Grant Code: New

Title: Wheat straw treated with lignocellulolytic enzymes: a new marketable coproduct

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Justification/Rationale: Wheat straw is the crop residue after harvesting wheat grain, which has been widely used to feed ruminants. However, wheat straw contains 8% lignin, leading to low fiber degradation and overall digestibility. Therefore, some strategies have been developed to increase the digestibility of this residue that allows its better utilization in animal nutrition. Thus far, in the United States, ammoniation is the most common strategy to treat and improve the digestibility of wheat straw. However, the ammoniation can lead to animal toxicity as well as high risk for the workers because ammonia can burn skin, eyes, and throat and lead to an explosion. Therefore, finding new strategies that can bring the same benefits, compared to ammoniation, while reducing the risks, must be considered. In addition, treating wheat straw can improve digestibility and develop a new marketable coproduct for wheat producers.

Hypothesis & Objectives: This project aims to develop a new marketable coproduct for wheat production settings. We hypothesize that enzymatically treated wheat straw will have a greater digestibility for cattle (and other ruminants such as sheep and goats) and consequently will allow better commercialization of wheat straw as a coproduct with greater nutritional value. We also hypothesize that the enzymatically treated wheat straw could potentially replace conventional forage in the diets, such as whole-plant corn silage, as well as to replace the ammoniation that can be hazardous to workers and animals.

Procedures/Plan of Work: The research project will include two experiments.

- Experiment I: We will evaluate two different sources of lignocellulolytic enzymes (enzymes synthesized at our laboratory and enzymes from the residue of mushroom production, a rich source of enzymes – spent mushroom substrate) in the same doses on the wheat straw. The wheat straw from grain production will be harvested from five locations, chopped, mixed with water (to assist enzymes activity and fermentation), and molasses (to provide sugars for the fermentation), and then homogenized with the enzymes. Six levels of enzymes synthesized in the lab or from the spent substrate will be tested at the packing, and they will be expressed in mg of lignocellulolytic enzymes per kg of fresh matter as follows: 0, 15, 30, 45, 60, and 75 mg/kg of fresh matter. Also, positive control, using anhydrous ammonia, will be included in the treatments. Each experimental unit will be treated individually with the respective levels, placed into nylon-polyethylene vacuum bags, and heat-sealed using a vacuum machine. The experiment will consist of a randomized complete block design, where each enzyme level from each enzymatic source will be used with five replicates (one from each field location), grouped by the location where straw was obtained. Bags will be opened 30 d after packing and weighed to determine the loss of dry matter (DM) and nutrients. Samples of fermented material will be collected to determine the fermentative profile (pH, lactic and short chain fatty acids, ammonia N), chemical composition, and *in situ* degradability. The *in situ* degradation will be performed using three cannulated dairy cows for 36 h to determine the DM and NDF disappearance rate and for 120 h to determine undigested NDF. The DM, ash, nitrogen, neutral detergent fiber, acid detergent fiber, and lignin will be determined in the material.

- Experiment II: The enzymatic level to treat the wheat straw in the animal's trial will be 20 mg of lignocellulolytic enzymes/kg of fresh matter, based on our previous studies (Agustinho et al., 2021a; Machado et al., 2020). We will treat wheat straw to feed dairy cows where the conventional forage will be partially replaced by treated wheat straw. Feed intake, digestibility, performance (milk yield and composition), and economical aspects will be evaluated. The wheat straw from grain production will be harvested, chopped, mixed with water and molasses, and then homogenized with inert material (negative control treatment) or ammonia anhydrous (ammoniation treatment) or 20 mg of lignocellulolytic enzymes/kg of fresh matter from spent substrate or enzyme produced at the lab. The experiment will be conducted at the Dairy Center at the University of Idaho. Seventy Holstein dairy cows will be distributed in a randomized block design. In the positive control, 100% of the fermented feedstuffs will come from whole-plant corn silage (WPCS), whereas, in all other diets, 50% of the fermented feedstuffs will consist of WPCS and 50% of fermented wheat straw. Samples of the diets, orts, and feces will be collected to determine intake and digestibility. Milk yield will be recorded daily, and milk samples will be collected to determine the concentration of milk composition. Spot urine samples will be collected to estimate the efficiency of microbial protein synthesis. Rumen fluid will be collected through an esophageal tube coupled to a vacuum pump to determine volatile fatty acids and ammonium nitrogen.

- Statistical analysis: Treatment will be the fixed effect and plot (field of wheat) the random effect in experiment I. In experiment II, animals and periods will also be included as the random effects. Differences will be declared significant at $P < 0.05$ and tendencies if $0.05 < P \leq 0.10$.

***Duration*:** Two years. In the first year, we will produce the enzymes and conduct the experiment I to evaluate the treated wheat straw packed in nylon-polyethylene vacuum bags. In the second year, we will treat wheat straw to conduct the experiment II (feeding dairy cows). **It is important to clarify that although the project consists of two years, we are just requesting funding for one year (Experiment I) at this time.**

Anticipated Benefits, Expected Outcomes and Impacts, and Transfer of Information: New treatment method of wheat straw will allow greater commercialization of it and lead to the development of a new marketable product. We expect that animals fed with treated wheat straw will have a similar intake, apparent total tract digestibility, and performance as those fed with the conventional forage, as well as a lower methane emission. Overall, feeding treated wheat straw will lead into a better margin for producers. We intend to transfer the information from this project as three or four abstracts, two journal articles, and several educational materials to producers via Extension and outreach efforts.

***Literature Review*:**

Wheat straw is the crop residue after harvesting wheat grain, and it has been used to feed ruminants as a source of fiber over the years. Wheat is one of the most cultivated crops in Idaho, leading to the high production of wheat straw. However, the profitability of the commercialization of wheat straw is still limited. Therefore, finding ways to aggregate more value to wheat straw can be the key to transforming it into a new marketable coproduct and improving the profitability of producers.

Wheat straw has around 8% lignin (Tuyen et al., 2012), leading to a low fiber digestibility. However, some strategies can enhance digestibility, such as ammoniation, enzymes, or sodium hydroxide treatment (Wang et al., 2004; Rodrigues et al., 2008; Tuyen et al., 2012). Until the present moment in the US, ammoniation has been the most common strategy used for this purpose. The ammoniation process consists of applying anhydrous ammonia to the material. However, ammoniation can lead to toxic effects on animals (Müller et al., 1998), and risk for the workers because ammonia can burn skin, eyes, and throat, as well as lead to an explosion. Therefore, finding new methods that can bring the same benefits, compared to ammoniation, while reducing risks must be considered.

One way to improve straw digestibility that is not commonly used in the US, is using a mushroom that produces enzymes. Previous studies using a beneficial fungus (e.g., *Pleurotus ostreatus*) to treat grain straw, such as wheat and rice straw, have shown improved fiber digestibility (Fazaeli et al., 2004) and allowing replacement of conventional ingredients with treated straws (Khattab et al., 2013; Kholif et al., 2014). However, using white-rot mushrooms to directly ferment the straw might risk losing carbohydrates (Adesogan et al., 2019). Therefore, using the enzymes from fungus without allowing them to grow would improve wheat straw digestibility and avoid nutrient losses.

Based on our research, enzymes produced from beneficial fungus (*Pleurotus ostreatus*) reduce lignin concentration in whole-plant corn silage (Machado et al., 2020; Agustinho et al., 2021a; b) and increase digestibility (Machado et al., 2020; Agustinho et al., 2021b). Therefore, we hypothesize that wheat straw treated with enzymes can be an effective way to improve digestibility and develop a new marketable coproduct for wheat producers.

References

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FY2023

COMMODITY COMMISSION BUDGET

Principal Investigator: *(Pedram Rezamand)*

Allocated by	____________ *(Commission/Organization)*	during FY2021	$ -
Allocated by	____________ *(Commission/Organization)*	during FY2022	$ -

REQUESTED SUPPORT: **Budget Categories**		*Awarded for FY2022*	*Requested for FY2023*
(10) Salary *(staff, post-docs, etc.)*	*NOTE: Faculty salary/fringe not allowed*	$ -	$ -
(12) Temporary Help		$ -	$ 4,000
(11) Fringe Benefits		$ -	$ 120
(20) Travel		$ -	$ 2,500
(30) Other Expenses		$ -	$ 73,500
(40) Capital Outlay >$5k		$ -	$ -
(45) Capital Outlay <$5k		$ -	$ -
(70) Graduate Student Tuition/Fees		$ -	$ -
TOTALS		$ -	$ 80,120

TOTAL BUDGET REQUESTED FOR FY2023:	$ 80,120

BREAKDOWN FOR MULTIPLE INDEXES: **Budget Categories**	*(Insert PI Name)*	*(Insert Co-PI Name)*	*(Insert Co-PI Name)*	*(Insert Co-PI Name)*
(10) Salary *(staff, post-docs, etc.)*	$ -	$ -	$ -	$ -
(12) Temporary Help	$ -	$ -	$ -	$ -
(11) Fringe Benefits	$ -	$ -	$ -	$ -
(20) Travel	$ -	$ -	$ -	$ -
(30) Other Expenses	$ -	$ -	$ -	$ -
(40) Capital Outlay >$5k	$ -	$ -	$ -	$ -
(45) Capital Outlay <$5k	$ -	$ -	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ -	$ -	$ -	$ -
			Total Sub-budgets	$ -