Evaluation of Two "*in vitro*" Digestibility Tests with the "*in vivo*" Test of Alfalfa (*Medicago sativa*) in Guinea Pig (*Cavia porcellus*) Feeding

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Abstract

The purpose of the present study was to compare two types of "in vitro" digestibility assays by using commercial enzymes and guinea pig cecalliquor with the in vivo assay, to identify the assay that most resembles the in vivo response. The alfalfa was used in two cutting times of 30 and 45 days. The "in vivo" digestibility of alfalfa was analyzed, obtaining that after 30 days of cutting the digestibility was 53.64% and at 45 days it was 47.72%, while for the digestibility "in vitro", the DaisyII®-Ankom Technology with cecal liquor and commercial pepsin; for the cecal liquor a value of 55.46% and 49.90% was obtained, for the alfalfa in the two cutting times, while the digestibility with enzymes was 71.01% and 66.34% respectively. It was determined that the method with more relation to the in vivo test corresponds to the trial with cecal liquor, because it presents a lower statistical difference ($p < 0.05$) for both cut-off times. At the same time, it is identified that the protein is the nutrient that has a higher digestibility coefficient, becoming an indicator of the nutritional quality of the food.

Keywords: Digestibility, cecal liquor, pepsin, guinea pig, alfalfa

Introduction

The use of legumes in livestock systems is a millenarian tradition, due to its high concentration of protein. In addition, they produce abundant green biomass throughout the year, fix nitrogen, enrich and protect the soil. The Leguminosae family includes more than 16,000 species (Rodriguez, 2010).

Alfalfa is one of the most valuable forage crops for livestock feeding worldwide (Juan, N., et al 1995), (Morales, et al., 2006). The value of alfalfa

lies mainly in its high dry matter production potential, high protein concentration and high percentages of digestibility (Juan, Romero & Bruno, 1995).

There are numerous parameters to characterize the quality of the alfalfa, but the main ones, from the practical point of view, are the digestibility of the dry matter (DMS), and voluntary animal consumption of dry matter (CDM) (Borrajo, 1965). The knowledge of the digestibility of food is basic to establish its nutritional value (Bochi-Brum, O., et al 1999). The digestibility tests allow estimating the proportion of nutrients present in a ration that can be absorbed by the digestive system (Church D., Pond W., 2002); (Lachmann&Febres, 1999), remaining available for the animal (Bondi, 1988); (Lachmann&Febres, 1999).

The evaluation of foods is frequently done in delayed and costly experiments based on in vivo determinations, requiring animals and large amounts of food (Pascual, J., et al 2000); (Bochi-Brum et al., 1999).

The use of the traditional method of total collection of feces (CHT) is laborious and implies some restrictions on the ordinary handling of animals. The difficulties of carrying out the total collection of the excreted feces ((Rodríguez, N. et al, 2007), presents a series of disadvantages from the practical point of view: it is laborious, it requires the availability of collection cages, of trained personnel in its management, the cost of maintenance of the animals (Lachmann&Febres, 1999).

Other methods have been developed to determine the nutritive value of foods using fast, easy and less expensive techniques (Pascual, J., et al 2000. So different in vitro methods have been proposed for their estimation. The procedure proposed by Tilley and Terry (Tilley and Terry, 1963) is with slight modifications, the most widely used in most laboratories (Pascual, J., et al 2000) .Despite the accuracy, modifications and adaptations to the in vivo method. , this continues to be a procedure that requires a considerable amount of time and work, in addition each of the foods to be analyzed must be incubated separately, thus limiting the number of samples to be analyzed per run.

The importance of the true digestibility concept is that it represents the part of the food available for the digestion of the animal or the microbial enzymes. In vitro methods are more related to true than to apparent digestibility, because they are incapable of estimating metabolic losses by feces of endogenous origin (García, 2012).

Both methods in vitro and in vivo have been presented to relate In vivo digestibility of dry matter and organic matter (Pascual, J., et al 2000).

The search to make the process to estimate digestibility more efficient, faster and cheaper, has led to the development of the in vitro method of Goering and Van Soest, using the DaisyII®-Ankom Technology (ANKOM

2017), which allows simultaneous incubation of up to 100 different samples, distributed in four 4-liter glass containers, maintains uniform temperature and constant agitation during the incubation procedure (Goering 1979). With this method, the material that disappears from the bags of digestion during incubation is considered digestible (Mabjeesh 2000).

This is a fast, safe and efficient method, the data obtained have a high correlation with the conventional method of Tilley and Terry. (Vared 1995).

On the other hand, the in vitro degradability using cecal liquor, is a procedure that I have been using it in a practical and routine way in our facilities for several years with quite reliable results in relation to those found in vivo.

Therefore, the object of the present investigation was to compare two types of in vitro digestibility from commercial enzymes (pepsin) and using cecal liquor (extracted from the caecum) of guinea pigs to compare with the values closest to the in vivo tests.

Material And Methods

The present research was developed in the Animal Nutrition and Bromatology Laboratory ofCienciasPecuarias ESPOCH in Riobamba city, Chimborazo province. Alfalfa was collected at two cutting times at 30 and 45 days, then dried in an oven at 65 ° C for 48 hours before grinding (AOAC 2005) and pelleting. For the in vivo analysis, male animals of the same species guinea pig, three months,were used, fed alfalfa at two cut-off times, after which the proximal analysis of the feed and faeces was carried out.

The first in vitro digestibility test used commercial pepsin and the second used cecal liquor obtained from 12 guinea pigs, subsequently incubated for 48 hours in the DAYSI II digester (Modified ANKOM 2017) with nitrogen-enriched buffer, for the experiment was used 24 samples of alfalfa for each cutt-off times, then the samples were washed and dried in an oven to determine digestibility parameters. The treatment of the experimental data was carried out by the analysis of variance (ANOVA) of one factor, when there were significant differences ($p < 0.05$) among means the Tukey test was used for comparison of both methods.

Results And Discussion

Digestibility analysis

In Table 1, the results of the in vivo and in vitro digestibility analysis are observed: cecal liquor and enzymes for the two cutting times.

Table 1. Percentages of digestibility in vivo and in vitro.

***Cutting times* (days)**	***Digestibility "invivo" (%)***	***Digestibility "in vitro" (%)***	
		Cecal liquor	***Enzymes (Pepsin)***
30 days	$53,65 \pm 0,20^{a}$	$55,47 \pm 0,25^{b}$	$71,02 \pm 0,28^{c}$

45 days	47,73 ± 0,59[a]	49,91±0,28[b]	66,34 ± 0,27[c]

a, bDifferent letters in the same row indicate significant differences (p <0.05).

The results of alfalfa digestibility at two cutting times obtained in the "in vivo" assay are statistically different with the "in vitro" treatments. 53.65% digestibility was obtained, the in vitro technique that is closest to this value is that of cecal liquor, through which 55.47% of digestibility was obtained, there being a difference of 1.82%. A similar pattern presented alfalfa results of 45 days of cut, the difference in the percentage of digestibility between the two techniques was 2.18%.

A greater difference was obtained with the enzymatic technique, obtaining 17.36% for the 30-day cut and 18.61% for the 60-day cut; guinea pig for being a post-gastric fermentor has microorganisms at the level of the cecum, allowing to obtain more real data considering that in the large intestine of guinea pig there are no anaerobic protozoa or fungi that degrade the fiber. (Álvarez D. 2009).

In the research of Chauca, L. (1997) of the production of guinea pigs, values of 69.40% were obtained in the digestibility "in vivo" this percentage is higher than the data of the investigation, being able to be attributed to the soil and climatic conditions , in addition to the cutting time of the species. In the comparative study of the forage digestibility by means of two laboratory methods, they used the enzymatic technique with cellulase from the fungus Penicillium funiculosum, reporting a digestibility of 68.84%, close to that obtained in the investigation. (Araiza 2013).

Table 2. *Reports the in vivo digestibility coefficients of the alfalfa nutrients in two cutting times*

Cutting times	**DMD**	**OMD**	**CPD**	**EED**	**CFD**	**ELND**
30 days	54,22±0,94	54,09±1,16	62,89±0,96	44,49± 0,21	35,78±0,82	67,70±0,21
45 days	46,72±0,59	47,34±0,38	55,58±1,16	16,34± 0,24	39,12±0,47	54,05±0,95

Different letters in the same row indicate significant differences (p <0.05).
DMD= Dry matter digestibility. OMD = Organic matter digestibility. CPD= crude protein digestibility. EED = fat digestibility, CFD = Crude fiber digestibility. ELND = Nitrogen-free extract digestibility.

This determination tells us the percentage of each nutrient that is absorbed by the animal, the data obtained in the research are within the parameters referred to by Castro and Chirinos, (1994) in the Abstracts of the annual scientific meetings of the Peruvian Production Association Animal (APPA). 1976-1993 cited by Chauca, L. (1997) of the production of the Guinea pig (Cavia porcellus) together with the FAO, determined that the protein is the nutrient with greater availability for digestion.

The digestibility of dry matter DMS and the digestibility of organic matter is greater in alfalfa of 30 days than that of 45 days due to its maturity time.

Conclusion

It was identified that the optimal in vitro method to compare with the in vivo test is the cecal liquor technique, because it presents a smaller difference in both cutting times

It was determined that the protein is the nutrient with the highest digestibility coefficient, which is why it can be considered as an indicator of the nutritional quality of the food.

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