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Laccase-Rich Enzymatic Pretreatment of Fibrous Waste to Improve Its Nutritional Quality for Animal Feed

Luis Rodrigo Saa [1,*], Maryen Alberto Vázquez [2,*], Bárbara Rodríguez Sánchez [2], Elaine Cristina Valiño Cabrera [2] and Lourdes Lucila Savón Valdés [2]

[1] Laboratorio de Sanidad Animal y Zoonosis, Departamento de Ciencias Biológicas y Agropecuaria, Escuela de Ingeniería Agropecuaria, Universidad Técnica Particular de Loja, Loja 110107, Ecuador
[2] Institute of Animal Science, P.O. Box 24, San José de las Lajas 32700, Mayabeque, Cuba; sabygen03@gmail.com (B.R.S.); elainevalino@gmail.com (E.C.V.C.); lourdeslsavonvaldes@gmail.com (L.L.S.V.)
* Correspondence: lrsaa@utpl.edu.ec (L.R.S.); maryne4e@gmail.com (M.A.V.)

Abstract

The feeding of monogastric species of productive interest is a problem for many countries, especially underdeveloped ones; therefore, it is essential to study alternative systems that do not compete with human food, particularly through the revaluation of agri-food waste by fungal fermentation processes, an eco-friendly and efficient strategy. This research evaluated the pretreatment of sugarcane bagasse with the laccase-rich crude enzyme extract from *Curvularia kusanoi* L7, whose laccase activity was the basis of the treatment, and its effect on the nutritional quality of bagasse and on in vivo apparent digestibility of the diets containing 10% of this substrate, either pretreated or untreated in broiler chickens and rabbits. Fiber modifications were assessed by Fourier transform infrared spectroscopy (FTIR), and in vivo digestibility was analyzed using twenty 60-day-old male Cuban Brown rabbits and 30 broiler chickens (HEEB55). Both studies showed similar results: bagasse pretreated with the enzymatic crude presented higher in vivo digestibility than the control, and in poultry its digestibility did not differ from that of the corn–soybean control (87.38%). FTIR evidenced modification of the bagasse cell wall structure. It is concluded that pretreatment with the enzymatic crude rich in fungal laccases from *C. kusanoi* L7 increases the digestibility of sugarcane bagasse in diets for poultry and rabbits, representing a new option for developing fibrolytic products in modern livestock farming within the framework of fungal fermentation of fibrous sources for animal feed.

Keywords: *Curvularia kusanoi* L7; enzymatic pretreatment; fiber; in vivo digestibility; broiler chicken; Cuban brown rabbit

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1. Introduction

The rapid growth of the world's population is leading to the depletion of many resources and exacerbating the international food crisis. For these reasons, the development of livestock production is essential as one of the key ways to reduce the negative impact of food shortages. Alongside this research, numerous studies are also being conducted to achieve more sustainable animal production capable of meeting international demand, while utilizing alternative sources of animal feed that do not compete with human nutrition. In this regard, the use of biotechnology offers great potential for improving the competitiveness of agricultural production, especially in developing countries. In this regard, the up-cycling of lignocellulosic biomass is one of the most promising solutions [1]. However,

the structural complexity of plant cell walls and the low digestibility of this type of feed source limit its use by species of productive interest, such as monogastric animals. In these cases, fiber pretreatment is one of the most effective solutions for improving its nutritional quality [2]. Among the various pretreatment processes, biofermentation stands out for its lower energy consumption, the absence of harsh chemicals, and its operation under conditions close to ambient conditions, in addition to enabling the reduction of inhibitors and toxic substances in the reaction medium [3]. This type of pretreatment involves the use of microorganisms and/or their enzymatic products. The use of enzymes, in turn, offers significant economic benefits by reducing the total duration of the process. Therefore, current studies focus on the search for new strains with high enzymatic productivity, with the goal of using their crude enzymes or purifying them [4].

Among the microorganisms that stand out for their high enzyme production are fungi, which are capable of producing cellulolytic enzymes and those that enable the modification of lignin, such as laccases. Among these enzymes, laccases are particularly attractive because they act on lignin and, by modifying or depolymerizing it, can release the polysaccharide matrix and thereby improve fiber accessibility [5]. However, although enzymatic pretreatment of lignocellulosic residues has been widely investigated, most approaches have relied on cellulases, hemicellulases, and xylanases, whereas laccases have been used mainly for other biotechnological purposes [6]. Consequently, their application as the main enzymatic basis for pretreating fibrous residues intended for animal feed remains largely unexplored, which makes the in vivo validation of these treatments essential to confirm their effectiveness in monogastric species. This study therefore addresses this gap by offering a new option for developing fibrolytic products within the framework of fungal fermentation of fibrous sources for animal feed. Accordingly, the aim of this study was to evaluate the enzymatic pretreatment of sugarcane bagasse using a laccase-rich crude enzyme extract from *Curvularia kusanoi* L7 and to assess how this pretreatment affects the in vivo digestibility of the diet in species of productive interest.

2. Materials and Methods

Microorganism: *Curvularia kusanoi* L7 strain, isolated from lemon tree, with number of nucleotide sequences registered in GenBank, and accession number KY795957. This microorganism is a filamentous fungus that has been previously evaluated at the Institute of Animal Science of the Republic of Cuba. It exhibits high laccase enzyme productivity, as well as significant results in the degradation of fibrous compounds [6].

Laccase-rich crude extract production process: The crude extract was obtained by solid-submerged fermentation of wheat bran with the *Curvularia kusanoi* L7 strain. This microorganism was inoculated from the pure culture on PDA at a rate of 3 cm^2 in flasks containing 3 g of All-bran-Kellogg's cereal based on wheat bran and 100 mL of citrate buffer (50 mM, pH 5.0). The flasks were incubated on an orbital shaker at 120 rpm for a period of 10 days at 30 °C. Fermentation samples were taken every 24 h, the contents of each flask were filtered through a Büchner funnel, and the resulting liquid was centrifuged (4 °C, 10,000 rpm, 3 min) to obtain the crude enzyme [7]. To obtain the laccase-rich crude extract, the fermentation time at which the highest production of this enzyme is reached was selected, and under the same fermentation conditions, a larger amount of the crude extract was obtained.

Determination of Enzymatic Activity: The crude extract was tested for cellulolytic and ligninolytic enzyme activities, using a volume of 100 µL of the crude extract for each enzymatic determination in a 1 mL reaction volume. CMCase (endo-1,4-β-glucanase) activity was determined on 2.2% (*w*/*w*) carboxymethylcellulose in 50 mM sodium citrate buffer, pH 5. PFase (exo-1,4 β-glucanase) activity was measured on Whatman No. 1 filter

paper (50 mg) in 0.6 M sodium acetate buffer, pH 6.0. All reactions were incubated at 50 °C for 30 min, and the released reducing sugars content was determined using the 3,5-dinitrosalicylic acid (DNS) method, with enzyme activities expressed as micromoles of glucose released per minute [8]. Laccase activity was determined by the degradation of syringaldazine (5 mM in ethanol). The substrate oxidation was monitored for 1 min under aerobic conditions at 530 nm. One unit of laccase activity (U) was considered the amount of enzyme that catalyzes the conversion of 1.0 µmol of syringaldazine per minute [9].

Enzymatic pretreatment of sugarcane bagasse and evaluation of fiber degradation by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT-IR): Finely ground sugarcane bagasse was spread on aluminum trays and 5 mL of crude extract per gram of dry matter was applied. The enzymatic product was applied by spraying it onto the surface and left in pretreatment for 72 h in an oven with temperature control at 40 °C. Untreated sugarcane bagasse was used as a control where the enzymatic crude was replaced with distilled water. The enzymatic degradation of the pretreated bagasse fiber was analyzed using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (ATR-FT-IR). A Pelkin Elmer device with a diamond ATR base and MCT/A detector was used. The scans were made from 4000 cm^{-1} to 400 cm^{-1} [10].

Apparent digestibility in broiler chickens of diets containing either untreated sugarcane bagasse or bagasse pretreated with a laccase-rich crude enzyme extract from *Curvularia kusanoi* L7: The study was conducted at the poultry experimental unit of the Institute of Animal Science, Mayabeque, Cuba. At 21 days of age, 30 broiler chickens (HEEB55) with an average live weight of 470 ± 50 g were randomly selected. The birds were individually housed in metabolic cages and allotted to a completely randomized design with three treatments and ten replicates per treatment, each bird (one per cage) being one experimental unit: (1) control treatment (corn/soybean), (2) inclusion of sugarcane bagasse without enzymatic treatment, and (3) sugarcane bagasse treated with a laccase-rich crude enzyme extract from *Curvularia kusanoi* L7. The feeding trial lasted 7 days in total, consisting of 4 days of adaptation to the experimental diets followed by 3 days of total excreta collection. Initial live weight was recorded at the start of the trial, and final live weight (LW) was recorded at the end of the collection period, immediately before the birds were removed from the metabolic cages. The total excreta collection method was used. During the collection period, the feed offered and the feed refused were weighed daily per cage to calculate daily feed intake, and excreta were collected daily from each bird, pooled per animal per day (morning and afternoon collections combined), oven-dried at 60 °C and ground prior to chemical analysis. Dry matter (DM) and total ash contents were determined [11]. For the extraction of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose, the Van Soest fractionation method was used [12]. Each chemical analysis was performed in triplicate on each feed and excreta sample; these triplicates are laboratory replicates and were averaged to obtain a single value per sample, and were not treated as independent experimental units in the statistical analysis.

Apparent digestibility was determined as describe in Equation (1):

$$\text{Apparent digestibility (\%)} = \left(\frac{\text{nutrient intake} - \text{nutrient exceted}}{\text{nutrient intake}} \right) \times 100 \quad (1)$$

where nutrient intake = feed intake × nutrient concentration in the diet, and nutrient excreted = excreta output × nutrient concentration in the excreta, calculated for each individual bird over the 3-day collection period.

Apparent digestibility in Creole brown rabbits of diets containing either untreated sugarcane bagasse or bagasse pretreated with a laccase-rich crude enzyme extract from *Curvularia kusanoi* L7: Twenty 60-day-old male Cuban brown rabbits with an average live weight of 1.4 ± 0.2 kg were used. They were randomly distributed in individual cages under

a completely randomized design with two treatments and ten replicates per treatment, with each animal constituting one experimental unit. The treatments consisted of: (I) control treatment (10% untreated bagasse) and (II) inclusion of 10% sugarcane bagasse treated with a laccase-rich crude enzyme extract from *Curvularia kusanoi* L7. The diets were prepared according to the recommendations of Machado et al. [13]. The study lasted 10 days, seven for adaptation to diet intake and three for total excreta collection. Drinking water was offered ad libitum, and feed was provided at a rate of 100 g daily, divided into two rations: morning and afternoon. During the experimental phase, the weight of the feed offered and the refusals were monitored daily to determine daily feed intake. Feces were collected individually from each animal every day, and the daily fecal samples from each animal (morning and afternoon) were pooled into a single composite sample per animal covering the entire 3-day collection period; 10% of this pooled sample was taken and stored frozen at −20 °C until analysis [14]. Dry matter (DM) and total ash contents were determined [11]. The Van Soest fractionation method [12] was used to extract neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, and hemicellulose. Each analysis was performed in triplicate on each feed and pooled excreta sample; these triplicates are analytical replicates and were averaged to obtain a single value per sample, and were not treated as independent experimental units. The apparent digestibility coefficient (%) was calculated for each animal from its pooled excreta sample as described in Equation (1), using the total intake and total excretion recorded over the 3-day collection period.

The chemical composition of the main fibrous components of untreated and pretreated bagasse is provided in Supplementary Table S1, and the ingredient and chemical composition of the experimental diets are provided in Supplementary Tables S2 (broiler chickens) and S3 (rabbits).

Statistical Analysis:

For the evaluation of enzymatic activities, the data from each kinetics were analyzed using simple analysis of variance, to assess the effect of fermentation time on enzyme production. The data were processed with the help of the statistical package InfoStat [15].

In the experiments carried out on animals of productive interest a completely randomized design was used in both experiments. In the case of broiler chickens, three treatments with ten replicates each were used, and in the study on rabbits, two treatments with ten replicates each were used. Each animal represented an experimental unit. The results were analyzed using the computerized statistical package InfoStat [15]. The mean values were compared using Duncan's test [16] where necessary.

3. Results

3.1. Evaluation of the Lignocellulolytic Activity of the Enzymatic Crude from Curvularia kusanoi L7

Figure 1 shows the lignocellulolytic kinetics of strain *C. kusanoi* L7 in solid-submerged fermentation of wheat bran.

The enzymatic activity results of *C. kusanoi* L7 crude evidence a significant fibrolytic capacity. The production of lignocellulolytic enzymes is combined, where cellulolytic enzymes such as exoglucanase and endoglucanase are produced in the first days of fermentation, followed by a later production of the enzymes that modify lignin. In this way, the degradation of the plant cell wall is a more exhaustive and efficient process [17]. It is important to note that although degradation studies have been carried out previously with this strain [6], it had never before been used as a fiber fermenter for animal consumption.

Based on the analysis of the enzymatic kinetics of the *C. kusanoi* L7 crude extract resulting from the solid-submerged fermentation of wheat bran, it was decided to use the enzymatic extract corresponding to the seventh day of fermentation, the time at which this strain reaches its highest laccase activity. Therefore, a larger amount of the crude extract was produced

for this time point, maintaining the same fermentation conditions as previously described in Materials and Methods. In this way, an extract enriched in laccases was obtained, with laccase, endoglucanase and exoglucanase activities of 2, 0.08 and 0.06 U/mL, respectively. The laccase-enriched extract was applied at 5 mL per gram of bagasse dry matter, which corresponds to 10 U of laccase, 0.4 U of endoglucanase and 0.3 U of exoglucanase per gram of bagasse dry matter, based on the measured activities of the extract.

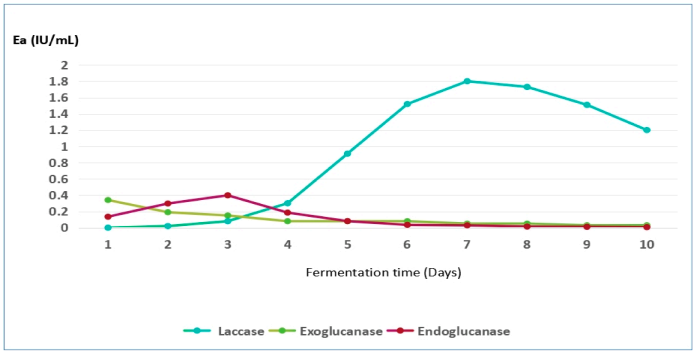


Figure 1. Lignocellulolytic kinetics of strain *C. kusanoi* L7 in solid-submerged fermentation of wheat bran. Exo-1,4 β-glucanase (SEM ± 0.011, $p = 0.0458$), Endo-1,4 β-glucanase (SEM ± 0.022, $p = 0.0003$) and Laccase (SEM ± 0.003, $p = 0.0018$). Values are means ± SEM of three independent fermentation replicates ($n = 3$). One-way analysis of variance was performed for each enzyme activity using InfoStat [15].

3.2. Enzymatic Pretreatment of Sugarcane Bagasse and Evaluation of Degradation by ATR-FTIR

Pretreatment of sugarcane bagasse with the laccase-rich crude enzyme extract from *Curvularia kusanoi* L7 resulted in noticeable spectral changes compatible with structural modification of plant cell wall components. Although no statistical analysis was performed between the spectra, these changes were qualitatively evident (Figure 2). The most pronounced changes were observed in the 2000–1000 cm^{-1} region. Within this region, the bands at ≈1730 cm^{-1} (C=O stretching of esterified uronic acids and acetyl groups), ≈1610 and ≈1510 cm^{-1} (aromatic ring vibrations of lignin), ≈1460 and ≈1370 cm^{-1} (CH and C–O vibrations), ≈1240 cm^{-1} (C–O of guaiacyl units) and ≈1030 cm^{-1} (C–O–C stretching of polysaccharides) showed increased transmittance, which corresponds to lower absorbance and is compatible with modification of lignin and of the polysaccharide matrix. Since the spectra are expressed as transmittance, these changes are described in terms of increased transmittance (i.e., reduced absorption) rather than as absolute decreases in signal intensity. This area of the spectrum being known as the lignin fingerprint [18].

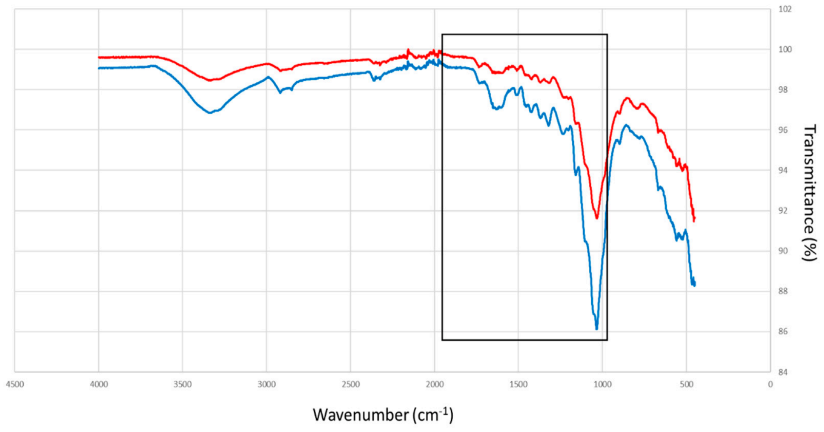


Figure 2. ATR-FTIR spectra of untreated sugarcane bagasse (blue line) and bagasse pretreated with the laccase-rich crude enzyme extract from *Curvularia kusanoi* L7 (red line), recorded from 4000 to

400 cm^{-1}. Spectra are presented as transmittance (%). A linear baseline correction was applied using the instrument software (PerkinElmer Spectrum); no normalization to a common reference peak was performed, and the spectra are shown with a vertical offset to facilitate visual comparison. The rectangle indicates the spectral region (2000–1000 cm^{-1}) showing the most pronounced changes compatible with structural modification of the fibrous fraction.

The results analyzed by FTIR suggest that the enzymatic pretreatment of bagasse successfully modified the plant fiber, an aspect of great importance to ensure better access of digestive enzymes in monogastric animals to this food, with the consequent improvement in its utilization.

3.3. Apparent Digestibility of Diets Containing Untreated or Pretreated Sugarcane Bagasse with Laccase-Rich Crude Enzyme Extract from Curvularia kusanoi L7 in Diets of Species of Productive Interest

The following Tables 1 and 2 present the results of the in vivo apparent digestibility evaluation of the biotransformed feed in diets of broiler chickens and Creole brown rabbits. It should be noted that the tabulated values correspond to the digestibility of the whole diet and not only to the untreated or pretreated bagasse.

Table 1. Apparent digestibility of diets containing untreated or pretreated sugarcane bagasse with Laccase-rich crude enzyme extract from *Curvularia kusanoi* L7. Study in broiler chickens.

Ind. (%)	Control Corn/Soybean	Control with 10% Untreated Sugarcane Bagasse	Diet with 10% Pretreated Bagasse	SD ±	*p*
DMD	87.38 [b]	80.09 [a]	87.34 [b]	±1.51	$p = 0.0031$
ADFD	75.61 [b]	65.37 [a]	77.90 [b]	±2.29	$p = 0.0007$
CD	81.82 [b]	65.78 [a]	81.22 [b]	±1.78	$p < 0.0001$
LW	1.79 [c]	1.51 [a]	1.71 [b]	±0.03	$p < 0.0001$

Values are means ± SD ($n = 10$ animals per treatment). Means were compared by one-way ANOVA followed by Duncan's multiple range test. Means with different letters in each row differ at $p < 0.05$ [16]. DMD dry matter digestibility, ADFD acid detergent fiber digestibility, CD cellulose digestibility, LW final live weight.

Table 2. Apparent digestibility of diets containing untreated or pretreated sugarcane bagasse with Laccase-rich crude enzyme extract from *Curvularia kusanoi* L7. Study in rabbits.

Ind. (%)	Control with 10% Untreated Sugarcane Bagasse	Diet with 10% Pretreated Bagasse	SD ±	*p*
DMD	85.23	87.02	±1.12	$p = 0.0016$
ADFD	82.56	85.22	±1.06	$p < 0.0001$
CD	83.91	88.03	±1.78	$p = 0.0009$

Values are means ± SD ($n = 10$ animals per treatment). Means were compared by one-way ANOVA followed by Duncan's multiple range test [16], *p*-values are shown for each variable. DMD dry matter digestibility, ADFD acid detergent fiber digestibility, CD cellulose digestibility.

As shown in Table 1, no statistically significant differences were found in the in vivo digestibility of the control diet (corn–soybean) and the diet containing enzymatically pretreated bagasse. However, this absence of significant differences should not be interpreted as evidence of nutritional equivalence, since equivalence was not statistically tested; the values should therefore be regarded as numerically similar rather than equivalent. Notably, the enzymatic pretreatment process increased the live weight of the animals at the end of the experiment. These values, although lower than those achieved with the corn–soybean

(traditional) diet, were significantly higher than those obtained with the alternative diet containing untreated bagasse. Taken together, these results indicate that laccase-rich enzymatic pretreatment substantially improves the nutritional quality of sugarcane bagasse, bringing its digestibility close to that of a conventional diet, although it does not fully match the performance obtained with the corn–soybean control.

The results obtained in rabbits are similar to the previous study with broiler chickens. As can be seen in Table 2, the treatment with enzymatically pretreated bagasse presents higher in vivo digestibility values than the control treatment with untreated bagasse.

4. Discussion

The use of lignocellulosic biomass in animal feed is presented as an important solution to the livestock feed shortage. Rising prices for cereals and other dietary components create the need to seek more economical alternatives that provide a food product with adequate nutritional value. Within lignocellulosic biomass, several agroindustrial residues have a chemical and physical composition that allows their use with satisfactory results in this field. Many of them are used in the production of animal feed for ruminants, poultry, pigs, and other species of economic interest [19]. The critical point in the bioconversion of fibrous substrates of different types is to access the polysaccharide matrix. In this research, it was decided to use smaller, short-cycle monogastric species such as broiler chickens and rabbits, as their production is a growing strategy to meet the need for animal protein. To this end, producers are increasing the amount of raw material used for animal feed and promoting options to reduce dependence on traditional foods that compete with human nutrition through fermentative processes that allow a more efficient use of the fiber components. However, the use of lignocellulolytic biomass depends largely on the fiber composition and, in particular, on the cellulose and lignin content [20]. The structural complexity of the fiber, as well as its close association and chemical cross-linking, make them recalcitrant biomolecules.

An analysis of the results obtained from infrared spectroscopy shows how the enzyme preparation induced spectral changes compatible with modification of the fibrous fraction of sugarcane bagasse. Even though the crude shows significant cellulolytic activity, its greatest fiber degradation effect is observed in the area associated with the lignin fingerprint. The reduced intensity of the lignin-associated bands is compatible with partial lignin modification and suggests the potential of this type of pretreatment of to increase its digestibility. This research coincides with similar studies by Alberto et al. [18] who used this type of enzymatic treatment for the degradation of raw wheat straw. They also found that the greatest reductions in spectral intensities were observed in the lignin fingerprint region, specifically in the characteristic triplet attributed to the vibrations of the aromatic ring of this molecule. Furthermore, a recent study by Banerjee et al. [21] (2025) reported similar results when laccase was used to delignify corn stalks, achieving 80.56% delignification, with the structural changes characterized by FTIR and SEM/XRD.

It is known that the mechanism of action of this enzyme itself involves the oxidative depolymerization of phenolic compounds through the formation of unstable phenoxyl radicals [22]. Therefore, variations in the intensity of these types of bands in the spectrum are indicative of enzymatic action on the phenolic units of lignin. In this regard, Perna et al. [23] analysed the degradation of lignocellulosic components by laccase activity using FTIR spectral fingerprinting, similar to the approach employed in the present study. Consistent with this, the spectra obtained support the structural modifications induced by the laccase-rich crude extract, which were qualitatively evident despite the absence of statistical analysis between spectra.

According to Mattinen et al. [24] and Kahar [25], the changes at 1610 cm^{-1} attributed to the vibrations of the aromatic ring of lignin due to the vibration of the aromatic skeleton (C=C), are indicative of changes in the lignin surface, so a lower intensity of this band demonstrates the ability of laccases to oxidize the phenolic units present on the surface of the fibers [26,27].

One plausible explanation for the improvements in digestibility observed with the pretreated bagasse is that, as lignin is modified the polysaccharide matrix becomes more accessible to the animal's digestive enzymes and to the caecal microbiota, which would account for the higher ADFD and cellulose digestibility values recorded for the pretreated diet. This mechanistic link is further supported by the fact that the untreated bagasse showed the lowest digestibility values, indicating that, when unaltered, the fiber structure limits nutrient utilization. Thus, the present study does not merely confirm that enzymatic pretreatment improves fiber digestibility; it also provides in vivo evidence, in two monogastric species, that a laccase-rich fungal extract can effectively modify the cell wall of sugarcane bagasse and translate this structural change into a nutritionally relevant improvement.

It should be noted that the crude extract used here also contained cellulolytic activities (endoglucanase and exoglucanase), so the observed improvements cannot be attributed exclusively to laccase. The contribution of each enzymatic component was not isolated in this experimental design, and the possibility of synergistic action between lignin-modifying and cellulolytic activities cannot be excluded. Therefore, the effect reported here corresponds to the crude preparation as a whole, and the specific role of laccase remains to be established in future fractionation or purification studies.

These results may also lead to the incorporation of higher levels of pretreated fiber into feeding systems for monogastric species while reducing the use of cereals such as corn and soybeans; however, these aspects were not directly evaluated in the present study and warrant further investigation.

The degradation of cell wall components such as cellulose and lignin improves the utilization of fibrous feed in the lower gastrointestinal tract of these species. In the specific case of rabbits, which are classified as hindgut fermenters due to the high capacity of the cecum relative to the stomach, the most important microflora belongs to the Bacteroides species, where the enzymatic activity in the bacterial flora of the cecum is pectinolytic, followed by hemicellulolytic and cellulolytic enzymes [28]. For these reasons, the partial removal of lignin through enzymatic pretreatment will allow more efficient and functional access of the animal's endogenous enzymes to the cellulose and hemicellulose strands present in the diet.

In broiler chickens, fiber can be incorporated into rations at levels of up to 7–10% without negative effects on biological performance [29]. López et al. (2012) [30] evidenced this effect, finding a decrease ($p < 0.05$) in weight gain in chickens that were given an increase in the content of *Tithonia diversifolia* from 10% to 15% in the diet, since excess fiber in the ration can cause negative effects on productivity in young monogastrics, related to palatability, reduction of nutrient digestibility and feeling of satiety, making the chicken unable to consume the amount of feed necessary to cover its energy needs [30]. In the present investigation, an inclusion level of 10% was used and, although an animal behavior study was not conducted, a significant increase in live weight was evident at the end of the experiments. However, final live weight alone does not allow feed conversion to be inferred, since feed intake and feed conversion ratio were not directly evaluated in this study. Therefore, the observed increase in live weight should be interpreted as a higher final body weight, and any effect of the pretreated bagasse on feed conversion requires direct assessment in future studies specifically designed for that purpose.

Although the results are important for feeding systems of monogastric species, the evaluation and incorporation of laccase-enriched enzymatic crude in feed pretreatment systems for ruminant species may also offer significant advantages, especially in dry seasons or when low-quality pastures are used. However, responses of ruminal fermentation and nutrient digestibility to plant-derived phenolic compounds and feed additives are strongly dependent on the basal substrate and on the compound tested [31,32]; therefore, the ruminant application of this crude extract should be verified with in vitro and in vivo studies using contrasting diets.

On the other hand, in the field of animal production, lacasse enzymes are not used to the same extent as cellulolytic and xylanolytic enzymes. In fact, most international companies that market fibrolytic enzymatic products for the livestock sector [33], lack lignin-modifying enzymes such as laccases in their composition. Considering that the combination of cellulolytic and ligninolytic activity achieves a more complete and exhaustive degradation of plant fiber [34,35], the inclusion of laccase enzymes in fibrolytic products for livestock could be expected to optimize formulations and achieve much more efficient technologies.

5. Conclusions

In general, the complex situation regarding the feeding of productive species, coupled with the increasing scarcity of many resources, has driven the scientific community to search for promising solutions that guarantee economical and environmentally sustainable animal feed. Therefore, new research is guided by the principles of the circular economy, where agroindustrial byproducts can constitute a new variant of animal feed through the right use of biotechnological tools that improve their nutritional quality.

Under the experimental conditions evaluated, the present investigation demonstrates that enzymatic pretreatment of sugarcane bagasse with a laccase-enriched crude extract modifies the structural components of the fiber, particularly the lignin fraction, as evidenced by FTIR, and improves the nutritional characteristics of this alternative feed, with a positive effect on its in vivo apparent digestibility in animals of productive interest such as broiler chickens and Cuban brown rabbits. This study thus provides in vivo evidence, obtained in two monogastric species, that a laccase-rich fungal extract can improve the nutritional quality of a fibrous agroindustrial residue, contributing experimental elements to the study and incorporation of this type of lignin-modifying enzymes in the pretreatment of fibrous sources for animal production and outlining new lines of thought in the formulation and study of fibrolytic products for current livestock farming. However, these findings should be interpreted within the conditions evaluated and do not support claims of nutritional equivalence with the corn–soybean control diet, nor a direct effect on feed conversion or on higher inclusion levels of fibrous materials, which remain potential applications requiring further investigation. Extrapolating this approach to other species requires caution: in ruminants, the response to plant-derived phenolic compounds and feed additives is strongly conditioned by the basal diet and the specific compound assayed, so the application of this crude extract in ruminant nutrition cannot be assumed from the present results and should be validated through further in vitro and in vivo research under contrasting feeding conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation12110536/s1, Table S1: Chemical composition of the main fibrous components of control bagasse and bagasse pretreated with the laccase-enriched crude extract from *Curvularia kusanoi* L7; Table S2. Ingredient and chemical composition of the experimental diets used in broiler chickens; Table S3. Ingredient and chemical composition of the experimental diets used in rabbits.

Author Contributions: Conceptualization and design of the study; M.A.V., L.R.S. and B.R.S.; Investigation, L.R.S., M.A.V., E.C.V.C. and B.R.S.; Methodology, M.A.V. and B.R.S.; Data curation, L.R.S. and M.A.V., Formal analysis M.A.V., L.L.S.V., E.C.V.C. and L.R.S.; Supervision, M.A.V.; Writing—original draft preparation, M.A.V. and L.R.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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