the industry. It turns out that this test used more broadly to make more far-reaching decisions for what it truly intended. Many people see the results of this test as "black and white," reliable and hassle free. In reality, the in vitro pepsin digestibility test is an approximation, an indicator correlated with the true state of affairs, but not a complete, unclear or definite picture. One gram of each sample weighed in a 250-ml Erlenmeyer, adding 75 ml of pepsin in solution. The flask capped and incubated for 63 hours at 45°C. The contents of the incubation flask transferred to a tube and centrifuged for 10 min. The supernatant then filtered through qualitative filter paper in 100 mL beaker. From the aliquot, 10 ml of the filtered extract transferred to a macro tube, and the protein content quantified by following the method of *Kjeldahl* (Sindirações, 2013).

The determination of Metabolizable Energy and Metabolizability values of nutrients/or ingredients is carried out by means of nutritional tests, including metabolism tests which require a period of adaptation for animals and facilities. Another more viable alternative for companies that need immediate results is the use of prediction equations, which in this case provides the Apparent Metabolizable Energy that made by calculating the difference in gross food energy by excreted corrected for metabolic and fecal energy losses endogenous urination. The Apparent Metabolizable Energy (AME) verified by indirect method using prediction equations. Sieves with 3.0 and 1.5 mm meshes used in the FAO grain size analysis, following a mesh-opening ascending order (bottom-up). One hundred grams of each sample weighed and sieved manually (30 abrupt moves within 10 min). Then, the sum of the mass retained on each sieve was determined, together with a granulometric percentage.

The data submitted to a descriptive statistical analysis. We opted to use the relative dispersion test, i.e. Pearson's coefficient of variation (CV), so we could analyze the dispersion of the means.

3. Result and Discussion

Table 1 describes the centesimal composition and energy values of the evaluated animal-origin meals.

Table 1. Bromatological composition and energy value of animal-origin meals (n=70 samples of each ingredient)

Ingredient	Moisture %	Crude Protein %	Ethereal Extract %	Ash %	Calcium %	Phosphorous %	AME[1] kcal/kg
HFM[2]	5.86	82.93	9.15	3.07	0.62	0.42	2.863
CV[3] (%)	14.80	1.34	6.64	10.42	24.92	37.78	10.20
POM[4]	7.11	55.10	17.16	18.45	5.67	2.98	3.008
CV[3] (%)	7.59	2.64	6.59	1.78	15.98	15.55	7.81
SOM[5]	3.31	49.17	16.19	18.72	6.36	3.52	3.722
CV[3] (%)	11.80	1.91	12.33	8.00	10.77	16.65	5.50

[1]AME = Apparent Metabolizable Energy. [2]HFM = hydrolyzed feather meal. [3]CV = coefficient of variation. [4]POM = poultry offal meal. [5]SOM = swine offal meal.

The crude protein (CP) percentage of the hydrolyzed feather meal (HFM) verified in this study was 1.73% higher than the value (Carvalho *et al.*, 2016), whose average was 81.2%. Studied the constitution of HFM and found 80.0% CP, 8.0% moisture, 2.0% ethereal extract (EE), and 4.0% mineral matter (MM) (França *et al.*, 2011).

Regarding apparent metabolizable energy (AME), the value obtained for HFM was similar to that reported in the literature. Eyng *et al*. (2012) presented 2.758 of AME_n for feather meal (FM),in the experiment the AME means verified for FM were: 1,772, 2,187, and 2,472 kcal/kg; The Brazilian Table for Poultry and Swine (2017) showed two FM, with 75 and 84% BP, whose AME were 2,656 and 2,666 kcal/kg, respectively.

According to Bellaver and Zanotto (2004), offal meal moisture (U) is regularly between 4.0-6.0%, and should not exceed 10%. POM presented 7.11% U, and the swine offal meal (SOM), 3.31%. The POM moisture was in conformity with the description by The Brazilian Table for Poultry and Swine (2017) 7.0% for the ingredient mentioned. The lower value observed for SOM may mean overcooking or overfrying, which in turn may be associated

with equipment wear, excessive retention time, and/or malfunction of gauges and thermometers.

In comparison, the results of CP, MM, calcium (Ca), phosphorus (P), EE, and AME of the meals tested were different from those (Eyng *et al.*, 2010; Silva *et al.*, 2012; Troni *et al.*, 2016; The Brazilian Table for Poultry and Swine (2017)). Silva *et al.* (2012) reported that the amounts of CP, MM, and EE of the offal meal might be influenced by the predominance of certain animal parts, such as head, back, and feet. Still, according to the authors, in Brazil, 47% of the meal has protein composition of 57.7-61.4%, ash of 11.8-16.20%, and EE of 10.1-16.1%. The Brazilian Table for Poultry and Swine (2017) described the POM constitution, with CP content of 55.50-57.70%, and MM of 11.60-15.20%; as well as SOM, with 47% CP, and 27.90% MM.

The mean values of AME (kcal/kg) for the POM reported were 3,020 (Eyng *et al.*, 2010), 3,545 3,241 and 3,340 (Troni *et al.*, 2016), and 3,241 and 3,682 (The Brazilian Table for Poultry and Swine (2017)). That is, in the literature, the descriptions showed 336 kcal more than what was found in this study. The higher mineral content of the POM probably reverberated to the lower energy content, since the feed had higher EE content.

Differently, the AME in SOM was 3,722 kcal/kg, higher than that found by Fialho *et al.* (2009), of 3,004 kcal/kg, by Eyng *et al.* (2010), of 2,145 kcal/kg and by The Brazilian Table for Poultry and Swine (2017) of 2,240 kcal/kg. According to De Marco *et al.* (2015), the CP and EE content and the composition of fatty acids and minerals contribute to variations in the feed energy values.

Feather meal (FM) consists of a by-product generated by pressure-cooking and subsequent drying of clean, non-decomposed feathers from poultry slaughter. FM hydrolysis is indispensable because due to its keratinized nature, it presents a great amount of sulfur amino acids and resistance to the action of digestive enzymes, making its use difficult for the animal organism (Vidmar & Vodovnik, 2018; Eyng *et al.*, 2012). For protein availability, acid or alkaline hydrolysis is required for a partial degradation of keratin filaments, making it soluble and digestible (Silva, 2016).

The protein and mineral contents, calcium (Ca), and moisture content of HFM were lower when compared to those described by Carvalho *et al.* (2012). The differences were probably due to the factors involved in the process of obtaining HFM, such as temperature, cooking time, and material drying time.

The poultry offal meal (POM) characterized as a by-product resulting from cooking, pressing, and grinding of these animals' offal, such as intestines, lungs, carcasses, feet, necks, and bones from mechanically separated meat. It should not contain feathers or residues from a hatchery or other materials that are foreign to its composition (Silva *et al.*, 2011).

The variables EE, calcium (Ca), phosphorus (P), and AME presented higher values when compared to those of Eyng *et al.* (2010), Silva *et al.* (2012), and Troni *et al.* (2016). The heterogeneity of the processing methods may justify the results. In the case of EE, it may be linked to the digesters' feed forms, as well as to the frying of the meals. It is important to

consider that some greases add approximately 3.57% fat to the mass, in order to improve the cooking of the offal. Consequently, this fat is added to that present in the raw material (Silva *et al.*, 2012). The distinct amounts of Ca and P presumably came from the proportions of parts (organs) of the birds and swine used. In addition, the technological level of the slaughterhouses may have influenced the minerals contained in the meal, and the greater the use of the meat adhered to the parts, the greater the possibility of the presence of small bone fragments, resulting in higher mineral content.

Table 2 describes the amino acid composition of the animal-origin meals evaluated.

Table 2. Animal meal amino acid composition percentage, (n=70 samples of each ingredient).

AOM[1]	Arg[2]	Cys[3]	Phe[4]	Ile[5]	Leu[6]	Lys[7]	Met[8]	Met+Cys[9]	Thr[10]	Val[11]	Trp[12]	His[13]
HFM	5.68	4.59	3.89	3.89	6.59	2.00	0.60	5.20	3.84	5.88	0.528	0.76
CV (%)	1.85	3.48	1.74	1.7	1.74	5.94	2.57	3.58	2.16	2.99	1.84	4.42
POM	3.33	0.60	1.78	1.77	3.15	2.61	0.88	1.52	1.75	2.13	0.38	1.00
CV (%)	6.33	15.79	7.15	7.77	7.26	8.83	8.6	8.32	7.95	7.58	7.91	5.85
SOM	3.00	0.46	1.62	1.41	2.80	2.38	0.77	1.11	1.54	1.99	0.34	1.01
CV (%)	4.63	9.55	5.33	6.31	5.70	7.52	19.43	18.90	5.84	5.74	8.05	6.70

[1]AOM = animal-origin meals. [2]Arg = arginine; [3]Cys = cystine; [4]Phe = phenylalanine; [5]Ile = isoleucine; [6]Leu = leucine; [7]Lys = lysine; [8]Met = methionine; [9]Met+Cys = methionine + cystine; [10]Thr = threonine; [11]Val = valine; [12]Trp = tryptophan; [13]His = histidine.

There were deficiencies of essential amino acids in HFM - lysine, methionine, tryptophan, and histidine; and higher amounts of methionine + cystine, threonine, and isoleucine when compared to the results Li & Wu (2018) and The Brazilian Table for Poultry and Swine (2017). Cystine had greater variation, and the content measured was higher than that verified by the researchers mentioned above. This fact may have been due to the thermal-sensitivity of the by-product, influenced by the processing conditions.

Carvalho *et al*. (2012) determined isoleucine (3.84%), leucine (6.71%), and valine (5.84%) in

FM. The authors' results were lower than those estimated for HFM in this study were. According to Silva *et al.* (2012), the relationship between valine, leucine, and isoleucine been increasingly discussed, since these amino acids show specificity for the same system of membrane transporters, and use the same enzymes for this purpose.

The amino acids constituting the POM had a lower percentage than those of Fernandes *et al.* (2011) for POM (64.14 and 68.33%), from Cao and Adeola (2016) for POM (63.42%), as well as from The Brazilian Table for Poultry and Swine (2017) for POM (55.3 and 57.7% CP). The SOM studied presented constitution similar to the one described by The Brazilian Table for Poultry and Swine (2017) for lysine, methionine, isoleucine, histine, and methionine + cystine. The other SOM amino acids maintained their amounts smaller than did those found by Eyng *et al.* (2010) and The Brazilian Table for Poultry and Swine (2017).

For many years, poultry and swine feeds formulated based on the CP concept, which, for the most part, caused diets to have imbalanced amino acid levels, resulting in an excess of many of them. With the advances in animal nutrition, it was possible to establish how much of these nutrients animals really need, and then provide them with adequate amounts without excess or deficiencies, constituting the ideal protein concept (Prabu *et al.*, 2017; Ribeiro and Oelke 2013). Thus, the need arose to evaluate the amino acids constituting the ingredients that will be part of the feeds, such as non-alternative feeds and/or by-products.

In general, the amino acid estimates varied according to the meal crude protein content, being different from those reported in the literature (Spranghers *et al.*, 2017; Eyng *et al.*, 2010; Carvalho *et al.*, 2012; Silva *et al.*, 2012; Cao and Adeola 2016; The Brazilian Table for Poultry and Swine (2017).

The feather meal (FM) has a high cystine content, which in turn is contained in feather keratin, which makes feed use difficult due to the hydrogen bonds, and this contributes to maintaining great protein stability when attacked by enzymes. The modification of the FM cystine amount is directly related to CP content, raw materials, and processing conditions (Maciel *et al*., 2017).

Protein sources are ingredients that contribute to the rising cost of bird diets. Therefore, it is necessary to use technologies that provide the best nutritional utilization of this ingredient by animals, such as proteolytic enzymes. The use of enzymes is one of the technologies used to improve the nutritional characteristics of FOA by increasing feed quality. Proteases can degrade proteins by releasing peptides and amino acids and other nutrients to animals (Silva *et al*., 2018).

Table 3 shows the grain size, digestibility, and microbiological quality of the AOM studied.

Table 3. AOM grain size, digestibility, and microbiological quality (n=70 samples of each ingredient)

Ingredient	Retention Sieves		Protein Digestibility in Pepsine[1] %	Acidity %	Peroxide / mEq	*Salmonella* / 25g
	Mesh 3.0 mm	Mesh 1.5 mm				
HFM	19.98	63.94	27.72	0.91	4.59	Absent
CV (%)	24.50	15.26	32.50	33.52	50.04	
POM	25.09	68.70	45.71	1.74	1.59	Absent
CV (%)	22.95	9.69	10.38	34.29	56.27	
SOM	37.89	56.32	50.60	1.34	1.74	Absent
CV (%)	17.52	12.33	13.66	18.48	32.98	

The AOM should not present pathogenic bacteria, and *Salmonella sp*. should be absent in 25 g of the sample considered (Brazil, 2008).

According to Sindirações (2013), the ideal texture for an AOM characterized by non-retention in a 3.5 mm mesh sieve, and a maximum of 10% of sieve accumulation with a 1.5 mm mesh. The samples evaluated were out of the desired specifications. The grain size analysis refers to a control parameter, which makes the performance of new grinding and/or adaptation of the equipment possible in order to comply with the requirements. However, it should be noted that adaptations and/or corrections generate additional costs to the production process (Costa *et al.*, 2008).

The oxidative and hydrolytic rancidity indicators of the different AOM studied, acidity, and peroxide index (PI) met the recommendations of Sindirações (2013). The results were lower than were those reported by Fernandes *et al.* (2011), who evaluated the quality of the POM produced by independent cold stores or industries, in the different seasons of the year. Researchers reported a PI higher than 10 mEq/g in spring and acidity higher than 3.0 mg NaOH/g in the summer when the AOM in this study analyzed.

The protein digestibility of offal meal was inferior to those described by Murakami *et al.* (2018) of 84.84% and by Kawauchi *et al.* (2014) of 67.5%. The lowest digestibility verified in the HFM, whose value was 27.72%.

The AOM characteristics make them susceptible to deterioration by pathogenic microorganisms when not properly treated (Ruis *et al.*, 2013; Fujihara *et al.*, 2014).The samples evaluated were in conformity with the national hygienic-sanitary standard. High

temperatures during AOM processing eliminate much or even all bacterial contamination. However, care should be taken with subsequent activities, such as handling and/or transportation, which can contaminate meals.

The occurrence of free fatty acids in feeds serves as an indication of hydrolytic rancidity (rancid). This occurs in situations of high moisture, favoring the elevation of the enzyme lipase produced by bacteria. Thus, high acidity usually associates with a high bacterial population (Coradi *et al.,* 2011; Matias *et al.,* 2012). According to the recommendations of Sindirações (2013), the maximum value for acidity should be 3.0 mg NaOH per gram of sample, and for the peroxide index, it should not exceed 5.0 mEq/1000g.

Carollo (2013) monitored the quality of meat and bone meal (MBM) during prolonged storage (10 weeks) and evaluated the addition of antioxidant (BHT - 500 mg/kg) to MBM. The authors did not verify changes in acidity; however, there was an increase in the IP of the control lot, which exceeded the maximum limit, reaching 80 mEq/kg at 56 days of storage. The effect of antioxidant addition on the preservation of MBM quality seen when BHT was added at day zero or seven. However, when the oxidation process had already started, the addition of BHT did not have the desired effect on PI.

The AOM studied in the present experiment had antioxidant addition, and storage time was less than one week. Furthermore, PI is questionable because it is a punctual analysis, which can be performed after the peroxide formation peak, in which these compounds are transformed into secondary ones, such as ketones and aldehydes (Valle, 2010).

The processing of animal meals in the grease, specifically temperature, pressure, and time employed, can compromise the product quality either by charring organic matter, reducing total digestibility or by making specific amino acids unavailable. These variations directly reflect on the protein quality of ingredients, which can cause large differences between by-products (Glencross *et al.,* 2019).

According Eyng *et al.* (2012), low biological values of HFM are based on protein content, in natura material, and processing conditions. Regarding operational procedures, low-pressure cases may require a long hydrolysis time and, if it does not occur completely, digestibility becomes compromised.

The lack of standardization of operational processes and the constituents of raw materials are the main causes of differences in the chemical and energy compositions of the animal-origin meal - AOM.

AOM are predisposed to damage by oxidative processes, as well as by contamination by pathogenic microorganisms. Thus, a routine of verification of components and quality of the AOM that will be used in feeds for monogastric animals must be established

The Brazilian Compendium of Animal Feed (CBAA, 2013) presents the quality specifications for animal meal. For poultry viscera flour, the values are 8% moisture (maximum), 55% crude protein (minimum), 60% pepsin digestibility, 10% ethereal extract (minimum) and 15% mineral matter (maximum). In porcine meal the specification is 8% moisture (maximum),

55% crude protein (minimum), 85% pepsin digestibility, 15% ethereal extract (minimum) and 33% mineral matter (maximum). For beef and bone meal, the specifications are 8% moisture (maximum), 55% crude protein (minimum), 30% pepsin digestibility, 10% ether extract (minimum) and 28% mineral matter (maximum).

4. Conclusion

The results found in this work demonstrated that the ingredients meet the quality requirements required by the Brazilian Legislation and, is essential since it makes the adequate and beneficial destination of the residues generated by the productive chains of the meat (slaughter) possible, mitigating possible impacts to the environment. Furthermore, it allows to reduce the livestock activity costs and to propitiate its sustainability.

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