IMPROVING THE NUTRITIVE VALUE OF LUPIN USING A COMBINATION OF PECTINASE AND XYLANASE

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Summary

The synergistic effect of two non-starch polysaccharide-degrading enzymes was tested *in vitro* with the aim of breaking down the cell wall content to improve the nutritive value of lupin for poultry. Lupin kernels were incubated without (control) and with enzymes (pectinase, xylanase or a combination of pectinase and xylanase) for 1 hour at 38ºC. The combination of pectinase and xylanase greatly reduced water-holding capacity, viscosity and cell wall content, compared to pectinase or xylanase alone. In addition, the pectin content, chain length of pectin and galacturonic acid concentrations were reduced by the combination of pectinase and xylanase more than the individual enzymes. It was concluded that pectinase and xylanase act synergistically to break down the non-starch polysaccharides in the lupin kernel, and thus may improve the nutritive value of lupins for poultry.

I. INTRODUCTION

Australia is the world leading producer and exporter of lupin (White et al., 2007). The Australian lupin varieties have very low alkaloid content of 0.01% and sweet lupins are currently used as a source of protein and energy in a range of monogastric diets in poultry, pigs, fish and rabbits (Inborr, 1990; Glencross et al., 2004). Sweet lupins (*Lupinus angustifolius*) are locally available and relatively inexpensive so are a potential alternative to high-priced feedstuffs such as soybean meal. Lupins have high content of protein (34%) and energy (18 MJ/kg) so can be used as the main source of protein and energy in poultry diets (Petterson and Mackintosh, 1994). However, the use of lupins in poultry diets is still limited to 5% by feed manufacturers and poultry producers because they contain high amounts of non-starch polysaccharides (NSPs, 35%) that cannot be digested by monogastrics due to the absence of endogenous enzymes that can break them down into simple sugars. The main problematic NSPs are pectins, galactans, rhamno-galacturonans, arabinans and xylans, (Cheetham et al., 1993; Choct, 2006), with structures shown in Figure 1. These NSPs increase the viscosity of digesta in the intestinal tract and inhibit the digestion of nutrients. This results in poor growth and low feed conversion efficiency (Kocher et al., 2000; Steenfeldt et al., 2003).

Supplementing legume grains with exogenous enzymes can break down the NSPs (Perez-Maldonado et al., 1999; Jia et al., 2008), releasing cell contents and overcoming the negative effects (Ali et al., 2009). Since lupins contain xylans that are attached to the main pectin chain, it seems likely that some enzymes will work synergistically (Ravindran et al., 1999; Wu et al., 2004) to break down pectins and xylans. For example, when pectinase breaks down the main chain of pectin, it gives xylanase access to the xylan side branches attached to main pectin backbone.

In the present research we tested whether the combination of pectinase and xylanase will break down the NSP in lupin kernel more effectively than pectinase or xylanase alone.

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This hypothesis was tested *in vitro* by incubating lupin kernel with enzymes and measuring the degradation of pectins and xylans.

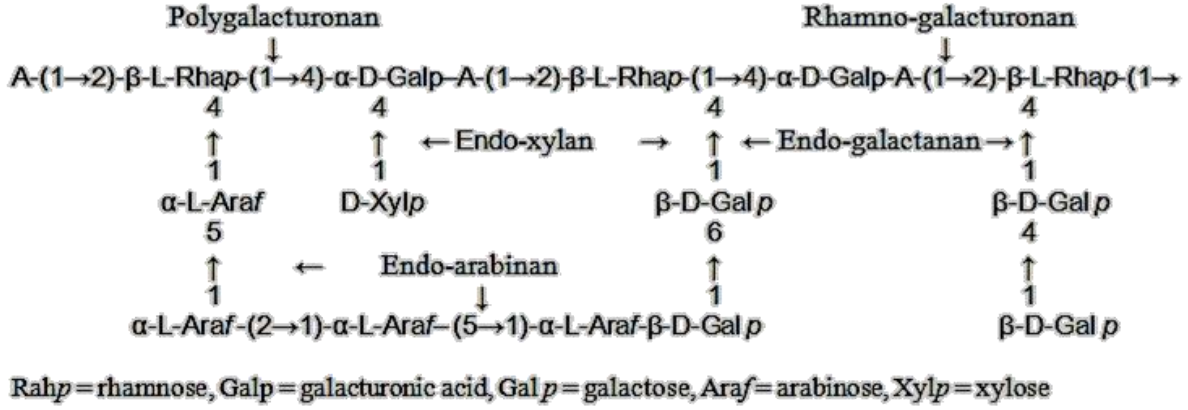

Figure 1 - Complex structure of pectin.

II. MATERIALS AND METHODS

Lupin kernels were incubated with three NSP degrading enzymes; pectinase (1.4 U/g polygalacturnose & 0.2 U pectinestrase, Novozymes Australia), xylanase (0.38 U/g, Rovabio) and a combination of mentioned pectinase and xylanase with the same doses.

Twelve lupin samples per treatment were dissolved in 25 ml deionized water and incubated without enzymes (control) and with enzymes in an incubator-shaker (150 rpm) for 1 hour at 38ºC. The samples were centrifuged at 15,000g for 15 min at 20ºC. The residues were then freeze-dried and water-holding capacity (WHC) was measured as gram of water per gram of organic matter (g:g). The filtration rate was calculated by measuring the volume of supernatant filtered through filter paper (8 μg, no. 41, Whatman) divided by the filtration time (μl/sec). Following this, the viscosity was measured using a Viscotester (HAAKE, PK 100, VT 550) by placing 0.50 ml supernatant on a cone plate PK5 at a shear rate of 3000/sec and speed rate of 500/min at a temperature of 23°C.

A half gram of each sample was placed in a filter bag in duplicate and digested in an ANKOM200 fibre analyzer (ANKOM Corporation Technology Fairport, NY). The xylan content was measured using the fibre analysis method (Van Soest et al., 1991). The galacturonic acid (GA) content was measured by spectrophotometer according to the method described by (El-Rayah and Labavitch, 1977).

III. RESULTS

The combination of pectinase and xylanase reduced viscosity by 21% ($P < 0.05$), WHC by 4% and the filtration rate by 50% (Table 1) as compared to control. This breakdown was manifested by reduction in cell wall (13%) and pectin (35%), but xylan breakdown was not statistically significant. In addition, the combination reduced the pectin chain length by 51% and galacturonic acid content by 40%. When pectinase and xylanase were used alone, the changes in physico-chemical properties of lupin kernel were less than that achieved by combination of two enzymes.

IV. DISCUSSION

The combination of pectinase and xylanase reduced the physico-chemical properties of lupin kernel more than the individual enzymes, more effectively reducing the content of galacturonic acid and cell walls. The superior effect of the enzyme combination supports the hypothesis of a synergistic interaction between pectinase and xylanase, since xylan is attached to the main chain of pectin.

Table 1 - Effect of enzymes on physical-chemical properties of lupin kernel *in vitro* (mean ± sem)

Parameters	Control	Pectinase	Xylanase	Combination
WHC (g:g)	$3.55^{a} \pm 0.01$	$3.50^{a} \pm 0.02$	$3.51^{a} \pm 0.09$	$3.42^{b} \pm 0.03$
Viscosity (mPas/sec)	$1.46^{a} \pm 0.01$	$1.35^{b} \pm 0.01$	$1.25^{c} \pm 0.01$	$1.15^{d} \pm 0.01$
Filtration rate (µl/sec)	$31.2^{a} \pm 1.5$	$26.7^{b} \pm 1.8$	$20.6^{c} \pm 1.2$	$15.4^{d} \pm 0.9$
Pectin (%)	$10.8^{a} \pm 0.3$	$9.02^{b} \pm 0.2$	$9.25^{b} \pm 0.1$	$6.92^{c} \pm 0.1$
Xylan (%)	$6.17^{a} \pm 0.1$	$6.09^{a} \pm 0.1$	$5.97^{a} \pm 0.1$	$6.01^{a} \pm 0.1$
Cell wall content (%)	$23.3^{a} \pm 0.2$	$22.1^{b} \pm 0.2$	$21.8^{b} \pm 0.2$	$20.4^{c} \pm 0.2$
Chain length of pectin (%)	$76.5^{a} \pm 3.5$	$58.9^{b} \pm 1.2$	$60.2^{b} \pm 2.9$	$37.3^{c} \pm 1.4$
GA concentration (µg/g)	$32.8^{a} \pm 1.4$	$26.9^{b} \pm 0.4$	$29.4^{ab} \pm 1.5$	$19.5^{c} \pm 0.7$

[abc] Means within rows with different superscripts differ (P<0.05).

These reductions in content of NSPs should improve nutritive value and digestibility of lupin kernel for monogastrics. For example, supplementing lupins with multi-enzyme preparation significantly improved feed consumption by 5% and chicken growth by 10% (Brenes et al., 1993; Ali et al., 2009; Olkowski et al., 2010). Similarly, using NSP-degrading enzymes such as xylanase or cellulase in lupin-based diets improves the production performance of chickens (Naveed et al., 1999).

In conclusion, the combination of pectinase and xylanase is more effective than the individual enzymes *in vitro*, so we will now test whether this outcome can be reproduced *in vivo*, testing for potential benefits in feed conversion efficiency and growth rate in quail. The synergistic interaction between the two enzymes could make inroads towards greater inclusion of up to 20% lupin kernel into diets for all poultry species without compromising production performance or increasing wet droppings.

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