NEW ADVANCES IN DIGESTIVE PHYSIOLOGY OF RABBITS WITH THE USE OF TROPICAL FORAGE IN CUBA

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ABSTRACT

The objective of this paper was to determine *in vivo* and *in vitro* nutrient digestibility of different tropical forage sources for rabbits. Results of four experiments conducted at the Instituto de Ciencia Animal were used. In the *in vivo* experiments, twenty-four White New Zealand rabbits of 60 days of age and 1.2 kg live weight were allocated in six individual metabolism cages. They were distributed in a completely randomized design into four groups with six replications. The *in vitro* experiment was conducted with the use of pancreatine-pepsine-chlorohydric method and cecal inoculum for semisynthetic diets. The use of cecal inoculum was compared with the use of *in vivo* method. There was higher digestibility ($P<0.001$) of dry matter (DM), neutral detergent fiber (NDF) and greater microbial growth using citrus meals diets with bacterial counts of 68.18, 45.17 and 2.56 x 10^{10} cfu/g *for Medicago sativa*, *Saccharum officinarum* and mulberry, respectively, while no differences in NDF digestibility between these forage sources and the citrus meal were observed. High correlation coefficient (R^2=0.71; $P<0.05$) between *in vitro* and *in vivo* NDF digestibility was obtained with the use of cecal rabbit inoculum and mulberry as the substrate. The NDF and ADF digestibilities increased in animals fed with 10 and 20% of dolicho forage meal. The NDF digestibility improved ($P<0.01$) by 15.21 and 13.29% in comparison with the control at 10 and 20% inclusion, respectively. It can be concluded that the citrus meal has higher nutritional quality than mulberry and sugarcane meal, and that the use of dolicho and mucuna forages at moderate level improves digestibility of diet fiber fractions.

Key words: Rabbit, Digestibility, Foliage, *In vitro* and *in vivo* methods.

INTRODUCTION

Rabbit production in Cuba and tropical countries is based on the use of different tropical forage sources with high percentage of soluble and insoluble fiber. The search for new feeds in developing countries is a good alternative due to the increase in the alfalfa price worldwide. The use of feeding sources with soluble fiber, as is citrus, improves the efficiency of digestion and microbial biomass production (Jehl and Gidenne, 1996). Other sources less digestible as those in the sugarcane meal offer perspective due to its content of energy and insoluble fiber that improves the speed of gastrointestinal transit (Dihigo *et al*., 2001). New sources of protein foliages as *Morus alba* and legumes as dolicho *(Lablad purpureum*) and mucuna (*Stizolobium niveum*) can be efficiently used in rabbits. However, their digestibility *in vivo* (total recollection method) or *in vitro*, with the use of chlorohydric- pepsin-pancreatin method or with the use of the cecal content of rabbits should be investigated. This would allow determination of their nutritive value and optimum dosing in rabbit diets. The aim of this paper was to determine *in vivo* and *in vitro* digestibility of different tropical forages for rabbits. Results from four experiments conducted between 2004 and 2006 in the Instituto de Ciencia Animal (ICA) were used.

MATERIALS AND METHODS

The meals were elaborated with the forages of dolicho (*Labblab purpureus)*, mucuna (*Stizolobium niveum)*, mulberry (*Morus alba*) sugarcane (*Saccharum officinarum) and* citrus (*Citrus cinensis, orange*). The alfalfa hay was the control. Table 1 shows the bromatological composition of diets.

Table 1: Chemical composition of diets (%)

Chemical analysis	Semisynthetic diets				Dólicho meal foliage (inclusion %)				Mucuna meal foliage (inclusion %)			
	Sugarcane	Citrus	Mulberry	Alfalfa	0	10	20	30	0	10	20	30
DM	80.60	86.60	88.97	90.14	84.44	84.3	85.28	86.84	89.87	87.97	83.25	87.23
CP	15.90	15.00	15.40	17.97	20.58	20.58	20.34	18.22	20.71	20.06	21.25	23.90
FC	22.89	13.15	19.48	14.83	-	-	-	-	-	-	-	-
NDF	34.38	13.43	21.00	32.5	29.56	30.20	32.76	37.99	29.56	30.20	32.73	37.99
ADF	-	-	-	-	18.78	22.91	23.32	26.71	20.94	20.67	23.39	28.43
Hemicellulose	-	-	-	-	10.78	07.29	09.44	11.28	8.62	9.53	9.34	9.56
Cellulose	-	-	-	-	14.36	15.34	18.33	19.51	15.64	15.12	17.15	20.13
Lignins	-	-	-	-	3.90	5.66	6.16	7.32	3.73	4.04	4.04	7.10
ME (kcal/kg)	2.870	2.570	2.500	2.640	2..520	2.530	2.632	-	2.500	2.551	2.683	2.700

Experimental procedure

For the *in vitro* experiment with semisynthetic diet the pepsin-pancreatine (Ramos *et al*., 1992) and cecal inoculum methods were employed, and the cecal inoculum method for mulberry. The cecal inoculum was prepared according to Pascual *et al*. (2000). The cecal contents of four rabbits (New Zealand x White Semigiant) were used. Eight samples of each forage (mulberry and alfalfa) and twelve in each semisynthetic diet were used according to completely randomized design. Samples were deposited in muslin bags with 48 µm openings, containing 2 g of a sample. Bags were put into incubation tubes of 120 ml, and 100 ml of inoculum were added into each tube. Cultures were incubated at 39^{o}C in a water bath for 48 h. After the incubation, the pH was measured and samples of the supernatant were taken to determine total bacteria counts. The bags were washed three times with distilled water and ethylalcohol at 90^{o}C and put into an oven at 60^{o}C for 48 h. One ml from each tube was taken for microbiological analyses under $C0_2$ atmosphere. For total anaerobic bacteria counting the Medium 10 of Caldwell and Bryant (modified) was used.

The *in vivo* experiment was performed to determine (i) effects of alfalfa meal substitution for *Lablab purpureus* forage meal on digestibility of nutrients in diets for rabbits, and (ii) effects of alfalfa meal substitution for different mucuna forage meal in diets for growing rabbits.
Diets, as well as different percentages of mucuna and dolicho foliage meal inclusion were evaluated in four treatments (Table 1). Size of pellets was 3 mm (Lebas, 1980). Twenty-four New Zealand rabbits of 60 days of age and 1.2 kg average weight were used in each experiment. The animals were allocated in individual metabolism cages. Feeding was *ad libitum* for 30 days. The last 5 days feces were collected for digestibility determination according to Pérez *et al*. (1995).

Chemical analyses and statistics

The pH was determined using a digital pH-meter, model PW9420. Dry matter (DM) and crude protein (CP) were assayed according to AOAC (1995). For the analysis of NDF, ADF, lignin, cellulose and hemicellulose, the fractionation of the fiber was performed. Statistical analysis was carried out in the SPSS system for Windows INFOSTAT, version 0.1 (2001). The Duncan´s (1955) multiple range tests were applied when necessary.

RESULTS AND DISCUSSION

Table 2 shows the coefficients of digestibility of DM, NDF, pH variation and total bacteria counts. The pH in the caecal simulation phase did not show differences due to the diets effect. Significant difference was found in the stomach phase (P<0.001) in mulberry diets due to its higher acid buffering capacity. Sugarcane meal had limited effect on pH variation. However, it exhibits the highest pH in the pancreatic phase.

Table 2: Coefficients of DM and NDF digestibility *in vitro*, bacterial counts and pH variation using semisynthetic diets with four forage plants as the only fiber sources

Indicators: Diets	pH variation Gastric	Pancreatic	Cecal	DMD (%)	NDFD (%)	Total bacteria ($cfu*10^{10}/g$)
Medicago sativa meal	4.48^b	6.29^b	6.86	61.82^a	35.09^a	1.37^b
Citrus meal	4.78^c	5.57^a	6.94	68.18^b	45.17^b	2.56^a
Mulberry meal	5.00^d	5.53^a	6.88	59.53^a	41.14^b	1.56^b
Sugarcane meal	3.44^a	6.56^c	7.20	60.15^a	31.57^a	1.31^b
SE(±)	0.05***	0.09***	0.20	1.10***	1.93***	0.13***

[abcd] Rows with different letters differ significantly at P<0.05, according to Duncan (1955)
*** (P<0.001)

Citrus diets showed the highest DM digestibility (P<0.001) in comparison with other sources due to greater fiber solubility and chemical characteristics. No differences were found in DM digestibility for other feeds. Citrus and mulberry diets had greater NDF digestibility (P<0.001) without difference between them. Sugarcane diet showed lower digestibility due to its fiber characteristics. The highest total bacterial counts (P<0.001) were found in cecal cultures with citrus meal. This corresponds to greater NDF and DM utilization.

The DM, CP and NDF digestibility coefficients of mulberry and alfalfa obtained with *in vivo* and *in vitro* method are shown in Figure 1. DM and CP digestibility in *vitro* were overestimated by 7.36 and 6.02%, respectively, compared to the *in vivo* method. Differences, however, were smaller as for the NDF digestibility was concerned. Correlation coefficient between *in vitro* and *in vivo* NDF digestibility was statistically significant (R^2=0.47; P<0.01).The inclusion of feed variables as DM, NDF, ADF and lignin improved the prediction equation and led to a multiple equation with greater precision, which explained 71% of the results:

NDFDvv = -104.52+1.65 NDF feed +0.70 NDFDv + 0.07ADF feed + 7.20 feed lignin + 0.15 DMDv, where NDFDvv is *in vivo* and NDFDv *in vitro* digestibility. Similarly with DM.

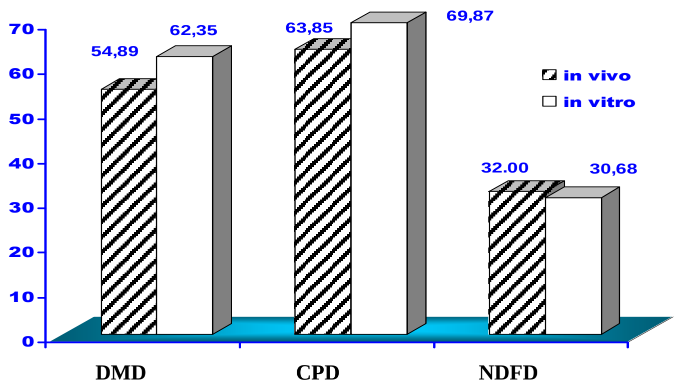

Figure1: Coefficients of digestibility of DM, CP and NDF using "*in vivo*" and "*in vitro*" methods for mulberry and alfalfa

Table 3 shows DM, CP, NDF, ADF and cellulose digestibilities with inclusion of different percentages of dolicho foliage meal. No differences were observed in DM digestibility until 20% of dolicho foliage meal were present in diets. A decrease in digestibility was observed (P<0.05) at 30% inclusion, probably due to increased cell wall content, mainly lignin. Dolicho foliage meal inclusion did not have remarkable effect on cellulose digestibility. Nevertheless, CP digestibility was higher (P<0.05) in animals that received diets with 10 and 20% of dolicho foliage meal compared to control and 30% inclusion. High CP digestibility in dolicho diets could be due to low presence of antinutritional factors in these meals (Scull, 2004), or due to the protein structure (Nielsen, 1991, Hajós *et al*., 1996) that was susceptible to proteases of endogenous and microbial origin.

Table 3: The DM, CP, NDF, ADF and cellulose digestibility at different dólicho meal inclusion

	Dólicho meal foliage inclusion (% DM)				
Variables	0	10	20	30	SE
CP	71.18[b]	79.37[a]	76.43[ab]	70.01[b]	2.43*
NDF	37.37[b]	49.30[ab]	56.73[a]	37.63[b]	4.86*
ADF	26.45[b]	45.56[ab]	59.89[a]	32.23[b]	4.25**
Cellulose	37.80	46.92	49.77	44.28	6.44

*P<0.05 and **P<0.01. Values with different letters in the same row differ (P<0.05) according to Duncan (1955)

Digestibility of NDF and ADF cellular wall components showed an increase (P<0.05 and P<0.01, respectively) in animals with 10 and 20% of dolicho foliage meal intake. These values were higher by 39.91 and 38.45% than those reported by Dihigo (2004) for trichantera and ramie, respectively. High fiber fractions digestibility could be due to inclusion of dolicho foliage meals (until 20%) in rabbit diets that stabilize or increase the microbial caecal activity (MCA) due to the presence of high pectin concentration and the starch in leaves (Minson, 1990), and as a consequence, it was observed a greater fiber digestibility because of greater concentration of pectinolytic flora in the rabbit digestive tract (Gidenne, 2002).Great lignin content of dolicho foliage meal may increase the passage rate and diminishes fiber components digestibility due to a lower retention time in the cecum, which is the main fiber digestion organ. At the same time a decrease in energy retention coefficient was observed with higher fermentation methane losses (de Blas *et al*., 1999).

Table 4 shows the DM, CP, NDF and ADF digestibility of rabbits receiving mucuna meal. No differences were observed in DM and the CP apparent digestibility. The NDF digestibility improved (P<0.01) by 15.21 and 13.29% in comparison with the control at 10 and 20% inclusion, respectively. The ADF digestibility pattern was similar. It is possible that cell wall constituents of this plant material positively influenced the cecum microbial activity (Gidenne *et al*., 2000).

Table 4: The DM, CP, NDF and digestibility at different mucuna meal inclusion

	Mucuna meal inclusion (% DM)				
	0	10	20	30	SE
DM	63.82	69.58	54.20	68.57	4.16
CP	70.64	74.41	70.77	75.03	3.32
NDF	35.99 [c]	51.20 [a]	49.78 [ab]	40.82 [bc]	3.36**
ADF	28.33 [b]	40.86 [a]	43.55 [a]	38.71 [a]	3.59*

* P<0.05 and ** P<0.01. Values in rows with different letters differ significantly at (P<0.05), according to (Duncan 1955)

CONCLUSIONS

Citrus meal had greater nutritional value than mulberry and sugarcane meals. High bacterial counts in cecal content using the citrus meal diet are consistent with greater degree of NDF degradation in rabbit digestive tract. Dolicho foliage meal inclusion until 30% did not affect DM, CP, NDF and ADF apparent digestibility. Alfalfa foliage meal replacement by mucuna foliage meal did not affect nutrient digestibility and performance. Inclusion of these legume foliage meals up to 30% DM improved cell wall constituents digestibility, however the DM and CP digestibilities were not affected. Thus, the optimal level of inclusion was the 20% DM intake. It is possible to use the *in vitro* digestibility of nutrients with rabbit caecal inoculum for *in vivo* NDF digestibility estimation.

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