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POLYPHENOL OXIDASE CONTAINING EXTRACT: SOURCES AND CONTRIBUTIONS IN ANIMAL NUTRITION

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ABSTRACT: Polyphenol oxidase (PPOs) which are found naturally from plants, fruits, and vegetables and agricultural by products are getting considerable attention in food processing industries and in animal nutrition due to their antioxidant activity. Even though synthetic antioxidants have been used widely; they become a major cause for nutritional losses and quality deterioration which subsequently results in health problem in humans. To substitute synthetic antioxidants with natural Polyphenol oxidase (PPOs) containing extracts, many researches are being conducted on characterization of PPO sources and on protection of polyunsaturated oils and protein against ruminal degradation using PPO. Among many sources of PPO, Apple *(malus sylevestris var domestica)*, Banana *(Musa cavendishii)* Mango (*mangifera indicacv.manila*) and Red clover *(Trifolium pretense L.),* Tomato stems *(Solanum lycopersicum L.)*, Potato (*Solanum tuberosum L.*) are mentioned by different researchers from fruit sources and other plant sources, respectively. The contribution of PPO for protein and lipid protection is also being investigated. The main aim of this paper is to document the existing information on different sources and contribution of PPO as a baseline for further investigation. Further laboratory investigation on the role of polyphenol oxidase is crucial to improve the productivity of livestock with minimum feed cost.

Keywords: Polyphenol oxidase, Rumen, Protein degradation, Lipid degradation

INTRODUCTION

Polyphenol oxidase (PPOs) are a group of metalloenzymes which includes: catecholase (EC 1.10.3.2), laccase (EC 1.10.3.1), and cresolase (EC 1.14.18.1) that catalyses' the oxidation of monophenols and o-diphenols to highly reactive o-quinones , which intern interact with proteins and oxygen to form reactive oxygen species (ROS) and typically brown-pigmented complexes (Boeckx et al., 2015; Getachew et al., 2009). It is known for the characteristic post-harvest browning of cut or bruised fruit or damaged plat tissue due to polymerization of PPO derived o-quinones with nucleophilic compounds (Araji et al., 2014). According to the research conducted on the factors affecting post cut browning of eggplant *(Solanum melongena)* in India the extent of browning depends primarily on specific activity of PPO and total soluble phenolics (Mishra et al, 2013). Interest on PPO containing crops and agricultural side stream wastes is growing very rapidly due to the association of browning reaction with reduced protein and oil loss in the silo and rumen (Lee, 2014a) and their role for production of safe human diet, due to their antioxidant properties (Balasundram et al., 2006). There are many sources of PPO and are reported to be available in many organisms including, plants, fungi, animals and bacteria (Bottino et al., 2009).

Potato tuber peels *(Solanum tuberosum L.)* and other related Industrial sidestreams sources of plants such as Apple *(malus sylevestris var domestica),* Pineapple Peels *(Ananas comosus L.) and* Tomato stems and leaves *(Solanum lycopersicum L.)* are among important unutilized sources of PPO (Gadeyne et al., 2016; Zhang, et al., 2009). According to (Hajaji et al., 2011), from the tree Carob, Barks contains the highest amount of polyphenol compounds. It was also indicated that the amount of PPOs were significantly affected by the verities of trees. PPO helps to reduce the extent of lipolysis and proteolysis within red clover feed to ruminates with increase in efficiency of nitrogen utilization and the level of beneficial polyunsaturated fatty acids in meat and milk (Michael and Tweed, 2008; Merry et al., 2006). It was also found that PPO is very important to reduces lipolysis in the silo and in the rumen, when the forage is damaged, it will result in activation of PPO which result in binding of phenols to protein (Lee et al., 2009). This review aims to document different sources of PPO and their contribution in animal nutrition as a baseline for further investigation.

Sources of Polyphenol oxidase (PPOs)

Differed researchers identified many sources of Phenolic compounds. Among major sources of phenolic compounds in human diets, vegetables, fruits and beverages are found. It is also indicated that food and agricultural products processing industries produces considerable amount of phenolics-rich by- products, which could be valuable natural sources of antioxidants (Balasundram et al., 2006). Despite there is a variation in PPO activity, isoforms and concentration, there are several plant sources of protein which are used for emulsification purpose but, the origin and concentration of PPO extract highly affects the degree of ruminal protection (Gadeyne et al., 2016).

According to Lee, (2014) among the screened forage grasses and legumes which are used for ruminant livestock feed only two, red clover (*Trifolium pratense*) and cocksfoot (*Dactylis glomerata*), have been found to have both a high PPO activity and substrate concentration. Camelina extracts especially camelina cake has also an important antioxidant properties which could be used as an easily accessible and cheap source of natural polyphenols, which could be eye-catching to the food and pharmaceutical industry (Terpinc et al., 2012). Some of sources of PPO which are characterized by different researchers are presented in (Table 1). Methods of characterization and the result found from each sources of PPO could be found from the corresponding references.

Table 1 - Sources of Polyphenol oxidase (PPO)

Sources of PPO	References
Apple *(malus sylevestris var domestica)*	(Holderbaum et al.,2010; Gadeyne et al., 2016; Fievez et al., 2016)
Anamur banana *(Musa cavendishii)*	(Unal, 2007)
Eggplant *(Solanum melongena)*	(Mishra et al., 2013; Gadeyne et al., 2016; Fievez et al., 2016)
Persimmon *(Diospyros kaki L)*	(Navarro et al., 2014)
Pineapple Peels *(Ananas comosus L.)*	(Gadeyne et al., 2016; Fievez et al., 2016)
Mango *(mangifera indicacv.manila)*	(Palma-Orozco et al., 2014)
Red clover *(Trifolium pretense L.)*	(Gadeyne et al., 2015; Gadeyne et al., 2016; Lee, et al., 2009;Yoruk et al., 2016; Webb et al, 2013; Fievez et al., 2016)
Tomato stems and leaves *(Solanum lycopersicum L.)*	(Thipyapong et al., 2004; Gadeyne et al., 2016)
Potato tuber peels *(Solanum tuberosum L.)*	(Ali et al., 2016; Ngadze, et al., 2012; Fievez et al., 2016)
Camelina sativa	(Terpinc et al, 2012)
Names in brackets are scientific names	

CONTRIBUTION OF POLYPHENOL OXIDASE (PPOS) IN ANIMAL NUTRITION

Role polyphenol oxidase (PPOs) for ruminal protection of polyunsaturated oils

Rumen microbes are important to utilize structural carbohydrates and non-protein nitrogen in ruminant nutrition. But they also lead to bio hydrogenation of oils such as polyunsaturated fatty acid (PUFA) and proteins which subsequently results in loss of nutrients for absorption which intern affects the health of animals leads to poor quality animal product (Fievez et al., 2016). Administration of PUFA oils in the diet of animal is stipulated to increase reproductive performance of animal and to increase healthier PUFA rich meat and milk (Gulliver et al., 2012).

Due to the aforementioned consequences polyunsaturated fatty acid (PUFA) degradation in the rumen several technologies have been created protect biohydrogenation of PUFA in the rumen, (Ashes et al, 1984; Sacakli & Tuncer, 2006; Gadeyne et al., 2015; Fievez et al., 2016). According to Lee et al., (2009) positive nutritional effect of red clover was observed with wilted and ensiled clover due to activation of the enzyme PPO during wilting followed by ensiling. In ruminant ration, polyunsaturated fatty acid (PUFA) which are important component of the diet are highly subjected to degradation by rumen microorganisms, which results in the loss of healthy value of PUFA (Gadeyne et al., 2015). Recently, a natural technology has been developed at Ghent University, Bioscience engineering laboratory to create rumen by-pass supplements. This technology relies on a process which is naturally occurring at ambient temperature and makes use of natural resources (Gadeyne et al., 2015). Formation of protein phenol matric is possible through an enzyme PPO extracted from a specific plants and addition of diphenol sources, these emulsion will protect the degradation of lipophilic nutrients by microbes in the rumen which leads to the transfer of non-degraded nutrients to the small intestine (Fievez et al., 2016). According to Gadeyne et al., (2015) ruminal BH is reduced when oils rich in PUFA are first emulsified in PPO extract of red clover with high PPO activity and presence of a diphenolic substrate is important to induce protection. The author suggests that the reason might be due to formation of denser cross linked protein layer in the interference and subsequent improvement in protected emulsions.

Role of polyphenol oxidase (PPOs) for ruminal protein protection

One of the major challenges in ruminant nutrition is improving the efficiency of nitrogen utilization (Dove and Milne, 1994 ; Hart et al., 2016). From the total ingested protein, up to 70 % of nitrogen is excreted which results in loss of

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productivity potential and sever land and water pollution (Hart et al., 2016). It is known that supplying extra quickly rumen-degradable protein (RDP) will not support rumen microbial metabolism and will result in inefficient use of crude protein (CP) by the rumen microflora and, subsequently, the animal rather it will result in formation of urea which in turn promotes the losses of N as urine (Lee, 2014a). For optimal performance in high producing animals there should be enough supply of rumen degradable protein to support microbial growth and bypass protein for normal photolytic digestion and absorption process in small intestine (Dove and Milne, 1994). Therefore, Increasing the rumen undegradable protein content of animal feed is very important to minimize the loss of nitrogen and consequently to improve nitrogen use efficiency and to avoid environmental pollution (Hart et al., 2016).

By-pass protein can be created through technological interventions like treatment with heat, Formaldehyde, acetic acid, tannic acid, lignosulfonate or xylose. The two most commonly used technologies are based on formaldehyde and xylose-heat treatment (Maillard reaction). However, the use of chemicals like formaldehyde is considered unhealthy and there is limited access to purchase. Heat-based treatments show disadvantages as they are energy demanding. Therefore, using PPOs for protecting ruminal protein degradation as a means of increasing undegradable protein (UDP) in the rumen has tremendous role to increase N-use efficiency (NUE) (Lee, 2014b).

The way for PPOs protection of plant protein in the rumen is not due to protease deactivation, rather it is the result of complexing protein. The complexed proteins which are the result of PPOs in the rumen minimizes protein degradability in the rumen which in turn increases undegraded dietary protein flow to the small intestine. However PPOs protection of plant protein and glycerol based PUFA due to deactivation of plant protease and lipase, rather than complexting protein (Kroll and Rawel, 2001; Lee, 2014).

If N intake is balanced in animal diet, PPOs protection of dietary protein could result in increase in N-use efficiency (NUE), but high amount of unbalanced dietary N with high amount of fermentable energy (FME) which could be supplied to maximize microbial protein production in the rumen will result in a loss of N and lower NUE (Lee, 2014b). It is also indicated that strategies of exploiting PPO mediated protein complexing could be very important to prevent the loss of dietary protein and to improve animal health through delivery of essential nutrients (Hart et al., 2016). According to the study conducted on red clover polyphenol oxidase: Activation, activity and efficacy under grazing, when the red clover tissues are damaged to a higher degree, rapid activation of latent PPO occurs with subsequent binding of phenols to protein. It is also indicated that wilting of red clover may assist the positive effect of red clover on plycerol based lipids and protiens in fresh forage and it would enhance the activation of *ortho*-quinone production and formation of protein–phenol complexes (Lee et al., 2009).

CONCLUSION AND RECOMMENDATION

From the document it could be concluded that proper utilization of Polyphenol Oxidase (PPOs) in the area of food processing industries and animal nutrition is vital for safe food production. It could be also drawn that PPO are vital enzymes to improve the productive performance of animals through their role in creation of rumen bypass supplements. To utilize PPO effectively and efficiently, further study on endogenous phenolic substrates available in various sources of the enzyme PPO and amount of diphenol to be added for each sources of enzyme is recommended.

DECLARATIONS

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Authors' contribution
All authors contributed equally to this work.

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Conflict of interest
The author declare no conflict of interest for the contents in the manuscript

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