



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.

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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; Ovrup-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 (Ovrup-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 (Pettersson, 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 Pregelised 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
Pregelatinised 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.

Table 3
Digestibility (%) specifications of each of the experimental diets.

	0	C10	C20	C30	S10	S20	S30	I10	I20	I30	Pooled SEM
Dry matter	84.3 ^{ab}	81.4 ^b	74.4 ^c	70.5 ^{cd}	87.2 ^a	86.2 ^a	84.9 ^{ab}	83.4 ^{ab}	77.5 ^b	67.7 ^d	1.00
Nitrogen	91.5 ^a	91.9 ^a	91.0 ^a	88.4 ^a	93.2 ^a	93.0 ^a	92.8 ^a	90.8 ^a	90.8 ^a	88.9 ^a	0.50
Energy	91.5 ^{ab}	87.8 ^b	81.5 ^c	77.9 ^c	92.4 ^a	94.2 ^a	90.8 ^{ab}	90.1 ^{ab}	84.7 ^b	77.2 ^c	0.90

Different superscripts within rows indicate significant differences between means among diets, but not between digestibility parameters ($P < 0.05$).

et al. (2005). Fish were netted from their respective tank, placed in a smaller aerated tank containing isoeugenol (0.002 mL/L) until they lost consciousness. The faeces were then removed from the distal intestine using gentle abdominal pressure. Care was maintained to ensure that the faeces were not contaminated by urine or mucous. Hands were rinsed in freshwater after the handling of each fish. After removal of the faeces from the fish, the faecal sample was placed in a small plastic vial and stored in a freezer at -20°C . Stripped faeces were collected during 0800 to 1000 over a four-day period, with each fish only being stripped twice and not on consecutive days. Faecal samples from different days were pooled within the tank, and kept frozen at -20°C before being freeze-dried in preparation for analysis.

2.4. Chemical and digestibility analysis

All chemical analyses were carried out by NATA (National Association of Testing Authorities) accredited analytical service providers (Chemistry Centre (WA), East Perth, WA, Australia). Diet and faecal samples were analysed for dry matter, yttrium, ash, phosphorus, nitrogen and gross energy content. Dry matter was calculated by gravimetric analysis following oven drying at 105°C for 24 h. Total yttrium and phosphorus concentrations were determined after mixed acid digestion using inductively coupled plasma atomic emission spectrophotometry (ICP-AES) based on the method described by (McQuaker et al., 1979). Protein levels were calculated from the determination of total nitrogen by LECO auto-analyser, based on $N \times 6.25$. Total lipid content of the diets and ingredients was determined gravimetrically following extraction of the lipids according to the Folch method. Insufficient faecal material was available for an accurate lipid analysis to be undertaken. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross energy was determined by adiabatic bomb calorimetry. Dietary fibres were determined by digesting the defatted sample with multiple washes of acetone and ethanol. The resulting residue was corrected for undigested protein and ash according to the method of the Champ et al. (1998). Neutral-detergent fibre (NDF) samples were boiled with buffered NDF solution. The residue is collected on a coarse sintered glass crucible (Van Soest and Robertson, 1981). The acid-detergent fibre (ADF) was determined following a sample being reacted in 0.5 M acid detergent solution and the residue is collected on a coarse sintered glass crucible after, the method of Van Soest and Goering (1970). Lignin is determined by reacting the ADF residue with cold 72% sulphuric acid. The sample was ashed and the residue was measured gravimetrically (Van Soest and Robertson, 1981). Total carbohydrate content was determined based on dry matter – (protein + lipid + ash) content. Cellulose content was determined based on the ADF-lignin. Hemicellulose content was determined based on NDF-ADF content (Pettersen et al., 1999; Hindrichsen et al., 2006).

Differences in the ratios of the parameters of dry matter, protein or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility (AD_{diet}) for each of the nutritional parameters examined in each diet based on the following formula (Maynard and Loosli, 1979):

$$\text{AD}_{\text{diet}} = \left(1 - \frac{Y_{\text{diet}} \times \text{Parameter}_{\text{faeces}}}{Y_{\text{faeces}} \times \text{Parameter}_{\text{diet}}} \right) \times 100$$

where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $\text{Parameter}_{\text{diet}}$ and $\text{Parameter}_{\text{faeces}}$ represent the nutritional parameters of concern (organic matter, protein or energy) content of the diet and faeces respectively. Digestibility values for each diet are presented in Table 3.

2.5. Statistical analysis

All values are means unless otherwise specified. Effects of fibre type and inclusion level on the digestibility of dry matter, protein and gross energy in each of the diets were examined by two-way ANOVA (Table 3). Levels of significance were determined using a least significant difference (LSD) test. Curve fitting and regression analysis were undertaken using Microsoft Excel. Limits for all critical ranges were set at $P < 0.05$.

3. Results

3.1. Ingredient composition

The isolation of the soluble and insoluble fibre fractions of lupin kernel meal produced two products with quite different compositional characteristics. The soluble fibre isolate also retained a significant amount of protein (574 g/kg), with the actual NSP content quite low (241 g/kg). Both the acid-detergent and neutral detergent fibre contents of the soluble NSP fraction were low at 2 and 9 g/kg respectively. In contrast, the isolation of the insoluble fibre was more successful in its preparation with an NSP content of 622 g/kg and protein limited to 232 g/kg. Fat content of each NSP preparation was similar at ~ 75 g/kg. The acid-detergent and neutral detergent fibre contents of the insoluble NSP fraction were higher at 94 and 148 g/kg respectively. Both lupin NSP samples had lower NSP and fibre levels than the cellulose (Table 1). All samples were low in lignin content, with the insoluble lupin NSP having the highest level at 2 g/kg, double that of the soluble lupin NSP and the cellulose.

3.2. Diet digestibilities

There were several significant differences among the digestibility parameters of the diets with different inclusion levels of each of the different fibre types (Tables 3 and 4, Fig. 1). Differences between the diets in terms of their dry matter and energy digestibility were most distinct, with more significant differences among the test diets with those two parameters. No significant differences among the diets were noted for diet protein digestibilities.

Two-way ANOVA analysis identified that there were significant effects of the different fibre types and also the inclusion levels of each

Table 4
MANOVA table of variable discrete and interaction terms.

	Value	F	Effect df	Error df	P
Intercept	0.001	109752.1	4	21.0	0.001
FIBRE	0.144	8.6	8	42.0	0.001
LEVEL	0.081	7.4	12	55.8	0.001
FIBRE*LEVEL	0.099	2.9	24	74.5	0.001

FIBRE: refers to the type of fibre used. LEVEL: refers to the inclusion level of each fibre isolate.

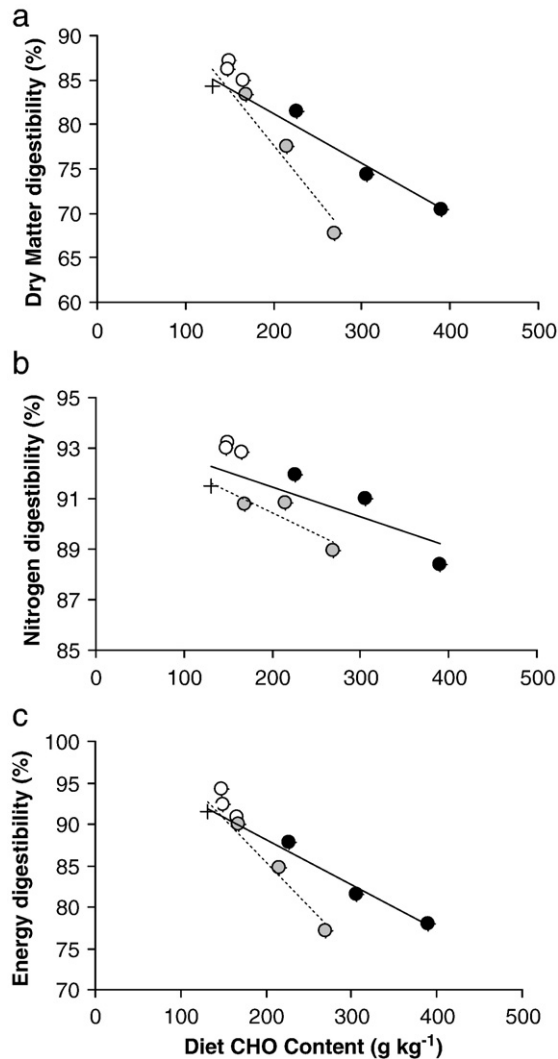


Fig. 1. a, b, c. Effect of diet carbohydrate level (%) on digestibility (%) specifications of dry matter, nitrogen and energy of each of the experimental diets. Each of the different NSP types is represented by a different shaded circle (cellulose ●; soluble NSP ○ and insoluble NSP ●). The reference diet is indicated with a +. Indicated regression lines are those for the entire dataset.

of the fibre types on the digestibility of dry matter and energy of each diet (Table 4). An interaction term was also defined between the type of fibre and the level of its inclusion (Table 4).

Regression analysis of the effect of each fibre type and inclusion level (of the specific fibre, not the test ingredient) graphically shows the effect of each fibre type more clearly (Fig. 1). The relatively low concentration of the soluble fibre provided little variability in the dietary soluble fibre content for any of the digestibility parameters to be extrapolated to any extent by regression. Comparison of the effects of the lupin insoluble NSP and the cellulose provides a more interesting comparison, with more profound negative effects of the lupin insoluble NSP on the digestibility of dry matter and energy than that observed of the cellulose on the diets. These are reinforced by the comparative significant effects reported in Table 3. Regression equations for lupin insoluble NSP and the cellulose on diet dry matter digestibilities were:

$$\text{ADC}_{\text{dry matter}} = -0.0668 * (\text{diet cellulose content}) + 95.981, R^2 = 0.9711 \quad (1)$$

$$\text{ADC}_{\text{dry matter}} = -0.1564 * (\text{diet insoluble NSP content}) + 110.2, R^2 = 0.9898 \quad (2)$$

Regression equations for insoluble NSP and the cellulose on diet nitrogen digestibilities were:

$$\text{ADC}_{\text{nitrogen}} = -0.0117 * (\text{diet cellulose content}) + 93.807, R^2 = 0.669 \quad (3)$$

$$\text{ADC}_{\text{nitrogen}} = -0.0185 * (\text{diet insoluble NSP content}) + 94.186, R^2 = 0.7599 \quad (4)$$

Regression equations for insoluble NSP and the cellulose on diet energy digestibilities were:

$$\text{ADC}_{\text{energy}} = -0.0603 * (\text{diet cellulose content}) + 100.94, R^2 = 0.9714 \quad (5)$$

$$\text{ADC}_{\text{energy}} = -0.1284 * (\text{diet insoluble NSP content}) + 111.89, R^2 = 0.9975. \quad (6)$$

4. Discussion

The increasing amount of plant protein raw materials being fed to different fish species is increasing the complexity of nutritional responses by fish to diets in which those raw materials are being included (Gatlin et al., 2007; Glencross et al., 2007). Apart from the potential introduction of anti-nutritional factors (ANF), the inclusion of different types of carbohydrates presents a different type of xenobiotic factor present in the diets of fish, particularly so for carnivorous species. One observation noted with the inclusion of different carbohydrate classes has been the occurrence of non-additive effects in digestibility studies and it has been suggested that the different fibre classes were one of the possible factors that caused these nutrient interactions resulting in loss of additivity (Glencross et al., 2007). The present study examined possible non-starch polysaccharide compounds from lupins and in particular the effect that they have on the digestibility of diets to fish. Earlier studies with salmonids have shown that the gross removal of these compounds can significantly improve diet digestibility characteristics (Refstie et al., 1998; Glencross et al., 2004).

4.1. Diet digestibility effects

Dietary fibre content has a clear effect on the digestibility of dry matter content of diets fed to rainbow trout (Fig. 1a). The clear response between insoluble fibre and cellulose addition, and diet dry matter digestibility is consistent with the fact that these fish are obtaining limited nutritive value from this fibre (NSP) content of the diet and it is acting largely as a bulking agent. This is similar to the effects observed by Ovrum-Hansen and Storebakken (2007), who also reported a significant effect of cellulose inclusion on organic matter

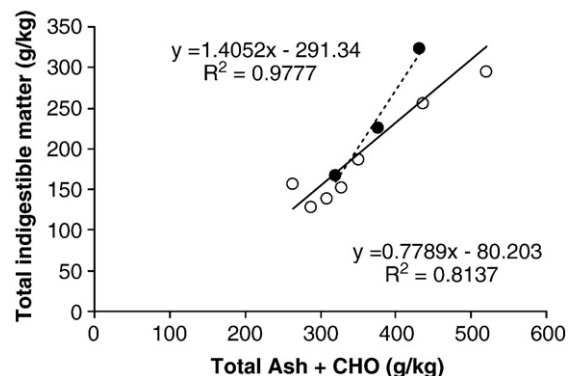


Fig. 2. The derived relationship between dietary ash + carbohydrate (CHO) content on the indigestible content of the diets. Shown are the combined effect of all treatments (○) and those of the insoluble NSP (●).

digestibility of diets fed to rainbow trout. However, in the present study the degree of reduction in dry matter digestibilities varies between comparable inclusion levels of insoluble fibre and cellulose addition suggesting that there are additional factors that can further exacerbate this reduced digestibility. An examination of the relationship between total dietary ash + carbohydrate content, and total indigestible matter from all diets produced a strong linear relationship ($y = 0.779x - 80.203$, $R^2 = 0.814$) (Fig. 2). Within this dataset, a similar examination of the data from the insoluble fibre series of diets also produced a strong linear effect ($y = 1.405x - 291.34$, $R^2 = 0.978$), but that the coefficient was greater than 1.0 indicates that there is an exacerbated effect from the addition of this fibre source on the level of total indigestible matter in the diet (Fig. 2). This indicates that this fibre source is therefore also reducing the digestibility of other dietary components that other fibre sources were not affecting. From an examination of the chemical differences between this and the other fibre sources in the study, the only notable difference is the higher inclusion level of lignin (albeit 1 g/kg cf. 2 g/kg). This fibre class has also been implicated as having significant effects on diet and ingredient digestibilities in other multivariate analysis studies even at very low inclusion levels (Glencross et al., 2008).

However, the lack of significant effects on diet protein digestibility in this study (as analysed by ANOVA) was limited by the overall extent of variability in the protein digestibilities of the diets (Fig. 1b). This lack of significant effects of fibre on protein digestibility is also consistent with the findings of Ovrum-Hansen and Storebakken (2007). Despite this limited variability, regression effects were significant on the observed protein digestibilities (Fig. 1b) and some differences between fibre sources could be inferred from that interpretation. However, further work specifically targeting certain fibre classes and more controlled levels than was achieved in this study may be warranted. Recent work by Glencross et al. (2008) has shown that the variability within lupin kernel meals can be largely explained by the crude protein and lignin content of the meals. In this regard, the difference of lignin content, albeit very small between the cellulose and insoluble lupin flour may account for the differences seen between those two fibre sources on the digestibility of dry matter and energy.

Dietary fibre content was also shown to have a clear effect on the digestibility of the energy content of diets fed to rainbow trout (Fig. 1c). The response between insoluble fibre and cellulose addition, and diet energy digestibility is also consistent with the fact that these fish are obtaining limited energetic value from this fibre content of the diet. However, that the degree of reduction in energy digestibilities also varies between comparable inclusion levels of insoluble fibre and cellulose addition further adding to the suggestion that there are additional factors in the insoluble fibre content of the lupins that can further exacerbate this reduced digestibility.

A study by Glencross et al. (2003) on the effects of lupin oligosaccharides, which are a class of soluble fibre, showed that by using both chemical and enzymatic removal methods that it was possible to measure the effect of this soluble fibre type on the digestibility of dietary protein, energy and organic matter in diets fed to rainbow trout. Work by Leenhouders et al. (2004) with soluble fibre (guar gum) also showed significant effects with as little as 40 g/kg inclusion of soluble fibre on the digestibility of organic matter, energy and protein of diets fed to European seabass (*Dicentrarchus labrax*). Similar effects on protein, fat and starch digestibility were also reported by Amirkolaie et al. (2005) with *Oreochromis niloticus* when guar gum was included in diets 80 g/kg, but no significant effects were observed with a similar inclusion level of cellulose. However, although the soluble fibre inclusion in diets in the present study reached inclusion levels of 72 g/kg, it still did not have a significant effect on any of the digestibility parameters. This suggests that different soluble fibre classes may exert different effects. Leenhouders et al. (2004) also noted significant effects of soluble fibre on digesta viscosities and suggested that this might be one mechanism by which soluble fibre impacts on nutrient digestibility.

It would be of value to revisit the lupin fibre isolates to examine their effect on digesta viscosities in this regard. Leenhouders et al. (2007) in a later study with *Clarius macrocephalus* also examined the effects of different cereal sources on the digesta viscosity and digestibility of various dietary parameters. They found that intestinal fermentation, digesta dry matter content and nutrient digestibility were affected only above critical level of digesta viscosity. However, these authors also noted that the observed changes in digesta characteristics were not necessarily accompanied by reduced fish performance.

5. Conclusions

The findings of this study confirm that there are different effects on diet digestibilities when different fibre types are included in diets fed to rainbow trout. In contrast to other works limited effect was observed from the inclusion of soluble fibre classes from lupins, but clear effects of both the lupin insoluble fibre and purified cellulose were observed.

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