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The influence of soluble and insoluble lupin non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

This study examined the effect of increasing inclusion levels of soluble and insoluble lupin (*Lupinus angustifolius*) fibres, and purified cellulose on the dry matter, protein and energy digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*). Soluble and insoluble fibre fractions from lupin kernel meal were produced using differential pH solubilities. There were significant differences among the digestibility parameters of the diets with different inclusion levels of each of the different fibre types, except for the soluble lupin fibre, which had limited effect on any digestibility parameters. Differences among diets in dry matter and energy digestibility were most distinct. Using an ANOVA analysis no significant differences were noted for diet protein digestibilities with any of the fibre types. However, regression analysis of the effect of fibre inclusion levels showed significant effects on all digestibility parameters, including protein digestibilities. The lupin insoluble NSP also had a greater effect on dry matter and energy digestibilities than that of cellulose, with findings suggesting that it also affected the digestibility of additional nutrients in the diet to a degree not seen with cellulose. These results show that different fibre classes can have distinctly different effects on diet digestibility parameters.

1. Introduction

There is now considerable use of grains in carnivorous fish feeds throughout the world. Key grains to be used at significant inclusion levels include wheat, soybean, lupins and rapeseed (Aslaksen et al., 2007; Gatlin et al., 2007; Glencross et al., 2007). Each of the different grains has advantages and disadvantages with their inclusion. The presence of anti-nutritional factors is one key limitation experienced with many plant protein options (Francis et al., 2001). The inclusion of a non-nutritive value, in the form of non-starch polysaccharides (NSP) is another potential issue.

The introduction of NSP with grain meals presents a problem in that different chemical classes of NSP may have different biological effects on the digestion process in animals. While some types of starch can be well digested (Bergot and Breque, 1983; Amirkolaie et al., 2006; Enes et al., 2008; Moreira et al., 2008), there are few NSP that succumb to the digestion processes in monogastric animals, fish included (Kraugerud et al., 2007; Ovrum-Hansen and Storebakken, 2007). There have been various reports on the effects of soluble and insoluble NSP in fish diets. Glencross et al. (2003) reported that the oligosaccharide content of whole seed lupin meal had an effect on energy and dry matter digestibilities of the test ingredients, and a minor effect on protein digestibility. The inclusion of purified oligosaccharides (guar gum) was shown to significantly impair the digestive function of *Dicentrarchus labrax* (European seabass) at low inclusion levels (Leenhouwers et al., 2004). In a study with tilapia (*Oreochromis niloticus*) the addition of soluble (guar gum) and insoluble (cellulose) fibres to diets was observed to have significant effects on diet digestibility (Amirkolaie et al., 2005). The inclusion of insoluble fibre did not affect protein digestibility, but the inclusion of soluble fibre did. Similarly, the inclusion of cellulose in diets for rainbow trout also did not affect the protein digestibility of the diet, but was shown to reduce the energy and dry matter digestibility of those same diets (Ovrum-Hansen and Storebakken, 2007).

Because of this acknowledged effect of different NSP types on the digestion process, one of the key elements in managing the inclusion of plant protein meals is to understand the carbohydrate complexity being added with each raw material and the implications of their inclusion. The carbohydrate composition of lupins has been well characterised by Carre et al. (1985) and Cheung (1990). Lupins are typically devoid of starch (<10 g/kg), but have high levels of galactose based polysaccharides (Petterson, 2000). Almost the entire carbohydrate content of lupins can be regarded as NSP. Within this NSP content however, lupins also have a significant soluble NSP component, characterised by the presence of oligosaccharides and other simple sugars (Cheung, 1990). Based on earlier work it is hypothesised that the inclusion of soluble lupin NSP will have a more profound effect on the digestion process within fish. However, it is expected that the insoluble NSP will largely act as a bulking agent, similar to cellulose,

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Table 1
Nutrient composition of the experimental ingredients (all values are g/kg DM unless otherwise indicated).

Nutrient	[a]Fish meal	[b]Pregelled wheat starch	[c]Lupin soluble fibre	[c]Lupin insoluble fibre	[d]Cellulose
Dry matter content (g/kg)	926	915	910	896	937
Crude protein	695	7	574	232	7
Crude fat	90	3	73	76	0
Ash	186	4	112	70	1
Total carbohydrate[e]	29	986	241	622	992
Lignin	0	1	1	2	1
Cellulose	0	0	1	92	777
Hemicellulose	0	19	7	54	196
Gross energy (MJ/kg DM)	22.4	18.4	20.2	18.9	16.7

[a] Fish meal: Chilean anchovy meal, Skretting Australia, Cambridge, TAS, Australia.
[b] Pregelatinised wheat starch: Manildra, Auburn, NSW, Australia.
[c] *L. angustifolius* soluble and insoluble NSP: produced from kernel meal obtained from Coorow Seed Cleaners, Coorow, WA, Australia.
[d] Cellulose: Sigma Chemical Company, St Louis, MO, USA.
[e] Calculated based on dry matter − (protein + ash + fat).

but will be otherwise relatively inert when fed to fish. This study examines the effect of the dietary inclusion of incremental levels of soluble or insoluble lupin NSP and purified cellulose on the digestible value of diets fed to rainbow trout, *Oncorhynchus mykiss*.

2. Materials and methods

2.1. Ingredient preparation

A batch of *Lupinus angustifolius* kernel meal was obtained from a commercial grain supplier (Coorow Seed Cleaners, Coorow, WA, Australia). Soluble fibre, protein and insoluble fibre were separated based on a modification of the protein isolation method of Lasztity et al. (2001). Ten kilograms of lupin kernel meal was solubilised in water and the pH raised to 10.0 with the addition of 2 M NaOH. The insoluble (insoluble fibre) and soluble materials (soluble fibre and protein) were then separated by centrifugation at 1000 ×*g* for 1 min after which the soluble material was decanted from the precipitate. The precipitate (insoluble fibre) was then neutralised to a pH of 7.0 by the addition of 2 M HCl. The soluble material was then resuspended in water and the pH reduced to 4.0 by the addition of 2 M HCl. The insoluble (protein) and soluble materials (soluble fibre) was then centrifuged at 1000 ×*g* for 1 min after which the soluble material was decanted from the precipitate. The remaining soluble material was then neutralised by the addition of 2 M NaOH. Each sample was frozen at −20 °C, after which it was freeze-dried. Each test ingredient was then milled using a Retsch rotor mill with a 750 μm screen. In addition to the test ingredients, each of the other ingredients used in this study were thoroughly ground such that they passed through an 800 μm screen. The composition of each test ingredient is presented in Table 1.

2.2. Diet development

The experiment design was based on a diet formulation strategy that replaced complete proportions of the diet with an allocated amount of the added fibre sources. For this, a basal diet was formulated and prepared to include approximately 500 g/kg DM protein, 210 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Table 2). Each test ingredient (fibre source) source was then added at to the test diets at 100, 200 or 300 g/kg inclusion to a reciprocal-sample of the basal mash (see Table 2). The diets were processed by the addition of water (about 30% of mash dry weight) to the mash whilst mixing to form a dough. The dough was subsequently screw pressed using a pasta maker through a 4 mm diameter die. The resultant moist pellets were then oven dried at 70 °C for 12 h and then allowed to cool to ambient temperature in the oven. The basal diet was prepared in a similar manner, but without the addition of any test ingredient. The source and composition of all ingredients is presented in Table 1.

2.3. Fish handling and faecal collection

Hatchery-reared rainbow trout (*Oncorhynchus mykiss*, Pemberton heat-tolerant strain) were transferred from grow-out ponds to experimental tanks (200 L). Freshwater (salinity<1 PSU; dissolved oxygen 8.0 ± 0.6 mg/L) of 16.3 ± 0.2 °C (mean ± S.D.) at a flow rate of about 4 L/min was supplied to each of the tanks. Each of the tanks were stocked with 20 trout of 210.6 ± 16.7 g (mean ± S.D.; $n = 40$ fish from the sample population). Each of the treatments was randomly assigned amongst 30 tanks, with each treatment having three replicates.

Fish were manually fed the diets once daily to apparent satiety as determined over three separate feeding events between 1500 and 1600 each day. The trout were allowed to acclimatise to the allocated dietary treatment for seven days before faecal collection commenced consistent with earlier studies by this group (Glencross et al., 2005). Faeces were collected using stripping techniques. Stripping techniques were based on those reported by Austreng (1978) and Glencross

Table 2
Formulations and composition of the experiment diets (all values are g/kg).

	0	C10	C20	C30	S10	S20	S30	I10	I20	I30
Fishmeal	700.0	630.0	560.0	490.0	630.0	560.0	490.0	630.0	560.0	490.0
Fish oil	150.0	135.0	120.0	105.0	135.0	120.0	105.0	135.0	120.0	105.0
Cellulose	–	100.0	200.0	300.0	–	–	–	–	–	–
Lupin soluble NSP	–	–	–	–	100.0	200.0	300.0	–	–	–
Lupin insoluble NSP	–	–	–	–	–	–	–	100.0	200.0	300.0
Pregelatinsied wheat starch	144.0	129.6	115.2	100.8	129.6	115.2	100.8	129.6	115.2	100.8
Vitamin and mineral premix[a]	5.0	4.5	4.0	3.5	4.5	4.0	3.5	4.5	4.0	3.5
Yttrium oxide	1.0	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7
Dry matter	954	959	958	956	944	949	943	954	951	943
Crude protein (g/kg DM)	517	454	410	365	518	538	537	501	475	441
Total Lipid (g/kg DM)	219	199	175	153	199	186	171	203	188	174
Ash (g/kg DM)	133	121	109	93	133	129	126	128	122	116
Carbohydrate (g/kg DM)	131	226	306	389	150	147	166	168	215	269
NSP (g/kg DM)[b]	0	99	198	298	24	48	72	62	124	187
Energy (MJ kg^{-1} DM)	22.9	22.2	21.5	21.0	22.2	22.0	21.8	22.3	22.0	21.6

Treatment annotations are based on C: cellulose, S: soluble NSP and I: insoluble NSP.
[a] Vitamin and mineral premix includes (IU/kg or g/kg of premix): vitamin A, 2.5 MIU; vitamin D3, 0.25 MIU; vitamin E, 16.7 g; vitamin K, 3, 1.7 g; vitamin B1, 2.5 g; vitamin B2, 4.2 g; vitamin B3, 25 g; vitamin B5, 8.3; vitamin B6, 2.0 g; vitamin B9, 0.8; vitamin B12, 0.005 g; biotin, 0.17 g; vitamin C, 75 g; choline, 166.7 g; inositol, 58.3 g; ethoxyquin, 20.8 g; copper, 2.5 g; ferrous iron, 10.0 g; magnesium, 16.6 g; manganese, 15.0 g; zinc, 25.0 g.
[b] Estimated from the carbohydrate content of each test ingredient and its inclusion level in each diet.