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TECHNICAL NOTE

Essential Aminoacid Contents of HydrolyzedProtein Supplements in the Diet of Laying Hens

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INTRODUCTION

The ideal aminoacid diet profile for layers has not been studied as much as for broilers and pigs. However, the use of the ideal protein profile to determine the aminoacid contents in the diet has advantages over empirically determined aminoacid requirements (Bregendahl and Robert, 2009).

Protein hydrolyzed (PH) supplements can be widely applied as ingredients in the formulation of special feeds (purified diets, protein supplements, etc.), as they improve protein digestibility and reduce allergenic traits. The functionality of peptides from hydrolyzedproteinsdepends mostly on controllinghydrolization (molecular size, structure and specific aminoacid sequences (Guerrero *et al*., 2012).

Protein-derived products were introduced in Cuba since the late 1970s. Previously, on many occasions these sources were wasted. Animal blood is one valuable source of proteins and essential aminoacids. According to Barboza *et al*. (1994), blood and its fractions are easily digested and have a quality aminoacid composition, which providesa high biological value to its proteins; hence, the formulation of different foods and feeds has included it.

PH is a derivative from bovines that contains 30% bovine blood, and it isproduced in LABIOFAM; it can be used as stimulant in calves and pigs with digestive and pneumonic disorders. It has also been useful in treating growing L1 chicks to gain weight, and has also been used on L1 hens along with a vitamin supplement to correct pecking.

It is composed of aminoacids, essential components of a number of biopreparation used in medicine and agriculture.

Aminoacid derivatization through gas chromatography is a process that can be done for hydrolizate analysis (Hušek,1991; Khan, 2014).

The aim of this study was to determine the content of essential aminoacids of a protein hydrolyzate, to be used as a supplement in layers.

DEVELOPMENT

This study took place in February 2015, using a protein hydrolyzate (Table 1), made at LABIOFAM, located in Valle de Yumurí, Matanzas, Cuba. The preparation was liquid and had 22% protein. The batch number used was No. 1409190 – 4.

The hydrolyzed-free aminoacid contents were determined at the Center of Genetic Engineering and Biotechnology (CIGB), using the kit from Phenomenex EZ: fastTM (Phenomenex, USA). Derivatization and measurement of free aminoacids in the sample were made according to the kit protocol. Norvaline (nNal) was added to each sample before derivatization as internal standard. Solid phase removal was made to capture the free aminoacids at the matrix, placed on the tip of the pipette. The matrix was washed to remove other reagents and contaminants. Then it was eluded and the volatile derivatives were prepared (alkyl chloroformates) from the free aminoacids. Determination was made by gas chromatography (Agilent Technologies 7890A, with a flame ionization detector).

A three-level calibration curve was made from the pattern aminoacid mixture concentration for identification and quatification. Estimations were made by the internal standard method. Injections of 2 µL were

made by triplicate, three volumes of the hydrolyzate (5, 10, and 20 µL), using an automatic injector (Agilent Technologies 7683), at 250º C, splitless. An EZFAAST column (10m long, 0.25 mm inner diameter, 0.1µm film thickness) was used. The carrier gas was H_2 (g), flowing at 1.2 mL/min. The initial temperature was 110º C, and final temperature was 320º C, at 20 º C/min. The detector was used at 320º C, H_2 (g) flow was 35 mL/min, and air flow was 350 mL/min. All the modules were controlled by the Agilent MSD Productivity ChemStation Software, B.02.02.

The data collected from aminoacid quantification of the PH sample with the three values, were analyzed through STATGRAPHICS PLUS, 5.1.

Fig 1 shows that out of the 15 essential aminoacids analyzed, valine, leucine, serine and phenylalaninewere above 1.0 nmol/µL; however, alanine, glycine, aspartic, glutamic,lysine and tyrosine were between 0.5-0.99 nmol/uL. Isoleucine, threonine, proline and histidine were below 0.5 nmol/µL.

The absence of methionine and the low values observed for tryptophan in the PH may be caused during the technical process, which is based on acid hydrolysis (hydrochloric acid 35-38%), are mostly destroyed (LABIOFAM, 2007). In that sense, Nicodemus *et al*. (2011), remarked that methionine and lysine are the limiting aminoacids in the diet (soy-corn) for layers, impacting on egg size and production. Along with cistein, the two are the main sources of sulphur for the animals. Also Joly (2008) referred that it is important to fix methionine and cistein in the diet.

Although low values of tryptophan and threonine were observed in the PH analysis, they were critical, and must be included in the diet of laying birds, because according to Dos Santos *et al*. (2013), threonine and tryptophan are limiting aminoacids in the diet of layers. These two play important roles in the synthesis of body proteins, particularly the so called plastic ones, which make up the animal´s body structure, such as muscles and feathers.

Jordao *et al.* (2006) noted that threoninewas even more demanding as layers grow older, to ensure their maintenance. Jansman (2005), said that tryptophan played a role as precursor of serotonin and melatonin. Accordingly, this aminoacid and derivatives had effects on feed consumption and behavioral events, like the time the animals were awake or asleep, their behavior, and pain perception.

CONCLUSIONS

Although no methionine (essential for egg production and quality) was found, other aminoacids were observed, which can be included in the diet of laying hens.

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Table 1. Principal components and basic proportions of the protein hydrolyzate

No.	Components	Basic proportions
1	Anticoagulant (sodium citrate)	3.8 g / 1 L blood for use
2	Antibiotic (oxytetracycline)	1 g / 1 L blood for use
3	Whole bovine blood	300 L / 1 000 L final product
4	Phenol (2.0 %)	2.83 L / 1 000 L final product
5	Hydrochloric acid (35-38 %)	4 L / 1 000 L final product
6	Water	694 L / 1 000 L final product

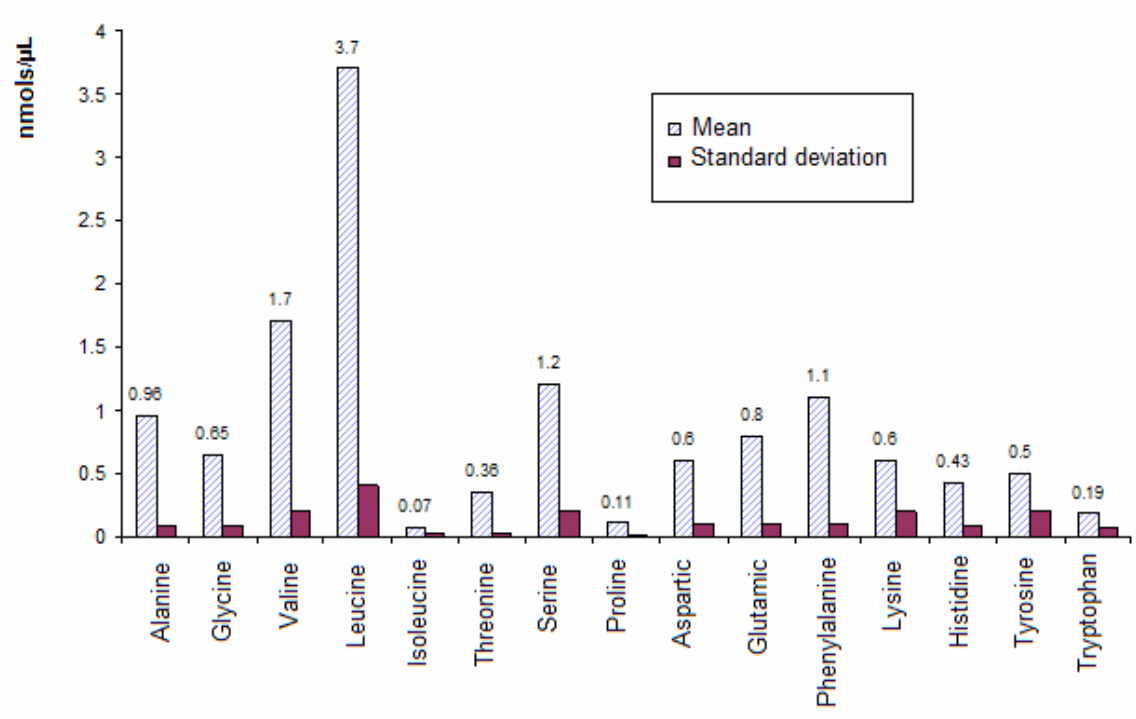

Fig 1. Average content of the three volumes of protein hydrolyzate