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# ENZYMATIC EXTRACT OF Trametes maxima CU1 ON PRODUCTIVE PARAMETERS AND CARCASS YIELD OF RABBITS



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# ENZYMATIC EXTRACT OF *Trametes maxima* CU1 ON PRODUCTIVE PARAMETERS AND CARCASS YIELD OF RABBITS

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ABSTRACT

The aim of this study was to evaluate the effect of the enzymatic extract (EnzE) of native fungus *Trametes maxima* CU1 on the productive parameters and carcass yield of rabbits. A total of 36 rabbits, 18 New Zealand and 18 California breeds were distributed randomly into two treatments; control (without EnzE supplementation) and EnzE2.5 (with 2.5% of enzymatic extract added to drinking water). All rabbits were fed with a commercial diet *ad libitum*. At 49, 71, and 91 d, data for body weight (BW), average daily feed intake (ADFI), feed efficiency (FE), and average daily gain (ADG) were collected. Moreover, dressing out percentage (DoP) and carcass fat yield (%CFY) were estimated. BW and ADFI were not different between treatments ($P > 0.05$). However rabbits supplemented with EnzE2.5 showed higher values compared to the control. Rabbits EnzE2.5 and New Zealand males showed the best productive efficiency at 49 d ($P < 0.05$). On the other hand, EnzE2.5 showed greater DoP that control; furthermore EnzE2.5 did not show any effect over %CFY. These results show the potential of *Trametes maxima* CU1 as a source of lignocellulases and amylases for the improvement of productive behavior and carcasses yield.

*Keywords:* laccase, native basidiomycetes, productive traits

## INTRODUCTION

The rabbit is a single-chambered stomach herbivore, adequate for a high cellulose content diet (Attia et al. 2012). It is capable of taking advantage of a wide variety of ingredients on diet due to its digestive physiology (Carabaño et al. 2010). Due to this characteristic, pre-hydrolyzed ingredients could be added on its diet to improve rabbit productivity and meat quality. Polysaccharides pre-hydrolyzation is achieved by a process involving exogenous enzymes, as cellulases, α-amylases, β-glucanases and β-xylanases. Exogenous enzymes are a natural option in animal nutrition, because they improve animal productivity complementing their endogenous enzymes activity (Cachaldora et al. 2004).

Researches have evaluated the effect of exogenous enzymes, such as proteases and xylanases, in diets for rabbits (García-Ruiz et al. 2006), and in commercial pelletized diets pre-treated with β-glucanases, cellulases, α-amylases, proteases and lipases (Attia et al. 2012). Others have tested *Ganoderma resinaceum* for the degradation of the cell wall components of olive lives trying to increase rabbit productivity (Ribeiro et al. 2012), and the most recent use of cellulases, hemicellulases, amylases and proteases within the product ZAD® (Abdel-Aziz et al. 2015), produced an improvement on the productive parameters of rabbits.

Commercially available enzymatic extracts are mainly obtained from bacteria and filamentous fungi (Mounsey 2006). White rot basidiomycetes are well known because of their lignin degradation capability, associated with the production of Laccases, Lignin Peroxidases (LiP) and Manganese Peroxidases (MnP) (Phan and Sabaratnam 2012). But just until the last years their potential as a source of cellulases, hemicellulases,

amylases and pectinases has been studied (Inglis et al. 2000; Valášková et al. 2007; Baldrian and Valášková, 2008; Younes et al. 2011). And even when white rot basidiomycetes are well known producers of enzymes that degrade the main cell wall components (Zaldrazil et al. 1995; Elisashvili et al. 2008a; Lynch et al. 2014), they have not been explored enough as a source of enzymatic extracts for the elaboration of additives in animal feed. Although it has even been observed that different patterns and levels of enzymatic production within the same species (Elisashvili et al. 2008b; Elisashvili et al. 2009b) are translated into changes on the chemical composition of the fibers present in the substrates (Rodrigues et al. 2008). *Trametes maxima* CU1 is a native basidiomycete from northeast Mexico known by its ability to produce high redox potential laccases (Hernández-Luna et al. 2008), cellulases, xylanases and amylases (Gutiérrez-Soto et al. 2015). Therefore, this fungus could be used to pre-treat some lignin rich ingredients applied in rabbit feed. So the aim of this work was to evaluate the effect of the enzymatic extract of *Trametes maxima* CU1 on the productive parameters and carcass yield of rabbits.

## MATERIALS AND METHODS

The study was performed at the Facultad de Agronomia of the Universidad Autonoma de Nuevo Leon (UANL), located in the Municipality of Aramberri, at the South of the state of Nuevo Leon, Mexico at an altitude of 2100 m above sea level and having an average temperature of 25°C. Management and care protocol of rabbits was performed according to the national policies established in the Norma Oficial Mexicana NOM-062-ZOO (1999). A total of 36 rabbits from breeds California (C) and New Zealand (NZ), 42 days old (weaned age) and average weight of 595.83 ± 43.05 g were used in the

research. Rabbits were placed individually in metal mesh cages (45 x 60 x 50 cm; wide, long and tall), provided with a feeder and a waterer.

A completely randomized experimental design was used in this work. Thirty-six rabbits were randomly distributed in cages. Two treatments were established in the drinking water that was going to be provided: Control (regular drinking water) and EnzE2.5 (Control + 2.5% enzymatic extract). Average daily water intake for each rabbit was 200ml. Assignment of breeds and sex of the rabbits by treatment was arranged, in such a way that the Control was integrated by 5 males and 4 females C and 4 males and 5 females NZ, meanwhile EnzE2.5 was composed by 4 males and 5 females C and 5 males and 4 females NZ. The diet used in the fattening of rabbits was a commercial feed (Conejina®, Purina), composed by 16% protein, 3.0% fat, 17% fiber, 10% ashes, 12% humidity, 42.5% NFE, 1.0% calcium and 0.55% phosphorous.

*Trametes maxima* CU1, the native strain used to produce the extract, was obtained from the Enzymology Laboratory of Facultad de Ciencias Biologicas, UANL. Inoculum preparation consisted of a 5 days old reactivated culture of the strain in YMGA solid medium as suggested by Hernández-Luna et al. (2008). A liter of solid medium contains 4 g glucose, 10 g malt extract, 4 g yeast extract and 15 g agar (Dickenson and Company BD, Le Pont de Claix, France). Six cylinders of 0.5 cm in diameter were taken from the periphery of a CU1 colony to inoculate wheat bran liquid medium [Kellogg´s Bran Flakes®, in 60 mM potassium phosphate buffer (Sigma-Aldrich, St. Louis, MO), adjusted to pH 6.0 with 1N KOH]. Enzymatic production was performed in 500 ml Erlenmeyer flasks containing 200 ml of wheat bran liquid medium. Inoculated flasks were grown in a rotary shaker at 28 °C and 200 rpm. In order to obtain cell free extracts, biomass was separated using Whatman N° 1 filter paper. Then, 10 ml aliquots were

taken, which were frozen until its analysis and enzyme quantification was done. The quantification of avicellases, CMCases, β-D-glycosidases, xylanases and amylases were estimated by the quantity of free reducing sugars, according to the method proposed by Miller (1959). Standard curves of glucose and xylose were utilized. The measurements of absorbance were made in a UV1800 spectrophotometer (Shimadzu, Japan). One unit of enzyme was defined as the amount of enzyme catalyzing the release of 1mmol of glucose or xylose equivalent per minute at 25 °C. The laccase determination was carried out according to the protocol described by Abadulla et al. (2000), using 20 mM 2,6-dimetoxyphenol (DMP) as substrate in 200 mM sodium acetate buffer, adjusting pH to 4.5. The measurements were made in a spectrophotometer at 468 nm (ε= 49,600 $M^{-1}cm^{-1}$; Shimadzu, Japan). One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of DMP per minute at 25 °C. In the enzymatic extracts produced by *Trametes maxima* CU1 cultures, the enzymes CMCases, avicelases, xylanases, β-D-glucanases, amylases and laccase were detected (Table 1).

All solutions were prepared with distilled water (Laboratorios Monterrey S.A., Monterrey, MX).

Studied variables were body weight (BW; kg) and average daily feed intake (ADFI; kg) at 49, 71 and 91 d of fattening. This data was used to estimate feed efficiency (FE; BW/ADFI) and average daily gain [ADG; ($BW_{current}$-$BW_{previous}$) / days]. Sacrifice was accorded to the method used by Dal Bosco et al. (2014) and Norma Oficial Mexicana NOM-033-SAG/ZOO (2014) at 91 days of fattening. Carcasses were kept at 4 ± 1.0 °C for 12 h. Slaughter weight and carcass weight were registered (n = 12 per treatment) to determine dressing out percentage (DoP). In the same way, carcass fat weight was

measured to estimate carcass fat yield (%CFY) in function of carcass weight. In this study, death and diseased animal were not observed in any treatments

The data obtained from the performance variables were analyzed with the instruction PROC MIXED of SAS (2006), using the statistical model according to Wang and Goonewardene (2004), considering initial weight (IW) as covariate in the statistical model and the fixed effects treatment (Control and EnzE2.5), breed (C and NZ), sex, fattening days and interaction between this parameters; the nested effect was considered, where each treatment, breed and the sex were nested in the cage in the days. When the effect of fixed parameters and their interaction was significant ($P \leq 0.05$), the instruction Adjust = Tukey of SAS (2006) was used to compare means. Dressing out percentage and carcass fat yield were analyzed with a variance analysis ($P \leq 0.05$) using the general lineal model (GLM; SAS 2006), where the treatment, breed and sex were the fixed effects. The comparison of means was performed using the Tukey test, when P-value was less than 0.05 in the fixed effects.

## RESULTS AND DISCUSSION

Treatment (Control; EnzE2.5), breed (C; NZ) and the interactions between them were not significant ($P > 0.05$) over the productive behavior of rabbits (Table 2). Similarly, Ribeiro et al. (2012) did not find any effect between treatments when they evaluated 50 g $kg^{-1}$ of olive leaves treated with fungi. But, fattening the rabbit fattening periods of were different ($P < 0.05$), and sex effect was significant only over BW and ADFI ($P < 0.05$). Similar behaviors in weight were obtained by Attia et al. (2012), using exogenous enzymes in the production of rabbits. These authors indicated that enzymes could partially hydrolyze polysaccharides, reducing intestinal viscosity and improving nutrient availability and absorption. According to our results, the enzymatic extract (avicelases,

CMCases, β-D-glucosidases and xylanases) used in the present study could hydrolyze the cell wall polysaccharides, improving their digestibility throughout the rabbits digestive tract. BW and ADFI had the same behavior for all treatments in the fattening period they were the lowest at 49 d and the highest at 71 d. Male rabbits and California breed showed the highest weights at 49, 70 and 91 d.

The productive efficiency of rabbits shows that FE and ADG were not affected, as can be seen in Table 3. While the trend was greater for rabbits treated with EnzE2.5 and male rabbits. FE and ADG were not different when compared at 49 and 71-91 d, in the treatments, breed and sex. Otherwise, Abdel-Aziz et al. (2015) found improvements by the use of exogenous enzymes in feed efficiency, indicating improvement in average daily feed intake. This difference could be due to a higher enzyme content used by those authors (50%).

At 71-91 days of fattening (Table 4), FE was higher in females than in males ($P< 0.05$) comparing the rabbits fed with EnzE2.5. With those of the Control treatment, which represents an improvement in the productive efficiency of rabbits (FE; ADG).The females of California breed consumed more feed, while males showed the lowest consumption in both breeds ($P< 0.05$), resulting to be more efficient than females. In general, rabbits treated with EnzE2.5 and New Zealand males showed the highest productive efficiency at 49 d of fattening. Similar results were found by Garcia-Ruiz et al. (2006). They evaluated enzymes (proteases and β-xylanases) in rabbit production. At 71-91 days periods these authors found improvements in ADFI and FE with enzymatic treatments, indicating that supplements with exogenous carbohydrolases may increase efficiency in the digestion of dry matter due to a high accessibility to the polysaccharides of plant cell walls partially pre-treated. Shanmuganathan et al. (2004), obtained similar

results between female and male rabbits in ADFI, FE and ADG in accordance with our research with EnzE2.5. These authors indicated an increase of growth rate because of the use of enzymatic (cellulases and proteases) and microbial additives, improving nutrients metabolism.

The DoP was different between treatments ($P< 0.05$), EnzE2.5 showed higher yield (55. 88 ± 0.72 %) compared to Control (52.28 ± 0.69 %). The effect of breed, sex and their interactions had no effects over DoP. On the other hand, %CFY (3.97 ± 0.33 %) was not different between treatments or affected by breed, sex and interactions of the evaluated parameters ($P> 0.05$). Similar results in rabbit dressing out percentage were obtained by Shanmuganathan et al. (2004). They found higher yield in treatments with enzymatic supplements, which was higher in females (60.62 ± 0.61 %). Abdel-Aziz et al. (2015), also found an effect in %HYC (55.3 and 54.3%) with ZAD® enzymatic product, these authors attributed their results to the pre-treatment of nutrients with the use of enzymatic complexes.

It is worth noting that commercially available enzymatic supplements have been used in higher concentrations (10-100 fold) to the ones reported here with *T. maxima* CU1. These commercial supplements also have higher enzymatic activities and are obtained from different filamentous fungi and bacteria. For example, Amylofeed®, which contains α-amylase (3100 U/g), β-glucanase (275 U/g) and β-xylanase (400 U/g), is obtained from *Aspergillus niger* (NRRL 25541) and *Aspergillus oryzae* (ATCC 66222) (Cachaldora et al. 2010). Another commercial product is Kemzyme (Attia et al. 2012), with an enzymatic composition of endo-1,3(4)-β-glucanase (2350 U/g), endo-1,4-β-glucanase (4000 U/g), α-amylase (400 U/g), bacillolysin (450 U/g), endo-1,4-β-xylanase (20,000 U/g) and 6-phytase (1000 FYT/g), obtained from *Aspergillus aculeatus* (CBS

589.94), *Trichoderma longibrachiatum* (CBS 592.94), *Bacillus amyloliquefaciens* (DSM 9553), *Bacillus amyloliquefaciens* (DSM 9554), *Trichoderma viride* (NIBH FERM BP 4842), and *Aspergillus oryzae* (DSM 14223), respectively. In the enzymatic extracts of *T. maxima* CU1, are also present endo/exo-cellulases, xylanases and amylases, but the lignolytic activity of laccase is unique, as laccase has not been reported in any of the commercial extracts described before. The synergic action of the laccase with the carbohydrases, as well as their thermostable functional properties and their ability for acting in a broad pH range could explain the positive effect on the productivity parameters found in this study.

In conclusion, *Trametes maxima* CU1 is a native basidiomycete isolated from northeast Mexico, which produces CMCases, avicelases, xylanases, β-D-Glucosidase, amylases and laccases that can be used in concentrations of 2.5% to improve rabbit productivity and meat yield. In this sense, enzymatic extracts obtained from fungus *T. maxima* CU1 are an applicable option to animal production. In further studies, we will evaluate the effect of the composition of culture medium on enzymatic production, the characterization of the enzymes operative and functional properties, as well as the effect of higher concentrations over rabbit productivity, and parameters related to rabbit meat quality, such as chemical composition, texture and sensory evaluation.

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**Table 1.** Enzymatic composition of *T. maxima* CU1 extracts.

| Enzyme | U $L^{-1}$* | U # |
|---|---|---|
| CMCases EC. 3.2.1.4 | 850.00 | 4.25 |
| Avicelases EC 3.2.1.91 | 1440.00 | 7.20 |
| Xylanases EC 3.2.1.8 | 825.00 | 4.12 |
| β-D- C 3.2.1.21 | 580.00 | 2.90 |
| Amylases EC 3.2.1.1 | 40.00 | 0.20 |
| LaccaseEC 1.10.3.2 | 10.00 | 0.05 |

* *Trametes maxima* CU1 enzyme production in 1L of liquid media
#Enzyme concentration in 200 mL of water per rabbit.

**Table 2.** Influence of Enzymatic extract, breed and sex on the weight and feed intake of rabbits.

| Parameters | Fattening period (Days) | | | |
|---|---|---|---|---|
| | 42-49 | 50-70 | 71-91 | SEM |
| BW (kg)/ nts | | | | 0.030 |
| Control | 0.857$^{C}$ | 1.505$^{B}$ | 2.146$^{A}$ | |
| EnzE2.5 | 0.898$^{C}$ | 1.560$^{B}$ | 2.172$^{A}$ | |
| Breed | | | | 0.029 |
| California | 0.891$^{C}$ | 1.562$^{B}$ | 2.190$^{A}$ | |
| New Zealand | 0.863$^{C}$ | 1.502$^{B}$ | 2.128$^{A}$ | |
| Sex | | | | 0.029 |
| Female | 0.862$^{C}$ | 1.470$^{B}$ | 2.101$^{A}$ | |
| Male | 0.893$^{C}$ | 1.594$^{B}$ | 2.217$^{A}$ | |
| ADFI(kg)/treatments | | | | 0.052 |
| Control | 0.483$^{C}$ | 1.837$^{B}$ | 2.761$^{A}$ | |
| EnzE2.5 | 0.475$^{C}$ | 1.928$^{B}$ | 2.770$^{A}$ | |

| | | | | |
|---|---|---|---|---|
| Breed | | | | 0.054 |
| California | 0.490$^{C}$ | 1.913$^{B}$ | 2.809$^{A}$ | |
| NewZealand | 0.469$^{C}$ | 1.852$^{B}$ | 2.721$^{A}$ | |
| Sex | | | | 0.052 |
| Female | 0.522$^{C}$ | 1.993$^{B}$ | 2.912$^{A}$ | |
| Male | 0.437$^{a;C}$ | 1.772$^{b;B}$ | 2.619$^{b;A}$ | |

BW: body weight; FE: feed efficiency; EnzE2.5: treatment with 2.5% enzymatic extract in drinking water; $^{a,b}$Means within the same column with different superscripts differ significantly ($P < 0.05$); $^{A,B,C}$Means within the same rows with different superscriptsdiffer significantly ($P < 0.05$) (through time); SEM: Standard error of mean.

**Table 3.** Effect of enzymatic extract, breed and sex on feed conversion ratio and daily weight gain of rabbits.

| Parameters | Fattening period (Days) | | | |
|---|---|---|---|---|
| | 42-49 | 50-70 | 71-91 | SEM |
| FE (kg gain $kg^{-1}$ intake)/t | | | | 0.050 |
| Control | $1.737^{A}$ | $1.239^{B}$ | $1.304^{B}$ | |
| EnzE2.5 | $1.909^{A}$ | $1.237^{B}$ | $1.276^{B}$ | |
| Breed | | | | 0.051 |
| California | $1.854^{A}$ | $1.175^{B}$ | $1.242^{B}$ | |
| New Zealand | $1.792^{A}$ | $1.301^{B}$ | $1.338^{B}$ | |
| Sex | | | | 0.051 |
| Female | $1.732^{A}$ | $1.268^{B}$ | $1.325^{B}$ | |
| Male | $1.914^{A}$ | $1.208^{B}$ | $1.255^{B}$ | |
| ADG(kg)/treatments | | | | 0.014 |
| Control | $0.039^{A}$ | $0.031^{B}$ | $0.031^{B}$ | |
| EnzE2.5 | $0.043^{A}$ | $0.031^{B}$ | $0.029^{B}$ | |
| Breed | | | | 0.015 |
| California | $0.042^{A}$ | $0.031^{B}$ | $0.030^{B}$ | |

| | | | | |
|---|---|---|---|---|
| New Zealand | 0.040$^{A}$ | 0.031$^{B}$ | 0.030$^{B}$ | |
| Sex | | | | 0.014 |
| Female | 0.043$^{A}$ | 0.033$^{B}$ | 0.030$^{B}$ | |
| Male | 0.039$^{A}$ | 0.029$^{B}$ | 0.030$^{B}$ | |

FE: feed efficiency; ADG: average daily gain; EnzE2.5: treatment with 2.5% enzymatic extract in drinking water; $^{a,b}$ Means within the same column with different superscripts differ significantly ($P < 0.05$); $^{A,B}$ Means within the same rows with different superscripts differ significantly ($P < 0.05$) (through time); SEM: Standard error of mean.

**Table 4**. Global productive behavior (42-91d) of rabbits by effect of enzymatic extract, breed and sex.

| Treatments | Breed | Sex | ADFI (kg) | ADG (kg) | FE (kg $kg^{-1}$) |
|---|---|---|---|---|---|
| Control | | Female | 5.594[a] | 0.033 | 2.508[a] |
| | | Male | 4.569[b] | 0.029 | 2.251[b] |
| EnzE2.5 | | Female | 5.260[a] | 0.032 | 2.403[a] |
| | | M | 5.086[a] | 0.031 | 2.384[a] |
| SEM | | | 0.175 | 0.001 | 0.063 |
| | California | Female | 5.549[a] | 0.033 | 2.525[a] |
| | | Male | 4.875[b] | 0.031 | 2.271[b] |
| | New Zealand | Female | 5.304[b] | 0.033 | 2.387[b] |
| | | Male | 4.780[b] | 0.029 | 2.364[b] |
| SEM | | | 0.180 | 0.001 | 0.065 |
| Control | California | Female | 5.804[a] | 0.032 | 2.662[a] |
| | | Male | 4.630[a] | 0.032 | 2.144[b] |
| | New Zealand | Female | 5.384[a] | 0.034 | 2.355[a] |
| | | Male | 4.508[a] | 0.027 | 2.359[a] |
| EnzE2.5 | California | Female | 5.295[a] | 0.033 | 2.388[a] |
| | | Male | 5.120[a] | 0.031 | 2.398[a] |
| | New Zealand | Female | 5.224[a] | 0.032 | 2.419[a] |
| | | Male | 5.051[a] | 0.031 | 2.369[a] |

| | | | |
|---|---|---|---|
| SEM | 0.252 | 0.001 | 0.091 |

ADFI: average daily feed intake; ADG: average daily gain; FE: feed efficiency; EnzE2.5: treatment with 2.5% enzymatic extract in drinking water; [a,b] Means within the same column with different superscripts differ significantly ($P < 0.05$); SEM: Standard error of mean.