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Research Article
Effect of Biologically Treated Wheat Straw with White-Rot Fungi on Performance, Digestibility and Oxidative Status of Rabbits

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Abstract

Background and Objective: Raising growing rabbits is an ideal solution to confront animal protein deficiency, especially in developing countries. The presence of lignin in wheat straw causes limitation of the digestion overall process. The biological delignification is a practical and promising alternative due to improving the digestibility of wheat straw. This study aimed to enhance wheat straw digestibility and enriching it with protein and use it as a growing rabbit feedstuff. **Materials and Methods:** Enzymes production of white-rot fungi was assayed in myco-straw and the mean value was recorded. Wheat straw has been treated with the three most effective fungal species with Biological Treated Wheat Straw (BTWS). After that, the myco-straw was grounded and included in diet to evaluate the growth performance, digestibility and blood parameters of a growing V-line rabbit. **Results:** The best three species for enzyme productions were *P. sajor-caju*, *P. columbinus* and *P. floridanus*. The optimum incubation period was 16 days. The fungal treatments showed significant enzymes activity of laccase, Mn-peroxidase, cellulase and xylanase. Body weight, body weight gain and feed conversion ratio of growing V-line rabbits had improved than those of the control. But, nutrients digestibility of the diet containing BTWS and Carcass traits of growing V-line rabbits were non-significant compared with the control one. In comparison with control, the lipid profile had no differences but the total protein was improved. **Conclusion:** White-rot fungal conversion of wheat straw is maybe one potential alternative providing a more practical, environmental-friendly and nutritionally enhancing as rabbits feedstuff. Rabbits fed BTWS-diets had significantly improved growth performance.

Key words: Wheat straw, white-rot fungi, solid-state fermentation, rabbit diet, V-line rabbits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lignocellulosic chemical properties make a suitable substrates of enormous biotechnological value[1]. Renewable bioconversion resources concerning animal feed is a good alternative to these chemically synthesized feed types. Wheat straw energy-rich cell walls utilization is hampered by the presence of lignin, causing limitation of the digestion overall process[2]. Pretreatment techniques can be divided into physical, chemical and biological. It also can be used individually or in combination[3]. High substrate digestibility and low inhibitor formation affect the efficiency of the pretreatment process[4]. The addition of microorganisms furnishes all the required hydrolytic enzymes making the minerals more available for absorption by the animal. The biological delignification is the practical and promising alternative among lots of pretreatment ways used to improve the digestibility of wheat straw[5]. Solid-State Fermentation (SSF) is considered as the most appropriate method for mushroom cultivation because they grow under conditions close to their natural habitats[6]. White Rot Fungi (WRF) are reported to degrade lignin under (SSF)[7,8]. White-rot fungi degrade polysaccharides by hydrolytic enzymes and lignin by oxidative ligninolytic enzymes (Laccases and peroxidases (LiP and MnP)). The inoculum type/seed culture has an essential part resulting in higher levels of lignin degradation under SSF[2].

To solve the severe shortage in meat production worldwide and particularly in the developing countries, raising semi-ruminant animals such as rabbits is considered to be one of the most useful ways. Of the total cost of rabbit production, the feeding cost represents about 60-70%. Therefore, the challenge for the feed formulation is to obtain the least cost diets that fully match the rabbit requirements. Through using untraditional cheaper feed ingredients or improving the utilization of common feeds by using some feed additives, the aim of cost reduction can be achieved. Wheat straw, which is ideal biomass energy, is the second-largest lignocellulosic material in the world[3]. As a feed ingredient, using such an agricultural by-product for fattening rabbits can participate as a solution for the problems of environmental pollution and the shortage of feedstuffs. Some recent studies had utilized some agricultural and agro-industrial by-products in rabbit diets representing a ratio (30-40%) of the pelleted diets of the rabbits[9-11].

Recently, microbial feed additives become one of the main additives during silage preparation and making. The daily weight gain, feed intake and feed conversion was indicated to be increased, while feed cost per unit gain was showed to be decreased in growing animals by microbial ensilage of crop residues[12].

Organisms that degrade cellulose and hemicelluloses are not preferable or may be of no use because of the depletion of the straw of valuable nutrients that the animal itself can digest[13-15].

The aim of this study was to achieve high digestibility and structure improvement of wheat straw as a growing rabbit feedstuff.

MATERIALS AND METHODS

Sources of fungi: This study was carried out during the period from November, 2018 to July, 2019. *Pleurotus sajor-caju* (Fr.) Sing (Strain 290), *P. columbinus* Quel. Ap. Bres (strain 250) were supplied from consultative commet company of mushroom cultivation, Giza-Egypt (CCCM). *P. floridanus* Sing (38539) was obtained from America Type Culture Collection (ATCC). The used fungal strains were routinely maintained on Potato Dextrose Agar (PDA) slopes at 4°C being subcultured at 3-month intervals.

Fungal growth on wheat straw: Flasks (250 mL) containing 5 g of wheat straw were moistened with 20 mL distilled water (1:4) and then autoclaved at 120°C and 14.7-15 psi in 15-20 min. Each sterilized flask is inoculated with 2 discs of a fungal species and incubated at 30°C for about a month. Some flasks with no fungal mycelia are used as control. The flasks are harvested in triplicates at regular intervals of 3 days and a fixed amount of wet fermented straw (5g wet weight) is taken out to extract enzyme[16].

Enzyme production: Enzymes were extracted from harvested straw by adding 20 mL acetate buffer (20 mM, pH 5.0) on shaker for 2 hours then centrifugation[16] at 6708×g for 20 min at 4°C.

The enzyme solution was assayed for various enzyme activities; Laccase (Lac) activity was measured in triplicates according to Leite *et al.*[17]. The reaction mixture contained 0.2 mL of crude extract and 2.7 mL. of 0.05 M 2,6-dimethoxyphenol (DMP) in 0.2 M citrate buffer solution (pH 3.4) and incubated at 30°C for 5 min. The absorbance was measured at wavelength 468 nm of 2,6-dimethoxyquinone produced after these 5 min of reaction. Laccase activity is expressed in U per each mL of the crude enzyme (one unit corresponds to the amount of enzyme which converts one μM of 2,6-dimethoxyphenol per minute ($\epsilon_{468} = 10^4$ M^{-1} cm^{-1}).

Manganese peroxidase (MnP) activity was measured by oxidation of (2,6-dimethoxyphenol DMP, $\epsilon = 27500$ cm^{-1} M^{-1}) in the existence of H_2O_2 and $MnSO_4$. The reaction mixture is

2.5 mL. containing (0.2 mM DMP, 0.5 mM $MnSO_4$, 25 mM sod. citrate buffer (pH 4.8), 0.1 mM H_2O_2 and the crude enzyme), 0.5 mL. of each component. Reactions were started by adding H_2O_2And the increase in absorbance was measured with spectrophotometer at A_{469} nm. One unit of enzyme activity (U) was defined as the amount of enzyme catalyzing the oxidation[18] of 1 µM of DMP/min.

Xylanase activity was determined by measuring the release of reducing sugar from Birchwood xylan (1.0% w/v) by dinitrosalicylic acid (DNS) method[19]. The assay mixture was incubated at 30°C for 20 min and then the absorbance was measured at 550 nm. One unit of xylanase was defined as the amount of enzyme required to release 1 µM of xylose from Birchwood xylan in 1 min under the standard assay conditions elsewhere[20,7].

Cellulase activity was determined by measuring the release of reducing sugar from cellulose (1.0% w/v) by dinitrosalicylic acid (DNS) method[19]. The assay mixture was incubated at 30°C for 20 min and then the absorbance was measured at 550 nm. One unit of cellulase was defined as the amount of enzyme required to release 1 µM of glucose in 1 min under the standard assay conditions.

Analysis of nutritional contents of mycostraw: The dried wheat straw samples treated with *P. sajor-caju* singly and that treated with the three fungal species (*Pleurotus sajor-caju, P. floridanus* and *P. columbinus*) together were analyzed for amino acids by the performic oxidation method [(21)] in the Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), in Giza. It was also analyzed for dry matter basis; Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), ash, moisture and carbohydrates according to AOAC.[22] in the Micro Analysis Unit-Department of Agricultural Chemistry, Faculty of Agriculture, Mansoura University.

Biological treatment of wheat straw

Experimental animals: In this study, forty two-day-old unsexed V Line rabbits, with nearly similar initial live body weights, were randomly distributed, in factorial arrangements, to four experimental groups, each with three replications. Each replicate group of rabbits (3 animals) was kept in a galvanized wie-cage (50×50×45 cm) and provided with a feeder and a nipple drinker and fed its respective experimental diet from 6 to 13 weeks of age. All experimental rabbits were kept in a naturally ventilated building and subjected to similar environmental, managerial and hygienic conditions. Pelleted feed and fresh water were provided on an ad libitum basis.

Experimental diets: Four experimental diets (Table 1) were formulated to meet the nutritional requirements of growing rabbits, according to the NRC.[23].

Treated experimental diets were formulated, the first was representing the control diet (Cont.) without adding wheat straw, the second representing the diet by replacement of berseem hay with Non-Treated Wheat Straw (NTWS), the third and the fourth treatments representing the biologically treated wheat straw (BTWS) with *Pleurotus sajor caju* singly and the three most effective species together (*P. sajor caju*, *P. columbinus* and *P. floridanus*) respectively as shown in Table 1.

Growth performance traits of rabbits: Individual Live Body Weights (LBW) of rabbits were recorded at the beginning of the experiment (6 weeks old) and on a weekly basis thereafter until the end of experiment (13 weeks of age). Weekly recorded on Feed Intake (FI) and Body Weight Gain (BWG) of rabbits were also maintained on a replicate group basis. Accordingly, Feed Conversion Ratio (FCR) was calculated as the amount of feed consumed per unit of BWG.

Digestibility trials of rabbits: During the 13th week of age, 4 digestibility trials were done using the same replicates groups of the experimental rabbits using the total collection method. Animals were fed their respective experimental diets for 3 days, in which daily feed intake and feces voided were quantitatively in polyethylene bags and stored at -20°C for later analysis[24].

Fecal samples were dried in a forced-air oven at 70°C for 72 h. (Air dried samples) then ground and placed in screw-top glass jars until chemical analyses. Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash content of the feces as well as those of feed were determined according to AOAC.[25] and expressed on a dry matter basis. Nitrogen free extract estimated as follows:

$$NFE = 100 - (CP + EE + CF + Ash)$$

The apparent digestion coefficients for DM, OM, CP, EE, CF and NFE were calculated.

Carcass traits of rabbits: At the end of the experiment (13 weeks of age), three rabbits from each treatment were randomly chosen and slaughtered after fasting for 16 hours. Just after estimating live body weight at slaughter (LBW), rabbits were carefully sacrificed, skinned and emptied. The individual weights of the eviscerated carcass (including head), liver (LI), heart (HE), kidneys (KI), digestive tract (DT), spleen

Table 1: Formulation and proximate analysis of the tested pelleted ration of weaning V-line rabbits from 6 to 13 week old

Feedstuffs	Control	NTWS	BTWS	BTWSmix
Yellow corn	9.7	9.7	4.7	7.7
Soybean meal, 44% CP	8	15	12	12
Wheat bran	27	20	20	20
Barley grain	17.5	17.5	17.5	17.5
Alfalfa hay	35	20	20	20
Non-treated Wheat straw (NTWS)	-	15	-	-
Biologically treated wheat straw with *P. sajor-caju* singly (BTWS)	-	-	15	-
Biologically treated wheat straw with *P. sajor-caju, P. floridanus* and *P. columbinus* together (BTWSmix)	-	-	-	15
Mint Straw	-	-	5	8
Limestone	1	1	1	1
Dicalcium phosphate	1.2	1.2	1.2	1.2
Vit. Min. Premix [§]	0.3	0.3	0.3	0.3
Sodium chloride	0.3	0.3	0.3	0.3
Total	100	100	100	100
Calculated analyses: As fed basis[23]				
Digestible energy (kcal kg^{-1})	2766	2733	2763	27.25
Crude protein (%)	16.43	16.35	16.35	16.34
Crude fiber (%)	13.05	15.2	12.38	13.52
Ether extract (%)	2.92	2.33	2.20	2.08
Lysine (%)	0.76	0.79	0.70	0.69
Meth. (%)	0.32	0.29	0.26	0.26
Total phosphorus (%)	0.77	0.69	0.67	0.66
Available phosphorus (%)	0.51	0.45	0.44	0.44
Calcium (%)	1.22	1.02	1.02	1.02
Determined analyses: As DM basis[25]				
Dry matter (%)	90.21	90.76	90.85	90.61
Organic matter (%)	81.34	82.31	82.34	82.08
Crude protein (%)	18.21	18.01	17.99	18.03
Crude fiber (%)	14.47	16.70	13.63	14.92
Ether extract (%)	3.24	2.57	2.42	2.30
Ash (%)	8.84	8.45	8.51	8.53
Nitrogen free extract (%)	56.24	54.27	57.45	56.22

[§] Each 3 kg premix contained 12,000,000 IU Vit. A, 2,500,000IU Vit. D_3,10,000 mg Vit. E, 2500 mg Vit K_3,1000 mg Vit B_1, 4000 mg Vit. B_2,1500 mg Vit. B_6, 10 mg Vit. B_{12}, 10,000 mg Pantothenic acid, 20,000 mg Nicotinic acid, 1000 mg Folic acid, 50 mg Biotin, 500 mg Choline chloride, 60 mg Manganese, 55 mg Zinc, 100 mg Selenium, 1000 mg Iodine, 35 mg Iron, 10 mg Copper, 250 mg Cobalt and Carrier $CaCo_3$ to 3 kg

(SP), lung (LU), fur plus legs (FPL) and testis (TE) were recorded. Relative weights were also calculated.

Blood parameters of rabbits: At 13 weeks of age, three blood samples per experimental group of rabbits were taken during slaughtering in heparinized test tubes. Blood samples were immediately centrifuged at 3000 rpm for 15 min in order to separate blood plasma. Plasma samples were frozen at -20°C until later analysis.

The concentrations of glucose (GLU)[26], total protein (TPR)[27], albumin (ALB)[28], triglycerides (TRI)[29], total cholesterol (CHO)[30] and high-density lipoprotein-cholesterol (HDL-C)[31] were determined in blood plasma using commercial kits. Blood plasma level of low-density lipoprotein-cholesterol (LDL-C) was also estimated by using the equation[32], as follows:

$$\text{LDL-C} = \text{Total cholesterol} - (\text{HDL - C} + \text{VLDL})$$

where, VLDL are very-low-density lipoprotein which was calculated as the concentration of plasma triglycerides divided by 5.

Statistical analysis: Data were analyzed using the GLM procedure of Statistical analysis Software[33] using a one-way design. The mean difference at $p \leq 0.05$ was tested using Duncan's Multiple Range Test[34].

RESULTS

Enzymes production and improvement of nutrients digestibility: The time course for enzymes production of *Pleurotus sajor-caju* and the three fungal species together using wheat straw as a substrate is given in Fig. 1 and 2.

P. sajor-caju and also the three fungal species together exhibited maximum laccase and cellulase on the 13th day.

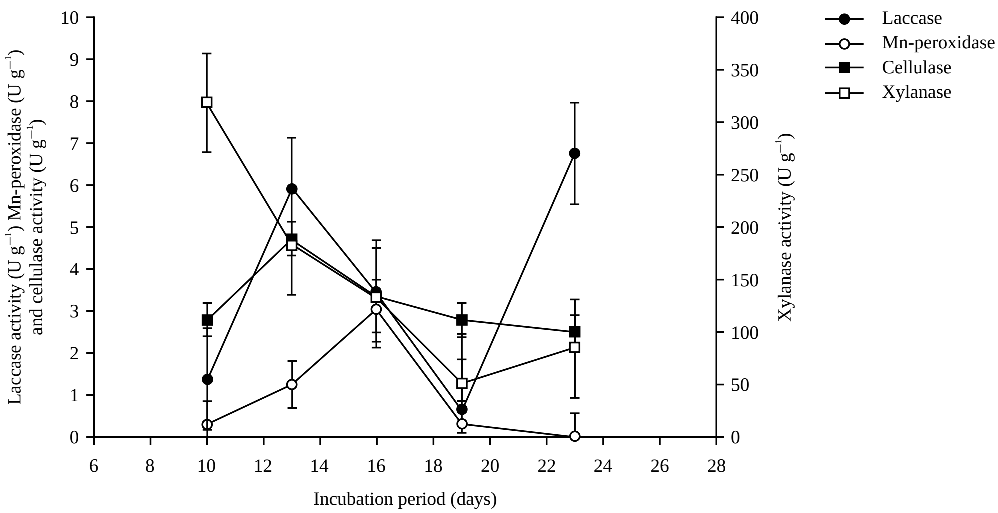

Fig. 1: Incubation period effect on enzymes production of *P. sajor-caju* on wheat straw; presented values are mean of 3 replicates, error bars represent standard error of the three values

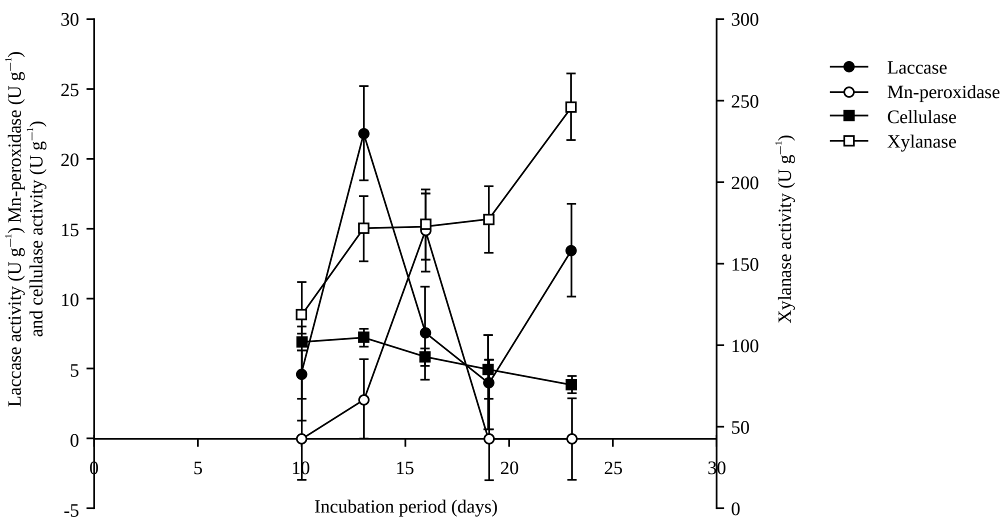

Fig. 2: Incubation period effect on enzymes production of the three fungal species together (*Pleurotus sajor caju*, *P. columbinus* and *P. floridanus*) on wheat straw, presented values are mean of 3 replicates, error bars represent standard error of the three values

Whereas maximum Mn-peroxidase activity of both *P. sajor-caju* and the three fungal species was on the 16th day. Xylanase production in the case of *P. sajor-caju* gave its maximum activity on the 7th day, then it had declined degradedly, while in the case of the three fungal species together it declined lightly then began to make another rise slowly giving better results.

The most efficient species in enzymatic activity among the three studied fungal species and the mixture were chosen for further analysis of nutritional content as follow.

Inoculation of wheat straw with *P. sajor-caju* and three fungal species increased protein content three fold and approximately three and half folds more than untreated wheat straw respectively and decreased lipids in case of *P. sajor-caju* but increased lipids in the treatment of the three species. The carbohydrates increased by one and half time of the untreated wheat straw. The fiber content of wheat straw highly decreased by fungal treatment (Table 2).

Amino acids content of wheat straw sample treated with *P. sajor-caju* had significantly increased than those of wheat straw without any treatment, while the content of amino acids of the sample treated with the three fungal species together had raised more than the untreated wheat straw and more than *P. sajor-caju* treatment according to Table 2.

Performance and carcass trail of growing rabbits as affected by wheat straw without or with biological treated (WS) containing diets: It was interesting to report that no morbidity or mortality of V-line rabbits were observed during the course of this study.

Live body weight of growing V-line rabbits: Means of Live Body Weight (LBW) of growing V-line rabbits at various ages affected by wheat straw are shown in Fig. 3. The results obtained stated that rabbits fed on BTWS (*P. sajor caju*) and BTWS (Mixture)-containing diets were significantly heavier than those fed on the control and the Non-Treated Wheat Straw (NTWS) throughout the whole experimental period (6-13 weeks of age).

Body weight gain of growing V-line rabbits: The effects of replacing dietary alfalfa hay with Wheat Straw (WS) on Body Weight Gain (BWG) of growing V-line rabbits are reported in Fig. 3. These results showed that body weight gain of rabbits fed on BTWS (*P. sajor caju*) and BTWS (Mixture) -containing diets were significantly higher when compared with Non-Treated Wheat Straw (NTWS) and their control group.

Feed intake of growing V-line rabbits: The effects of replacing dietary alfalfa hay with WS on feed intake of rabbits are illustrated in Fig. 3. Results revealed that rabbits fed on BTWS (*P. sajor caju*) and BTWS (Mixture) diets had insignificant difference as compared to their control groups.

Feed conversion ratio of growing V-line rabbits: Data on the effects of feeding the experimental diets on feed conversion of growing V-line rabbits are reported in Fig. 3. The statistical analysis of these results indicated that growing rabbits fed on the experimental diets containing BTWS (*P. sajor caju*) and BTWS (Mixture) highly significantly enhanced the feed conversion ratio values in comparison with their control counterparts.

Table 2: Values of chemicals analysis (Dry M. B.) and Amino acids analysis of the wheat straw samples treated with *P. sajor-caju* (BTWS) and with fungal mixture (*P. sajor-caju*, *P. floridanus* and *P. columbinus*) (BTWSmix)

	Treatments (%)		
Parameters	Control	BTWS	BTWSmix
Nutrients digestibility and Amino acids (%)			
Moisture	11.34	9.0	7.0
Crude protein (CP)	3.3	10.6	11.2
Crude fiber (CF)	39.7	11.0	12.0
Ether extract (EE)	1.5	1.0	2.0
Ash	12.05	6.0	4.0
Carbohydrates	43.45	62.4	63.8
Aspartic (ASP)	----	0.30	0.33
Threonine (THR)	----	0.14	0.17
Serine (SER)	----	0.14	0.17
Glutamic (GLU)	----	0.59	0.82
Glycine (GLY)	----	0.17	0.24
Alanine (ALA)	----	0.22	0.25
Valine (VAL)	----	0.16	0.19
Isoleucine (ILE)	----	0.13	0.16
Leucine (LEU)	----	0.25	0.31
Tyrosine (TYR)	----	0.10	0.14
Phenylalanine(PHE)	----	0.12	0.19
Histidine (HIS)	----	0.06	0.08
Lysine (LYS)	----	0.12	0.17
Arginine (ARG)	----	0.10	0.16
Proline (PRO)	----	0.19	0.30
Cystine (CYS)	----	0.08	0.12
Methionine	----	0.04	0.16

BTWS: Biologically treated wheat straw with *P. sajor-caju* singly, BTWSmix: Biologically treated wheat straw with *P. sajor-caju, P. floridanus* and *P. columbinus* together

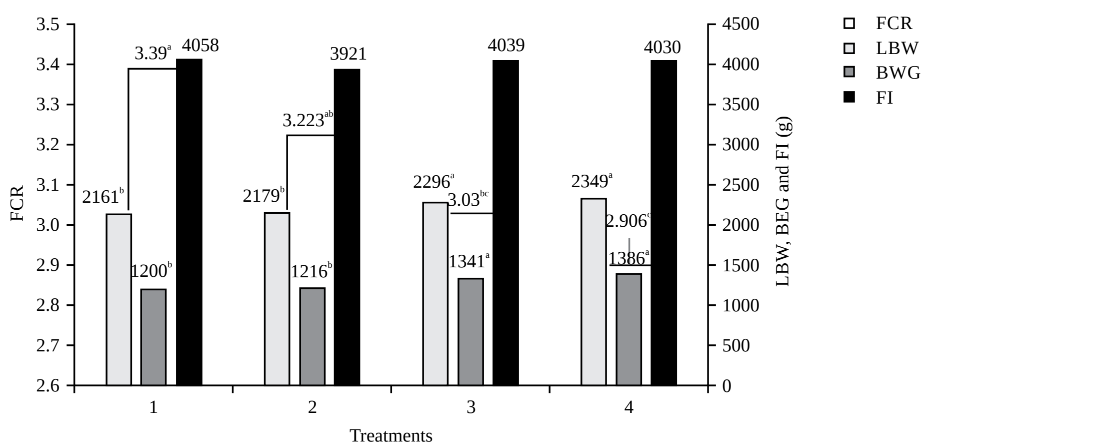

Fig. 3: Live Body Weight (LBW) , Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) of growing V-line rabbits as affected by wheat straw (WS) at 13 weeks of age

1-4: Refer to control, NTWS: Non-treated wheat straw, BTWS: Biologically treated wheat straw with *P. sajor-caju* singly, BTWSmix: Biologically treated wheat straw with P. sajor-caju, P. floridanus and P. columbinus together, (a-c): Means in the same row bearing different superscripts are significantly different

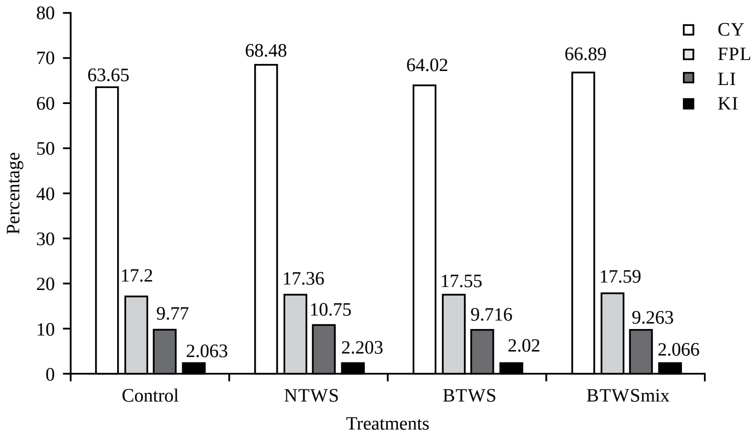

Fig. 4: Carcass traits of growing V-line rabbits as affected by wheat straw (WS) from 6 to 13 weeks of age

NTWS: Non-treated wheat straw, BTWS: Biologically treated wheat straw with *P. sajor-caju* singly, BTWSmix: Biologically treated wheat straw with *P. sajor-caju, P. floridanus and P. columbinus* together, CY: Carcass yield, FPL: Fur plus legs, LI: Liver and KI: Kidney

Table 3: Nutrients digestibility (%) of 13-wk-old V-line rabbits as affected by wheat straw (WS)

	Treatments					
Parameters	Control	NTWS	BTWS	BTWSmix	SEM	Significance
Nutrients digestibility (%)						
DM	68.65	70.02	69.69	69.83	0.431	NS
OM	70.66	71.75	71.56	71.71	0.485	NS
CP	70.25	70.53	70.99	71.76	0.460	NS
CF	50.32^{b}	52.14^{a}	51.44^{ab}	51.81^{a}	0.384	*
EE	76.36	76.49	76.60	76.59	0.493	NS
NFE	74.23	74.15	74.27	74.22	0.289	NS
NDF	57.23^{c}	75.57^{b}	81.24^{a}	81.76^{a}	0.850	*
ADF	57.26^{c}	78.18^{b}	81.71^{ab}	84.94^{a}	1.119	*
NFC	88.45^{c}	93.44^{b}	96.27^{a}	97.06^{a}	0.784	*

NTWS: Non-treated wheat straw, BTWS: Biologically treated wheat straw with *P. sajor-caju* singly, BTWSmix: Biologically treated wheat straw with *P. sajor-caju, P. floridanus and P. columbinus* together, CF: Crude fiber, NDF: Non- detergent fiber, ADF: Acidic detergent fiber, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NFE: Nitrogen free extract, NFC: Non-Fibre Carbohydrates, $^{a-c}$: Means in the same row bearing different superscripts are significantly different, NS: Not significant. *Significant, SEM: Standard errors of the means

Table 4: Blood constituents of growing V-line rabbits as affected by Wheat Straw (WS) from 6 to 13 weeks of age

	Treatments					
Parameters	Control	NTWS	BTWS	BTWSmix	SEM	Significance
Blood constituents						
TP[1] (g dL^{-1})	5.306^{b}	5.393^{b}	6.250^{a}	6.243^{a}	0.232	*
ALB[2] (g dL^{-1})	2.600^{b}	2.640^{b}	3.453^{a}	3.143^{ab}	0.190	*
Glob[3] (g dL^{-1})	2.706	2.753	2.796	3.100	0.272	NS
Tlipids[4] (mg dL^{-1})	209.3	213.2	202.0	206.2	8.104	NS
Chol[5] (mg dL^{-1})	80.83	81.76	76.80	77.86	2.603	NS
TriG[6] (mg dL^{-1})	53.16	54.60	51.96	50.60	2.107	NS
HDL[7] (mg dL^{-1})	25.03	26.13	26.26	27.50	1.047	NS
vLDL[8] (mg dL^{-1})	10.63	10.92	10.39	10.12	0.421	NS
LDL[9] (mg dL^{-1})	45.16	44.71	40.14	40.24	1.799	NS
AST[10] (U dL^{-1})	61.15	51.94	53.05	53.85	3.461	NS
ALT[11] (U dL^{-1})	12.59	11.09	11.08	12.22	0.607	NS
Creat[12] (mg dL^{-1})	1.246^{a}	1.173^{ab}	1.186^{ab}	1.063^{b}	0.042	*

NTWS: Non-treated wheat straw, BTWS: Biologically treated wheat straw with *P. sajor-caju* singly, BTWSmix: Biologically treated wheat straw with *P. sajor-caju, P. floridanus and P. columbinus* together, TP: Total protein, ALB: Albumin, Glob: Globulin, Tlipids: Total lipids, Chol: Cholesterol, TriG: Triglycerides, HDL: High density lipoprotein-cholesterol, vLDL: Very low-density lipoprotein, LDL: Low density lipoprotein-cholesterol, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase and Creat: Creatinine, $^{a-b}$: Means in the same row bearing different superscripts are significantly different, NS: Not significant, *: Significant., SEM: Standard errors of the means

Carcass traits of rabbits: Data on carcass traits of rabbits fed diets containing BTWS are given in Fig. 4. Feeding diets containing BTWS (*P. sajorcaju*) and BTWS (Mixture) showed no significant differences between the experimental groups in most measured percentages of organs.

Digestibility traits of rabbits: The effects of replacing dietary alfalfa hay by BTWS on nutrient digestibility of growing rabbits are given in Table 3. According to the variance analysis, these results showed that digestibility coefficients of Crude Fiber (CF), Non-Detergent Fiber (NDF), Non-Fiber Carbohydrates (NFC) and Acidic Detergent Fiber (ADF) were significantly affected. On the other hand, Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE) and Nitrogen-Free Extract (NFE) of 13 weeks old V-line rabbits were non-significantly affected by feeding the BTWS diets compared with their control counterparts.

Blood parameters of rabbits: The effects of feeding diets containing on blood plasma parameters of V-line rabbits are illustrated in Table 4. It was indicated that feeding the BTWS containing diets did not affect plasma concentrations of cholesterol (CHO), triglycerides (TRI), globulin (GLO), HDL-C, LDL-C, VLDL, Total Lipids (TL), AST and ALT of 13-week-old V-line rabbits compared with the control groups.

DISCUSSION

As a feed ingredient, using an agricultural by-product for fattening rabbits can participate as a solution for the problems of environmental pollution and the shortage of feedstuffs. Some recent studies had utilized some agricultural and agro-industrial by-products in rabbit feeding representing a ratio (30-40 %) of the pelleted diets of the rabbits[9-11].

Our results suggested (as a substrate is given in Fig. 1 and 2) that *Pleurotus sajor-caju*, *P. columbinus* and *P. floridanus* are good sources of protein content when grown on wheat straw as a substrate which agreed with many types of research[35,36]. These spp. are good producers of enzymes (cellulases, xylanases, Mn-peroxidase and laccase)[37] making wheat straw easy to be digested by other types of animals, not only ruminants[38-41].

Thus, the final product, in addition to the good content of carbohydrates, protein and other components of the mushroom, is more digestible and is free from any chemical additives that may lead to animal health problems affecting the livestock and human health. Many researchers have used several kinds of mushrooms for the same purpose[16].

Mn-peroxidase activity was found to have its highest value on the 16th day and decreased thereafter. Any notable decreasing, after the highest values, might have been done due to the depletion of the nutrients or denaturation of the enzyme that may be caused as a result of the interaction with other components in the medium or the changing in the pH of the medium as recorded by Veerabhadrappa *et al.*[42] and Jain and Naik[43].

Maximum activity of the xylanase production was found on the 7th day and after that there was a continuous decrease of xylanase production where culture reached towards the death phase as mentioned by Roy *et al.*[44] and Kshirsagar *et al.*[45]. The mixture of the three fungal species showed xylanase activity decline lightly on the 10th day then began to make continuous rise which can be attributed to mycelial autolysis and release of mycelial bound enzymes or it may be due to the lack of nitrogen in the second stage of cultivation and this agreed with Gupte *et al.*[46] and Shrivastava *et al.*[16].

Amino acids content of wheat straw sample treated with the three fungal species together had significantly increased than those of wheat straw without any treatment and than in the case of *P. sajor-caju* singly. Ruminal oxidative deamination and decarboxylation of valine, leucine and isoleucine which were increased by growing the wheat straw with the three *Pleurotus* spp. improved BCVFA which improve apparent dry matter digestibility and microbial growth and enhance microbial functions and enzyme activities[47]. Branched-chain volatile fatty acids (BCVFA: isobutyric, isovaleric and 2-methylbutyric) are essential nutrients and increase growth of rumen cellulolytic as well as some non-cellulolytic bacteria [(48)].

The improved growth rate of rabbits fed on BTWS experimental diets coincided with a lack of mortality indicates that BTWS is a safe feed ingredient in rabbit diets.

The present results are in agreement with the findings of Gaafar *et al.*[49], evaluated the growth response of rabbits to partial replacement of berseem hay by ensiled and dried Sugar Beet Tops (SBT) and reported that rabbits fed SBT-containing diets had significantly higher final body weight than their control counterparts. In the same line, Galal *et al.*[11], found that rabbits fed ration containing 20, 40 and 60% strawberry vine instead of clover hay had significantly heavier body weight compared with the control group. The improved BWG of rabbits fed on the diets containing BTWS agrees with the findings of these results are in agreement with those obtained by Omer *et al.*[50], who reported that rabbits fed strawberry by-products as a partial or complete substitute of dietary clover hay showed significantly better total body weight gain and average daily gain as compared to their control group. Better performance (BWG and FCR) of rabbits fed diets containing only 20% GW (D2) were significantly improved than control group as recorded by Kamel *et al.*[51]. On the other hand, Asar *et al.*[52], found that BWG of growing rabbits was not influenced by feeding corn-cob meal-containing diet instead of alfalfa hay.

Contrary to current results Abo Elmaaty *et al.*[53] evaluated that rabbits fed diets containing different dietary levels of both fennel and basil hay mixture without or with symbiotic in place of alfalfa hay had significantly lower feed intake than those of the control groups. The observed insignificant differences in feed intake of rabbits harmonize with those obtained by Omer *et al.*[54] reported that feed intake was insignificantly decreased by addition of 0.5% dried yeast to rabbit diets.

In accordance with the present results, Abo Elmaaty *et al.*[55], reported that feed conversion of rabbits fed diets containing sugar beet tops hay without or with prebiotic improved as compared to the control group. Similarly, addition of effective microorganisms to rabbit diets improved feed conversion as mentioned by Thayalim and Samarasinghe[56].

The results observed herein due to feeding the BTWS-containing diets agree with the findings of Abo Elmaaty *et al.*[55] who reported that there was no detectable negative effect on carcass dressing of NZW rabbits had fed SBT with probiotic supplemented diets. Also, Abo Egla *et al.*[57], observed that the effects of partial or complete substitution of Cucumber Vines Straw (CVS) for dietary clover hay on carcass characteristics of rabbits and reported that carcass traits of rabbits fed the diets containing up to 22.5% CVS (equivalent to replacement value of 75% of clover hay) were comparable to those of the control group but when the dietary level of CVS reached 30% (completely replaced clover hay) the relative weights of empty carcass and total edible parts were negatively affected.

EL-Tahan[58] pointed that the chemical composition of wheat straw, fungally treated with *Agaricus bisporus*, decreased slightly the DM content compared with those in raw wheat straw. El-Marakby[59] treated wheat straw with white-rot fungus *A. bisporous* reporting a great decrease in content of OM, Crude Fiber (CF), neutral detergent fiber, Acid Detergent Fiber (ADF), cellulose and hemicellulose and improvement for (CP) and ash content. Mahrous[60] found that treated cotton stalks by *Trichoderma viride* decreased the OM, CF, NDF, ADF and increased CP and ash. Supplementation with protein can increase the intake and digestibility of cereal straws[61]. The improved nutrient digestibility coefficient (CF), reported herein, agrees partially with Abo Elmaaty *et al.*[62], who reported that feeding growing rabbits on diets containing dried SBT with prebiotic in place of dietary alfalfa hay significantly improved the digestibility coefficients of DM, OM, CP, CF, EE and NFE.

The improvement in the protein content (TP) of the carcass, stable rate of lipids and also higher efficiency of liver and kidney function are good indicators. In the same line, since no morbidity or mortality were recorded throughout this study, these results may indicate that complete replacement with BTWS is safe and had no adverse effect on the metabolic functions or the health status of rabbits. Within the normal range, all hormone tests results yielded better results of BTWS than the untreated and the control as well as the sodium and calcium levels. As a result, the immune system and many vital functions work better. This improvement in the activity of the immune system is a result of the addition of *Pleurotus* species as they have a good effect on immunity.

In agreement with our findings, similar results were found by El-Gohary and Abo Elmaaty[63], who found that replacing the clover hay in rabbit diets with *Phaseolus vulgaris* straw had no significant effect on blood plasma constituents (total protein, albumin, total lipids, cholesterol and creatinine).

The present results also harmonize partially with Piva *et al.*[64], who found that adding 10g/d yeast culture to rations of cows increased total protein, globulin and albumin. Similarly, rabbits received Non-Treated Apple Pomace (NTAP) or Biologically Treated Apple Pomace (BTAP) at level of 25 or 50% had insignificant differences in globulin, albumin and A/G ratio of blood serum.

On the other hand, ALT and AST values reflected the impairment of liver function when their values increased[65]. Generally, our data observed that biological treatment of complex lignocellulosic agricultural wastes is one of the best treatment methods than physical and chemical treatments as the practical use of these treatments is still restricted in terms of safety concerns, costs and potentially negative environmental consequences, while using ligninolytic fungi, especially white-rot fungi species including their enzymes (laccase, Mn-peroxidase, xylanase and cellulase), maybe one potential alternative to provide a more practical and environmental-friendly approach for enhancing the nutritive value of wheat straw as an animal feed not only for ruminants. Growth performance of growing V-line rabbits fed diets biologically treated with fungi was significantly improved. These results confirmed that BTWS is safe with no adverse effects. BTWS and BTWSmix enhanced not only the live performance but also the immune responses of the growing V-line rabbits.

CONCLUSION

In conclusion, biologically treated wheat straw (BTWS and BTWSmix) not only enhanced the live performance but also the immune responses of the growing V-line rabbits. These results confirmed that BTWS is safe with no adverse effects.

SIGNIFICANCE STATEMENT

This study discovers the possible use of wheat straw treated biologically with white-rot fungal spp. in rabbits' diets that can be beneficial for improving the performance of fed rabbits. This study will help in the field of biological feeding for rabbits, dispensing with chemical compounds and their side effects on animals and in the long term on humans.

This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination and possibly other combinations, may be arrived at.

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