Effect of Fermented Water Hyacinth (*Eichhornia Crassipes*) on Palatability, Performance and Spermatozoa Quality on Male Sheep (*Ovis aries*)

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ABSTRACT
This experiment aimed to investigate the effects of fermented water hyacinth (Eichhornia crassipes) on palatability, performance and spermatozoa quality on male sheep. Fermented water hyacinth was mixed with the basal ration at various levels according to the following dietary treatments: (R1: 0% fermented water hyacinth (fwh) + 100% basal ration (br), R2: 10% fwh + 90% br , R3: 20% fwh + 80% br , R4: 30% fwh + 70% br) . These dietary treatments were given to four groups of sheep, each group consisted of five male sheeps served as the replicates. The rations were given twice a day at 6.7% of their body weight. Palatability was determined based on selection index using cafeteria-method in one day, while body weight gain and spermatozoa quality were evaluated after three months of feeding. Data were analyzed by ANAVA and Duncan's. The results showed that R3 had the highest palatability ($P<0.05$). Body weight gain were similar among the treatments. Increasing levels of fermented water hyacinth improved spermatozoa quality .Male sheep given R4 produced the best spermatozoa quality ($P<0.05$). In conclusion, inclusion of fermented water hyacinth in the ration improves reproductive performance in male sheep, but has limited effects on performance.

Keywords: *Fermented water hyacinth, Palatability, Performance, Spermatozoa quality, Male sheep.*

1. INTRODUCTION

Nutritious feed is needed to maintain farming of various livestock. Fermented feed can be produced to fulfil the nutrition requirement of livestock at lower cost, as it can use various types of organic materials as ingredients. One kind of feed is a fermented feed made of water hyacinth that is enriched with corn (*Zea mays*) cob. Both are organic materials commonly thought as residue. Corncob was found to contain 5.6% protein, higher than that of rice straw (4.9%) [1], thus have potential to be used as livestock feed. Several studies had examined its utility for feeding poultries, pig, goat [2], buffalo [3], fish [4], and ruminant [5].

Water hyacinth (*E. crassipes*) is an invasive species of water weed with rapid rate of growth [6]. This plant has spread a lot in the local water bodies. They can be found covering surface of water, affecting aquatic ecosystem in the river. Water hyacinth contains high level of protein (11.87%), calcium and phosphorous, thus it can stimulate milk production when combined at suitable concentration [7]. However, as with corncob, rough fibre and protein contained in water hyacinth make them difficult to be digested by livestock [8]. Thus, fermentation can be performed to break downfibreand protein, thus the feed will be easier to be digested and its palatability increased [9].

Fermentation of certain organic materials can be performed using specific and selected microorganism [10], as the specify of microbial enzymes are needed for specific organic materials. For example, fungi *Rhizopus* could significantly lower crude fibre content due to its lignocellulolytic enzymes [11] while several *Bacillus* species could produce protease and cellulase enzymes to break down complex organic matter in plant [12][13][14].

Fermented feed has several advantages, including increased digestibility, nutrient absorbance level, balance of rumen microflora, and lowered pH in stomach which subsequently reduced pathogenic microorganism, especially the ones carried in by foodstuff [15]. Administration of fermented feed to chicken could raise its body weight and strengthen egg-shell without lowered the egg production, but also induce more aggressive behaviour [16]. Previous study found that goats fed fermented water hyacinth had their bodyweight increased [17]. Molasses-fermented water hyacinth could also improve flesh quality of common carp [18], while fermented corncob flour could significantly affect weight gain of local rabbit [19].

Fermentation process or composting of organic materials consist of several steps, including mesophilic reaction, thermophilic reaction, cooling, and maturing [20][21]. Other study explained latent phase, which occurred before mesophilic reaction take place [22]. Various physical and chemical changes happened during the course of fermentation, resulting in development of different microorganism community in every phase. In aerobic degradation of solid waste, bacteria, fungi, and Actinomycetes were especially abundant [22].

Palatability has working definition based on adapted guideline of National Research Council as physical and chemical properties of the diet which are associated with promoting or suppressing feeding behaviorduring the pre-absorptive or immediate post absorptive period. In other word, it is the unconditioned response before any metabolic or condition effects on food intake could occur. There are two classes of palatability tests; consumption and non-consumption tests [23]. The non-consumption tests are autonomic or conditioned response tests, while consumption tests measure food intake; one a monadic or single-bowl "acceptance" test and the other a two-pan or forced choice "preference" test [23].

As one of main commodity in local farming, maintaining population of sheep is important. Thus, reproduction performance must be considered, not only in female sheep, but also in male sheep. Reproduction performance in male sheep is reflected mainly in the spermatozoa quality. Sperm qualityplayscritical role in fertilization; in fertile ewe, higher quality of spermatozoa correlates to higher number of successful fertilizations. Feed quality affects the quality of spermatozoa produced; thus, fermented feed can be given to add more nutrition to livestock, subsequently improving spermatozoa quality.

High content of fermented feed can possibly possess danger in the form of pathogenic bacteria and dangerous unidentified by products of microbial fermentation. Thus, this study was designed to evaluate the palatability of rationed feed mixture of dried water spinach (Ipomoea aquatica), which is commonly fed as fodder to local goats, and various level of fermented water hyacinth, which is enriched with fermented corncob and its effect to spermatozoa quality.

2. METHODS

2.1. Feed Fermentation and Ration Composition

Water hyacinth was collected randomly from Keputih river, Surabaya, while corncob was taken from rice mill in Tebel Village, Ngoro, Jombang, East Java. Both sources of raw materials were tested to check whether it contained chemicals and heavy metals contamination. Only raw materials with zero contamination were used in fermentation process. Both materials were rinsed, cut into small pieces of 1-3 cm, and air dried. Both were steamed separately until soften then dried under sun until water level below 30%. Water hyacinth and corncob were mixed at ratio 1:1 before starter powder was added and mixture was incubated for 15 days. Starter was made based on preliminary study, in which 1% consortium of 3 fungi (*Rhizopus stolonifer*, *Penicillium* sp1., *Trichoderma* sp1.) and 4 bacteria species (*Bacillus cereus*, *Burkholderia* sp., *Enterococcus* sp., and *Bacillus aerius*) with 75% bran and 25% cornstarch as filler was freeze-dried into powder. Temperature and pH of fermentation were measured daily until the feed deemed ready to use. The feed ready to be used was characterized by darker colour, tapai-like acidic fragrance, acid-sweet taste, and soft texture, protein level of fermented feed was at 12-17% while water content was 5-10%.

The feed that contained fermented water hyacinth and corncob was then mixed with dried water spinach (*Ipomoea aquatica*), which was common fodder used locally, to compose four different ration feeds; (R1: 0% fermented water hyacinth (fwh) + 100% basal ration (br), R2: 10% fwh + 90% br, R3: 20% fwh + 80% br, R4: 30% fwh + 70% br).

2.2. Palatability Test of Ration Feeds

Sample animals were made of 4 groups based on ration feed given (R1-4); each group consisted of 5 male sheep weighted ± 25 kg. Palatability was tested using cafeteria method; each group was given ration mixes twice daily; once in the morning and once in the afternoon. Each ration feed was given at 6.7% weight of body weight (±2 kg) during respective feeding session based on preliminary study. Feed consumed by each sheep was determined based on weight difference of remaining feed and initial feed weight. Palatability was evaluated for a day of feeding in all sheep sample. Selection index was calculated as ratio of feed consumed from total weight of feed given. Ration feeds were given continuously for 3 months to respective groups. Accretion of body weight was measured based on the difference on initial body weight of sheep and body weight after given ration feeds for 3 months.

2.3. Evaluation of Spermatozoa Quality

Spermatozoa sample was taken at the end of feeding from each sheep and analysed of its quality based on various parameters, including semen volume, colour and consistency of semen, semen acidity, viability, individual motility, mass motility, and concentration. From each goat, two ejaculate samples were taken by using assistance of artificial vagina. Directly after taken, volume, pH, colour, and consistency were evaluated. Viability was evaluated from semen smear stained with eosin-negrosin and observed under microscope. Percentage of viability was determined based on the number of unstained cells out of 200 spermatozoa observed [24]. Individual motility was evaluated based on following criteria; 90% if cells were highly active and fast with large wave motion, 70-85% if cells were active or fast with large wave and fast mass movement, 40-65% if cells moved slightly active or fast with thin rare wave and slow mass movement, 20-30% if cells were less active or not as quick with sperm moved individually and no visible wave, and 10% if cells moved very little as individual [24].

Mass motility was determined based on following categories; very good (+++) when a number of large active dark wave motion was observed, good (++) when thin small slow wave motion was observed, adequate (+) when no wave motion was observed and only individual movement was found, and necrospermia (0) when only small individual movement was observed [24]. Meanwhile, concentration was evaluated from spermatozoa count after semen was diluted up to 109 in saline medium [24].

2.4. Statistical Analysis

Statistical analysis was performed for all quantitative data collected ($p<0.05$). Selection index was tested using Kruskal-Wallis and post-hoc Mann-Whitney u tests, while body weight increase was tested using Brown-Forsythe test. Spermatozoa viability and motility were tested using Brown-Forsythe and post-hoc Games-Howell tests, while sperm concentration and semen volume were tested using one-way ANOVA and post-hoc Duncan test, pH of semen was examined statistically with one-way ANOVA.

3. RESULTS AND DISCUSSION

3.1. Palatability of Ration Feed

Feed palatability was determined based on selection index. Higher index meant that the ration was preferred by sheeps. Ration feed selection index and increase of body weight after given ration feeds are presented in Table 1. Based on selection index, R3 was the most preferred ration feeds over the other three mixes significantly compared to other ration feeds. However, body weight gain of sheep given different ration feeds were not statistically different.

3.2. Quality of Spermatozoa

Spermatozoa quality was evaluated from ejaculate taken at the end of feeding period (3 months). Various parameters evaluated is presented in Table 2. Based on various parameters observed, the best quality of spermatozoa was found from R4 group. Viability, individual motility, concentration, and volume of R4

Table 1. Selection index and body weight increase of ration feeds

Ration	Selection Index*	Body weight increase(kg)*
R1	0.1023 ± 0.0018^a	5.50 ± 0.45
R2	0.1040 ± 0.0011^a	5.64 ± 0.42
R3	0.4571 ± 0.0037^b	5.96 ± 0.20
R4	0.1028 ± 0.0021^a	5.82 ± 0.19
P-value	0.007	0.245

(R1: 0% fermented water hyacinth (fwh) + 100% basal ration (br), R2: 10% fwh + 90% br, R3: 20% fwh + 80% br, R4: 30% fwh + 70% br)

*) different superscript letters indicate significant difference based on statistical test ($p<0.05$)

Table 2. Evaluation of spermatozoa quality from each ration feeds

Parameters	Group			
	R1	R2	R3	R4
Viability (%)	36.90±1.20^{a}	44.80±1.32^{b}	47.30±1.34^{c}	52.20±3.39^{c}
Motility (%)	33.20±1.32^{a}	36.40±3.75^{ab}	41.70±1.16^{b}	48.10±3.03^{c}
Concentration ($x10^{9}$ cells/ml)	2.40±0.52^{a}	2.70±0.48^{ab}	3.00±0.67^{b}	3.70±0.48^{c}
Volume per ejaculation (ml)	0.55±0.08^{a}	0.66±0.11^{a}	0.88±0.11^{b}	1.05±0.12^{c}
pH	6.84±0.09^{a}	6.83±0.12^{a}	6.85±0.14^{a}	6.86±0.11^{a}
Mass motility	+	++	++	+++
Color	Milky white	Milky white	Light cream	Cream
Viability (%)	36.90±1.20^{a}	44.80±1.32^{b}	47.30±1.34^{c}	52.20±3.39^{c}
Motility (%)	33.20±1.32^{a}	36.40±3.75^{ab}	41.70±1.16^{b}	48.10±3.03^{c}
Concentration ($x10^{9}$ cells/ml)	2.40±0.52^{a}	2.70±0.48^{ab}	3.00±0.67^{b}	3.70±0.48^{c}
Volume per ejaculation (ml)	0.55±0.08^{a}	0.66±0.11^{a}	0.88±0.11^{b}	1.05±0.12^{c}

(R1: 0% fermented water hyacinth (fwh) + 100% basal ration (br), R2: 10% fwh + 90% br, R3: 20% fwh + 80% br, R4: 30% fwh + 70% br)

were higher significantly compared to other groups, however pH, mass motility, and colour only slightly differed from other groups.

Fermented feed is studied extensively to reduce the use of antibiotic growth promoters (AGP) in livestock husbandry. Fermented feed has potential to maintain intestinal health and homeostasis gastrointestinal microflora, suppress growth of pathogenic microbes, and as immunomodulator via diet manipulation [25]. The feed in this research was fermented feed designed for ruminant produced from water hyacinth (*Eichhornia crassipes*) and corncob. Water hyacinth is an invasive species that cause problem in aquatic environment due to its rapid growth and propagation, while corncobs are by product of farming activity. Both can be used as feed for livestock by reducing complex fibre contained within and increasing nutritive content by means of fermentation.

Based on results, it was found that R3 (20% fermented water hyacinth and corncob) was the most preferable among other rationed feeds given to sheep as indicated by highest selection index (0.4571±0.0037) compared to other rations. Palatability is affected by physical and chemical factors, such as material hardness, colour, shape, texture, taste, water content, protein, fat, and odour. Goats rely on smell, vision, and touch to response feed. Olfactory and taste stimulation determine whether feed will be consumed by livestock or not [23]. Goats generally do not prefer one type of feed for a long time, as they can distinguish tastes and have high tolerance for bitterness [23].

In addition to palatability, feed consumption is also affected by protein quality. Fungi and bacteria species used in the starter are known to produce various enzymes able to breakdown complex organic matters. *Rhizopus stolonifer* is known to be isolated from rotten fruit and able to produce various extracellular enzymes to degrade plant organic matter [26]. *Penicillium* was reported to produce cellulolytic and xylanolytic enzymes which are able to hydrolyze lignocellulosic materials of plant cell walls [27], as well as *Trichoderma* species which produced β-glucosidase, cellulase, and xylanase [28]. *Bacillus aerius* was reported to have extracellular endoglucanase activities [12], while *Bacillus cereus* could produce alkaline protease [13] and cellulase enzymes [14]. *Burkholderia* sp. was also found to secrete extracellular protease [29]. These enzymes produced by starter consortium of fungi and bacteria resulting in breakdown of organic matter complexes in water hyacinth and corncob, making feed could be digested easier by sheep. However, there was no significant difference of weight increase of sheep fed various level ration feeds.

Nutrient contained in the feed digested by sheep affect various physiological systems. Level of dietary lipids were found to affect various aspects of male reproductive system. A certain level of fatty acid intake is required to maintain optimal range of gamete composition and male reproductive capacity [30]. In the current study, based on various parameters evaluated, sheep fed R4 produced sperm with the best quality compared to other rationed feeds. R4 contained the highest level of fermented feed among ration mixes used in the study. This indicated that the higher the level of fermented feed added to sheep rations, the higher the level of dietary lipids that could be digested to support reproductive performance of male sheep, thus resulting in higher quality of spermatozoa compared to other ration feeds. Higher quality of spermatozoa is strongly correlated to rate of successful fertilization.

Production of fermented feed from both materials is expected not only to reduce environmental impact, but also to increase feed digestibility and quality for ruminant. The use of fermented feed should also be studied for other kind of ruminants. In addition, various kind of waste organic material can be tried and fermented to produce better feed.

4. CONCLUSION

Ration mix with 20% fermented water hyacinth and corncob (R3) have the highest palatability in male sheep compared to other mixes. Male sheep fed various rations with different level of this feed content showed weight gain, but no significant difference was found among different mixes. Sheep fed 30% fermented water hyacinth and corncob (R4) produced spermatozoa with highest quality.

AUTHORS' CONTRIBUTIONS

Author's Contribution. All authors discussed the research design. Isnawati : drafting the manuscript , data analysis, Ni'matuzahroh : editing the manuscript. Tini Surtiningsih : reviewing the manuscript. Firas Khaeyla : translating and proofreading the manuscript. Roni Afif Hidayat : Layouting and templating the manuscript.

REFERENCES

[1] E.B. Belal, Bioethanol Production from Rice Straw Residue, Biotechnol Biofuels 7 (2013) 139-142.

[2] Z.B. Sarian, "Corn Cobs Converted Into Nutritious Animal Feed" Version: 30 October 2016. http://www.zacsarian.com/category/agri-ideas.'

[3] M. Wanapat, R. Pilajun, S. Kang, K. Setyaningsih, and A.R. Setyawan, Effect of Ground Corn Cob Replacement for Cassava Chip on Fermentation and Urinary Derivatives in Swamp Buffaloes, Asian-Australian Journal of Animal Science 25 (8) (2012) 1124-31. https://pubmed.ncbi.nlm.nih.gov/25049671/

[4] R. Rostika and R. Safitri, Influence of Fish Feed Corn-Cob was Fermented by Trichoderma sp., Aspergillus sp., Rhizopus oligosporus to the Rate of Growth of Java Barb (Puntius gonionitus), APCBEE Procedia. 2 (2012) pp 148-152. https://doi.org/10.1016/j.apcbee.2012.06.027

[5] G. Lardy and V. Anderson, Alternative Feeds for Ruminant, NDSU, 2019. https://www.researchgate.net/publication/325654090_Alternative_Feeds_for_Ruminants

[6] H.T. Tham, Water Hyacinth (Eichhornia crassipes)-Biomass Production, Ensilability and Feeding Value to Growing Cattle, PhD Thesis. Swedish University of Agricultural Sciences. Uppsala. Thailand, 2012. URL: https://www.researchgate.net/publication/288653657_Water_Hyacinth_Eichhornia_crassipes_-_Biomass_Production_Ensilability_and_Feeding_Value_to_Growing_Cattle/citations#fullTextFileContent

[7] A. Kumar, P.C. Sharma, A. Kumar, and V. Neg, A Study on Phenotypic Traits of Candida Species Isolated from Blood Stream Infections and Their In Vitro Susceptibility to Fluconazole, Al Ameen Journal of Medical Science, 7(1) (2011) 83-91. https://www.semanticscholar.org/paper/A-study-on-phenotypic-traits-of-Candida-species-and-Kumar-Kumar/07012f8d5f589a25489968dc9c2a4e72248c46f0

[8] T. Saputro, 'Pakan untukTernak Domba. IlmuTernak.', 2015, Version: 30 October 2016. http://www.ilmuternak.com/2015/03/pakan-untuk-ternak-domba.html.

[9] J. Seo, J.K. Jung, and S. Seo. Evaluation of Nutritional and Economic Feed Values of Spent Coffee Grounds and Artemisia Princeps Residues as a Ruminant Feed Using in-Vitro Ruminal Fermentation, Peer Journal 3 (2015) 1343. https://pubmed.ncbi.nlm.nih.gov/26528409/

[10] I.Z. Boboescu, M. Ilie, V.D. Gherman, I. Mirel, B. Pap, A. Negra, E. Kondorosi, T. Biro, and G. Maroti, Revealing the Factors Influencing A Fermentative Biohydrogen Production Process Using Industrial Wastewater as Fermentation Substrate, Biotechnology for Biofuels 7 (2014) 139- 154. https://doi.org/10.1186/s13068-014-0139-1

[11] M.A. Belewu and F.T. Babalola, Nutrient Enrichment of Waste Agricultural Residues after Solid State Fermentation using Rhizopus oligosporus, Biotechnol Biofuels 13 (2009) 695-699. https://www.cabdirect.org/cabdirect/abstract/20093214461

[12] M. Oke, M. Annuar, and K. Simarani K, Enhanced Endoglucanase Production by Bacillus aerius on Mixed Lignocellulosic Substrates, Bio Resources

11(3) (2016) 5854-69. DOI: https://doi.org/10.15376/biores.11.3.5854-5869

[13] S. Sundarajan, C. Kannan, and S. Chittibabu S, Alkaline Protease from Bacillus cereus VITSN04: Potential Application as A Dehairing Agent, Journal of Bioscience and Bioengineering 111(2) (2011) 128-133. doi: https://doi.org/10.1016/j.jbiosc.2010.09.009

[14] A.M.B. Croos, S. Rajendran , and K. Ranganathan, Isolation of A Cellulase Producing Bacillus cereus from Cow Dung and Determination of The Kinetic Properties of The Crude Enzyme, Journal of the National Science Foundation of Sri Lanka 47(2) (2019) 261-267. DOI: http://doi.org/10.4038/jnsfsr.v47i2.9168

[15] A.M. Missotten, J. Michiels, J. Degroote, and S. De Sme, Fermented Liquid Feed for Pigs: An Ancient Technique for The Future, Journal of Animal Science and Biotechnology 6 (2015) 4-13. https://doi.org/10.1186/2049-1891-6-4

[16] R.M. Engberg, M. Hammersh, N.F. Johansen, M.S. Abousekken, S. Steenfeldt, and B.B Jensen, Fermented Feed for Laying Hens: Effects on Egg Production, Egg Quality, Plumage Condition and Composition and Activity of The Intestinal Microflora, Journal British Poultry Science. 50 (2) (2009) 228-239 https://doi.org/10.1080/00071660902736722

[17] H. Fitrihidajati, E. Ratnasari, Isnawati, and G. Soeparno G, Kualitas Hasil Fermentasi Pada Pembuatan PakanTernak Ruminansia Berbahan Baku Eceng Gondok (Eichhornia crassipes), Journal of Biosaintifika. 7 (1) (2015) 62-67 https://doi.org/10.15294/biosaintifika.v7i1.3540

[18] J.K. Sadique, A. Pandey, S.O. Khairnar, and N. Kumar N, Effect of Molasses-Fermented Water Hyacinth Feed on Growth and Body Composition of Common carp, Cyprinus carpio, Journal of Entomology and Zoology Studies. 6 (4) (2018) 1161-65 https://www.entomoljournal.com/archives/?year=2018&vol=6&issue=4&ArticleId=3993

[19] I. Arswandi, S. Yunilas, S. Umar, A.H. Daulay, and M. Tafsin, Utilization of Fermented Corncob Flour with "MOIYL" Probiotics on Local Rabbit Performance, J. Peternakan Integratif. 7 (1) (2019) 1888-94.

[20] K. Ishii, M. Fukui, and S. Takii, Microbial Succession during A Composting Process as Evaluated by Denaturing Gradient Gel Electrophoresis Analysis, Journal of Applied Microbiology 89 (2000) 768-777 https://doi.org/10.1046/j.1365-2672.2000.01177.x

[21] H. Yu, G. Zeng, H. Huang, X. Xi, R. Wang, D. Huang, G. Huang, and J. Li, Microbial Community Succession and Lignocellulose Degradation during Agricultural Waste Composting, Biodegradation. 18 (2007) 793-802. https://doi.org/10.1007/s10532-007-9108-8

[22] A. Bhatia, A. Rajpal, S. Madan, and A.A. Kazmi, Techniques to Analyze Diversity during Composting—A Mini Review, Indian Journal of Biotechnology 14 (2015) 19-25 https://www.semanticscholar.org/paper/Techniques-to-analyze-microbial-diversity-during-Bhatia-Rajpal/41e2fadaba67cd18b87e5869483345f2fe29f2c6

[23] G.C. Aldrich and K. Koppel, Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations, Animals, 5 (2015) 43-55 https://doi.org/10.3390/ani5010043

[24] T. Susilawati T, Pedoman Inseminasi Buatan pada Ternak, Universitas Brawijaya (UB) Press), 2013.

[25] J. Wang, G. Liu, and A. Merkoci, Electrochemical Coding Technology for Simultaneous Detection of Multiple DNA Targets, Journal of American Chemistry Society 125 (11) (2011) 3214–15 https://doi.org/10.1021/ja029668z

[26] S. Parveen, A.H. Wani, M.Y. Bhat, J.A.K. Koka, and M.A. Fazili, Variability in Production of Extracellular Enzymes by Different Fungi Isolated from Rotten Pear, Peach and Grape Fruits, Brazilian Journal of Biological Sciences 4(8) (2017) 259-264 http://dx.doi.org/10.21472/bjbs.040804

[27] K.B.R. Krogh, A. Mørkeberg, H. Jørgensen, J.C. Frisvad, and L. Olsson, Screening Genus Penicillium for Producers of Cellulolytic and Xylanolytic Enzymes. In: Finkelstein M., McMillan J.D., Davison B.H., Evans B. (eds) Proceedings. The Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals Held, Breckenridge, May 4–7, 2003 https://doi.org/10.1385/ABAB:114:1-3:389

[28] M.A. Horta, J.A.F. Filho, N.F. Murad, E.O. Santos, C.A. Santos, J.S. Mendes, M.M. Brandao, S.F. Azzoni, and A.P. Souza, Network of Proteins, Enzymes and Genes Linked to Biomass Degradation Shared by Trichoderma Species. Scientific Reports. 8 (1) (2018) 1-11. https://doi.org/10.1038/s41598-018-19671-w

[29] L. Vial, M. Groleau, V. Dekimpe, and E. Deziel, Burkholderia Diversity and Versatility: An

Inventory of the Extracellular Products, Journal of Microbiology Biotechnology 17(9) (2007) 1407-29 https://pubmed.ncbi.nlm.nih.gov/18062218/

[30] F. Saez, and J.R. Drevet, Dietary Cholesterol and Lipid Overload: Impact on Male Fertility. Oxidative Medicine and Cellular Longevity, 2019, article ID 4521786. https://doi.org/10.1155/2019/4521786