Dynamics of the physical properties and the fiber fractioning of the meal of dolic integral forage (*Lablab purpureus*), biotransformed with *Trichoderma viride* for feeding monogastrics

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The dynamics of the physical properties and the fiber fractioning of the meal of integral forage of *Lablab purpureus*, innoculated with M5-2 and 137 MCX-1 strains of the lignocellulitic fugus *Trichoderma viride*, was studied. The objective of this study was to evaluate the nutritional quality of the fibrous fraction of the meals submitted to a solid state fermentation (SSF). Fifty grams of the substratum were used to determine the indicators of fiber fractioning (neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose and cellulose), as well as the physical properties: volume, solubility, and water adsorption ability (WAC) at 0, 48, 72, 96 and 120 h after the inoculation. A split plot was carried out in order to study the measuring. There was a significant interaction treatment x time of sampling ($P < 0.001$) for NDF, ADF, cellulose, hemicellulose, solubility and volume. There was a decrease of the NDF ($P < 0.001$) from the integral meal inoculated at 24 and 48 h with the 137MCX-1 and M5-2 strains, respectively. The highest decrease of NDF (8.3 percentage units), inoculated with the M5-2 strain, was obtained at 72h. This coincided with the highest increase ($P < 0.001$) of cellulose and the decrease of hemicellulose, from 23.37 to 19.15 %. There was low content of lignin ($P < 0.001$), regardless of the strains, at 72 h. The results indicated an increase of the nutritional quality of the fibrous fraction for the meal of dolicho integral forage, inoculated with both strains. This makes possible the consideration of its inclusion as an alternative food for monogastric species.

Key words: *nutritional quality, fiber fraction, Trichoderma viride, integral forage meal, Lablab purpureus*.

It is known the scarcity of traditional food destined for monogastric species, due to, mainly, the development of new energy sources produced from the grains (biocombustibles). This reality increases the prices and represents an obstacle for stability and profitability of meat production in livestock fields (Espinosa 2008). The challenge is to develop alternatives that allow to use available resources in tropical areas.

In Cuba, researches carried out by Díaz (2000), Díaz *et al*. (2002) and Díaz *et al*. (2004) demonstrated the possibility of using meals of integral forage of tropical leguminous in rations for poultry and pigs. However, one of the main characteristics of this meal in the fiber content which affects the nutritional quality because it negatively influences on the digestibility of nutrients within the food.

The application of fermentative processes that allow to eliminate most of these components, as a consequent increase of the quality of the resulting fiber fraction, could be a solution before this limitations (Pandey 2000 and Ibarra *et al* 2002).

The solid state fermentation (SSF) with lignocellulitic fungi is a process that has been used for recycling voluminous materials. During these fermentations, biochemical and structural changes occur, which result in an increase of the biological value of the dietetic protein and in the improvement of the structure of the lignocellulitic compounds (Kiers *et al*. 2000, Zang and Lynd 2006, Faria *et al*. 2008, Sipos *et al*. 2010, and Wilson 2011). According to this statement, the composition of several compounds varies depending on the source, which was reported by Van Dyk and Pletschke (2012), among other authors.

There are no studies, among the available literature, that analyses the dynamics of the fermentation of the fibrous fraction and the physical properties of the meal of integral forage of *Lablab purpureus* (dolicho), bio-transformed with strains of the lignocellulitic fungus *T. viride*. Starting from this point, the objective of this study was to evaluate the nutritional quality of this meal for feeding monogastric species.

Materials and Methods

The meal of integral forage of *Lablab purpureus* (dolicho) was developed according to Savón *et al* (2004).

Microorganisms. Two strains of the conidial cellulolitic fungus *Trichoderma viride*, 137MCX-1 and M5-2, were used, both from the strain collection of the "Laboratorio de Producción de Alimentos del Instituto de Ciencia Animal". The strains produce cellulase enzymes, which have hydrolytic capacity in fibrous substrates, and meals from temporal leguminous through the solid state fermentation (SSF) (Valiño *et al*. 2004 a).

Fermentation process. Conic flasks of 500 mL were used with 50 g of dolicho integral forage meal. These flasks were moistened with distilled water up to 70 % W/V of the growth requirement. The humid substrate was sterilized at 1 atmosphere of pressure, at 121 ºC during 20 min., and it was inoculated with 1cm^3 of Agar Malta. The mixtures were homogenized and the conic flasks were put into an incubator at 30 ºC during seven

days. Each flask constituted an experimental unit.

Samples of the solid material of the same flask were taken every 24 h of fermentation, until 120 h. this procedure was repeated four times for each strain. The fiber fractioning (neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and lignin) was carried out according to Goering and van Soest (1970). The physical properties (volume, solubility, and water adsorption capacity) were analyzed according to Savón *et al*. (1999).

Statistical analysis. A split plot design was applied to find the interaction treatment x time of sampling for the studied indicators. Later, ANAVA was carried out to the split plots for the measuring that showed a significant interaction. The main plot with the hours, and the subplot were the treatments. For the non significant indicators, a linear model with the effects of treatment and time of sampling was used. The multiple range test of Duncan (1955) was applied for the necessary cases. The statistical program Infostat, version 1 (Balzarini *et al*. 2001) was used for analyzing the results.

Results and Discussion

The dynamics of the fiber fractionings and the physical properties of the meal of dolicho integral forage (table 1) showed that there was an interaction treatment x time of sampling for the indicators of the fiber fraction (NDF, ADF, hemicellulose and cellulose) and the physical properties (solubility and volume) of the integral forage meals, inoculated with the M5-2 and 137MCX-1 strains of the cellulolitic fungus *Trichoderma viride*.

A decrease ($P < 0.001$) of the NDF with the M5-2 strain was confirmed, at 24 h after the beginning of the fermentation process. At 72 h, there was a decrease of the content of the cell wall for this strain, around 8.30 percentage units (83.24 vs. 74.94). This coincided with the highest increase of the cellulose, 3.77 percentage units (38.34 to 42.10), and later it decreased until reaching a similar concentration to initial one. The decrease of the hemicellulose concentration (4.22 percentage units) is because the fungus uses, preferably, the components of easiest degradation (Valiño *et al* 2004a). Later, the concentration of hemicellulose continued to decrease until reaching the level that had after 48 h of started the fermentative process. This confirmed the great cellulolitic activity of this strain, contrary to the stated by Valiño *et al* (2010). These authors state a scarce mycelial growth and low sporulation in the meal of dolicho integral forage, up to 144 h of fermentation. Due to this cellulolitic activity, which favored a breaking of the cell wall at 72 h, with a decrease of the NDF and ADF regarding the initial values, the solubility evidenced an increase that was not regular until the 120 h. the ADF experienced the highest decrease, 4.08 percentage units ($P < 0.001$), at 72h of fermentation. This suggests the ability of the M5-2 strain for also producing polyphenoloxidase enzymes (Valiño 2010), which is based on the evident decrease experienced by the ADF, formed by cellulose and lignin, at 72 h due

Table 1. Interaction between time of sampling (h) and treatment (integral dolicho + *T. viride* M5-2 and integral dolicho + *T. viride* 137MCX-1) for indicators of fibrous fractioning and physical properties

Indicator	Strain	Hours						SE ± Sign
		0	24	48	72	96	120	
NDF	M5-2	83.24^{j}	80.12^{i}	75.44^{de}	74.94^{cd}	76.10^{ef}	74.16^{bc}	$P < 0.001$
	137MCX-1	78.69^{h}	77.80^{gh}	69.53^{a}	73.69^{b}	80.97^{i}	76.99^{fg}	± 0.46 ± 0.43
ADF	M5-2	59.87^{f}	57.24^{e}	59.23^{f}	55.79^{d}	57.89^{e}	57.21^{e}	$P < 0.001$
	137MCX-1	52.65^{c}	48.57^{a}	52.27^{c}	50.37^{b}	52.77^{c}	52.91^{c}	± 0.54 ± 0.52
Cellulose	M5-2	38.34^{bc}	37.10^{b}	34.23^{a}	42.10^{de}	38.13^{bc}	37.46^{b}	$P < 0.001$
	137MCX-1	41.56^{d}	39.22^{c}	42.28^{def}	41.15^{d}	43.60^{f}	43.36^{ef}	± 0.63 ± 0.65
Hemicellulose	M5-2	23.37^{d}	22.88^{d}	16.21^{a}	19.15^{c}	18.21^{bc}	16.96^{ab}	$P < 0.001$
	137MCX-1	26.05^{e}	29.24^{f}	17.27^{ab}	23.33^{d}	28.20^{f}	24.07^{d}	± 0.66 ± 0.64
Solubility	M5-2	16.66^{e}	15.74^{cd}	17.53^{f}	17.56^{f}	15.26^{bc}	17.86^{f}	$P < 0.001$
	137MCX-1	14.16^{a}	16.45^{de}	15.90^{cd}	16.23^{de}	15.38^{c}	14.64^{ab}	± 0.33 ± 0.34
Volume	M5-2	5.13^{a}	5.34^{a}	6.69^{b}	8.36^{d}	7.32^{c}	8.62^{d}	$P < 0.01$
	137MCX-1	9.63^{e}	10.48^{f}	10.48^{f}	10.56^{f}	11.45^{g}	11.18^{g}	± 0.22 ± 0.22

^{abcdefghi}Values +with different letter for each indicator differ at $P < 0.05$ (Duncan 1955)

The first SE corresponds to the hours at the same level of the treatment

The second SE corresponds to the treatments at the same or different level of the hours

to the decrease of the present lignin, as it was later demonstrated.

The 137MCX-1 strain had a decrease ($P < 0.001$) of nine percentage units of NDF, at 48h after the beginning of the process of fermentation. However, the concentration of this indicator increased later on, while the values of ADF and cellulose were invariable, and the hemicellulose experienced a decrease of eight percentage units regarding the initial concentration. Afterwards, it increased until achieving, at 120 h, concentrations of two units less than the initial value. The volume also increased after 24h, and reached 2.55 percentage units more at the end of the process. This corresponded with the decrease of solubility.

De la Mata (1991) stated that the lingocellulosic materials have a very resistant structure, mainly due to the crystallinity and the capillary structure of the cellulose, as well as the rate of polymerization and the lignin barrier that surrounds it. Analyzing the previous results, the M5-2 and 137MCX-1 strains are able to exert a positive effect on the rupture of the cell wall of the forage meal of integral dolicho. Their only difference is that M5-2 has a more immediate, higher and more sustained effect due to its mutant nature, and it is a hyper-producer of enzymes cellulase and phenoloxidase (Valiño *et al*. 2004b) that provides a decrease of the hemicellulose more degradable than cellulose, which kept until the end of the fermentation. Unfortunately, the reducing sugars were not measured, which could have explained better the mechanism of this process.

The 137MCX-1 strain had a latter effect. It showed, at the end of the 120 h, a less marked decrease of the cell wall, regarding the initial concentration, the increase in the cellulose (which accumulates) and the low decrease of the hemicellulose, while the ADF almost did not varied. In general, the highest values of solubility of the meal of dolicho integral forage were obtained with the inoculation of the M5-2 strain. The lowest content of lignin influenced on this fact (table 2). Table 2 shows the main effects for lignin and the water adsorption ability.

There was an opposite effect between the lignin and the water adsorption capacity (WAC) for the treatments which in this case were the solid state fermentation of integral forage meal with the strains M5-2 (treatment 1) and 137MCX-1 (treatment 2). As a result of the fermentative process, the inoculated meals differed ($P < 0.001$) in the content of lignin (17.20 % vs. 8.47 %) for the 137MCX-1 and M5-2 strains, respectively. This demonstrates that the first strain has the highest lignin activity while the second has the highest cellulolitic activity, according to Valiño *et al* (2010). Lignin was lower at 72 h of fermentation of the forage meals of integral dolicho, apart from the inoculated strain. Regarding the WAC, and according to Seoane *et al*. (1981), it is known that it is related to fiber content and reflects the fiber ability of swelling, which depends on the relative proportions of the polysaccharides that compose them and the lignin. The hemicellulose has higher hygroscopic level than the cellulose, because the lignin of phenolic nature has a strong hydrophobic character. Later, the WAC of the dolicho forage meal, inoculated with the 137MCX-1 strain, decreased while the lignin was being released, due to the fermentative process. Meanwhile, the decrease of this indicator with the inoculation of the M5-2 strain in the meal of the integral forage of dolicho provides an increase of the WAC. The hours of fermentation had no effect on the WAC for the integral meals, apart from the inoculated strain. The results showed the possibility of improving the quality of the fiber fraction in the meal of dolicho forage, inoculated with the M5-2 and 137MCX-1 strains of the cellulolitic fungus *Trichoderma viride*. Therefore, it could be considered as a potential alternative to be included in the food of monogastric species.

Table 2. Results of the main effects

Indicator	Treatment	Effects						SE± Sign
		1	2	3	4	5	6	
Lignin	Strain	8.47^{a}	17.20^{b}					P < 0.001 ± 0.28
	Hours	14.29^{d}	13.39^{cd}	14.60^{d}	10.74^{a}	11.56^{ab}	12.42^{bc}	P < 0.001 ± 0.49
WAC	Strain	11.11^{b}	9.77^{a}					P < 0.001 ± 0.17
	Hours	10.31	10.42	9.94	10.73	10.89	10.33	NS ± 0.29

^{abcd}Values with different letters differ at $P < 0.05$ (Duncan 1955)
Treatments 1) M5-2; 2) 137MCX-1. Hours: 0, 24, 48, 72, 96 and 120.
NS: Non significant. WAC: Water absorption capacity

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Received: May 30. 2013