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Evaluation of dietary inclusion of yellow lupin (*Lupinus luteus*) kernel meal on the growth, feed utilisation and tissue histology of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study assessed the use of yellow lupin kernel meal (YLKM) in diets for rainbow trout. Increasing level of inclusion (0%, 12.5%, 25%, 37.5% and 50%) of YLKM were provided at the expense of fish meal in diets formulated to be isonitrogenous (383 g/kg) and isoenergetic (20 MJ/kg) on a digestible basis. Growth of rainbow trout (initial weight, 35.8 ± 0.7 g; mean ± S.D.) fed the experimental diets for 42 days was significantly reduced at the 50% YLKM inclusion level, but linear regression modeling suggested a decline in growth at each inclusion level. However, the level of inclusion of the YLKM did not significantly affect feed intake. Food conversion ratio also deteriorated significantly at the 50% inclusion level, with a linear regression model also suggesting a decline in FCR at each inclusion level. Retention efficiency of nitrogen was unaffected by level of inclusion though a significant decline in the efficiency of energy retention was observed with increasing inclusion of YLKM. No significant effects of ingredient inclusion on body composition were noted, though some alterations in organ somatic indices were identified. Minor effects were a trend for an increase in relative size of the gastrointestinal tract, and decreases in hepatocyte lipid droplets with increasing levels of YLKM inclusion. This ingredient presents as a highly useful feed ingredient for aquaculture rations.

Keywords: Yellow lupin; Plant protein; Fish meal replacement; Rainbow trout

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1. Introduction

Most modern, nutrient-dense, aquaculture diets use some inclusion of plant protein ingredients. Many such ingredients have been assessed experimentally, but with the notable exception of soybean meal, few are used commercially (Carter and Hauler, 2000). Lupins (*Lupinus* spp.) are one ingredient that have been shown to provide some potential as a useful feed ingredient in fish diets and have been used in commercial diets in some countries (Burel et al., 1998; Glencross et al., 2002; 2003b).

Lupins are members of the Leguminosae family, which also includes beans and peas. There are more than 200 species in the genus *Lupinus*, although only three have been sufficiently domesticated to be considered as feed grains. These are the European white lupin (*Lupinus albus*), the narrow-leafed lupin (*Lupinus angustifolius*) and the yellow lupin (*Lupinus luteus*) (Gladstones, 1998; Petterson, 2000).

Several reports exist on the nutritional and biological evaluation of lupin grain in aquaculture diets where there have been detailed examinations of the use of either *L. angustifolius* or *L. albus* meals (De la Higuera et al., 1988; Hughes, 1988, 1991; Gomes et al., 1995; Burel et al., 1998, 2000; Farhangi and Carter, 2001). There are few reports however, on the nutritional or biological value of yellow lupin kernel meal (YLKM) when fed to a fish species (Glencross et al., 2002; Glencross and Hawkins, in press). While some problems with high inclusion levels of lupins in fish diets have been reported, only minor aberrations in the levels of thyroid hormones or a loss in feed intake have been suggested as reasons for this deterioration in performance (Burel et al., 1998; Farhangi and Carter, 2001).

Of those lupin species being grown commercially, the yellow lupin has the highest protein content compared to other lupin varieties. There are some preliminary studies on the inclusion of YLKM in fish diets and more detailed information on its digestible nutrient value, but there is no clear study examining the influence of increasing inclusion levels of this ingredient in diets for fish (Glencross et al., 2002; Glencross and Hawkins, in press).

This study examines the influence of incremental inclusion of YLKM in a diet fed to rainbow trout with a view to defining maximal inclusion levels before deleterious effects are observed. An examination is made of the nutritional, biochemical, organ somatic indices and histological influences of the inclusion of this protein source in diets for rainbow trout.

2. Materials and methods

2.1. Experimental ingredients and diet development

A single batch of yellow lupins was processed to remove the seed coat (dehull) and milled to produce a kernel meal with a maximum particle size of 800 μm. The composition of the meal and key ingredients used in this study are presented in Table 1.

All experimental diets were formulated to be isonitrogenous (383 g/kg) and isoenergetic (20 MJ/kg) on a digestible nutrient basis. Digestibility coefficient values for key

Table 1
Composition of ingredients used in experiment diets (all values are g/kg DM unless otherwise indicated)

	Fish meal[a]	Wheat gluten[b]	Yellow lupin[c]	Pregelled starch[b]	Cellulose[d]
Dry matter content (g/kg)	920	900	905	849	933
Crude protein	754	813	578	4	5
Digestible protein[e]	660	813	549	0	0
Crude fat	96	11	73	1	2
Ash	150	47	43	4	2
Nitrogen-free extractives + Fibre[f]	0	130	306	991	990
Phosphorus	23	1	6	0	0
Gross energy (MJ/kg DM)	21.6	21.9	21.1	17.3	17.3
Digestible energy (MJ/kg DM)[e]	18.9	19.9	13.7	8.6	0.0

[a] Ingredients sourced from Skretting Australia, Cambridge, TAS, Australia.
[b] Ingredients sourced from Weston BioProducts, Henderson, WA, Australia.
[c] Ingredients sourced from Department of Agriculture, South Perth, WA, Australia.
[d] Ingredients sourced from ICN Biomedical, Costa Mesa, CA, USA.
[e] Calculated values based on data from Glencross and Hawkins (2004).
[f] Based on dry matter minus protein, ash, and fat content.

ingredients were based on those reported by Glencross and Hawkins (in press). Diets were processed by the addition of water (about 30% of mash dry weight) to all ingredients while mixing to form a dough, which was subsequently screw-pressed through a 4-mm diameter die using a pasta maker. The antioxidant butylated hydroxy-toluene (BHT) was added as a solution in ethanol. The resultant moist pellets were then oven dried at 90 °C for approximately 9 h before being air-cooled, bagged and stored at −20 °C. A commercial extruded salmonid diet was used as the final treatment group. Formulations and proximate composition for all diets are presented in Table 2.

2.2. Fish handling

Eighteen shallow-conical bottomed 250 l tanks, with flow-through freshwater (4 l/min, salinity < 1 ‰ and 16.9 ± 1.3 °C, dissolved oxygen 7.5 ± 0.3 mg/l, mean ± S.D., $n = 42$), were each stocked with 24 juvenile (9 months, 35.8 ± 0.7 g; mean ± S.D.) hatchery reared rainbow trout (*Oncorhynchus mykiss*; Pemberton Heat-tolerant Strain). Treatments were randomly assigned in triplicate to the tank array. Photoperiod was maintained at 12L:12D.

The fish were fed to apparent satiety once daily at about 08:00 h for 42 days. Apparent satiety, a determined by a loss in feeding activity, was reached after three feeding sessions over a 1-h period. Uneaten feed was removed from each tank 1 h later and the uneaten portion dried and weighed to allow the determination of daily feed intake.

Fish were individually re-weighed after 3 and 6 weeks, with all fish within each tank used to determine the average weight gain per tank and treatment. Five fish were taken as an initial sample for composition analysis. At the end of the study two fish were taken from each tank (6 per treatment) for whole body analysis. An additional two fish from each tank were sampled for blood biochemistry, within 1 min of capture, by direct heart

Table 2
Diet formulations and composition of experimental diets (all values are g/kg unless otherwise indicated)

	0%	12.5%	25.0%	37.5%	50.0%	Commercial*
Ingredient composition						
Fishmeal	650.0	530.5	411.0	291.5	172.0	
Fish oil	163.0	165.5	168.0	170.5	173.0	
Wheat gluten	28.0	31.5	35.0	38.5	42.0	
L. luteus kernel meal	0.0	125.0	250.0	375.0	500.0	
Pregel starch	90.0	90.0	90.0	90.0	90.0	
Cellulose	64.0	50.5	37.0	23.5	10.0	
L-Lysine[a]	0.0	0.8	1.5	2.3	3.0	
DL-Methionine[a]	0.0	1.3	2.5	3.8	5.0	
Vitamin and mineral premix[b]	5.0	5.0	5.0	5.0	5.0	
BHT[c]	0.01	0.01	0.01	0.01	0.01	
Diet proximate composition						
Dry matter	943	934	907	939	913	912
Crude protein (g/kg DM)	516	542	494	481	465	522
Crude fat (g/kg DM)	233	245	235	230	208	246
Ash (g/kg DM)	137	127	87	70	53	74
Phosphorus (g/kg DM)	16	14	11	10	9	13
Energy (MJ/kg DM)	22.6	23.2	23.6	23.8	23.6	24.3

Ingredients sourced from: [a]WESFEEDS, Welshpool, WA, Australia. [b]Rhône-Poulenc, Goodna, QLD, Australia. [c]Butylated Hydroxytoluene (BHT): ICN Biomedical, Costa Mesa, CA, USA (Vitamin and mineral premix includes (IU kg^{-1} or g kg^{-1} of premix): Vitamin A, 2.5 MIU; Vitamin D_3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K_3, 1.7 g; Thiamine (Vitamin B1), 2.5 g; Riboflavin (Vitamin B2), 4.2 g; Niacin (Vitamin B3), 25 g; Calcium Pantothenate (Vitamin B5), 8.3; Pyridoxine HCl (Vitamin B6), 2.0 g; Folate (Vitamin B9), 0.8; Cyanocobalamin (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).

*Commercial salmonid diet provided by Skretting Australia, Cambridge, Tasmania.

puncture using a 1-ml syringe fitted with at 20G needle. These same fish were later dissected for organ weight determination. Growth was assessed as mean weight gain and daily growth coefficient (DGC). DGC was calculated as (Kaushik, 1998):

$$\mathrm{DGC} = (W_f^{\frac{1}{3}} - W_i^{\frac{1}{3}})t \times 100$$

2.3. Chemical analysis

Fish tissue and diet samples were analysed for energy, dry matter, nitrogen, fat, ash, calcium and phosphorus content. Energy was determined using adiabatic bomb calorimetry. Dry matter was calculated by gravimetric analysis following oven drying at 105 °C for 24 h. Protein levels were calculated from the determination of total nitrogen by LECO analysis, based on %N × 6.25. Fat levels were determined using the Soxhlet method (AOAC (Association of Official Analytical Chemists), 1990). Phosphorus and calcium levels were determined using by ICP-Atomic Emission Spectrometry (McQuaker et al., 1979). Levels of tri-iodothyronine (T_3) and thyroxine (T_4) were determined by a competitive immunoassay method using chemiluminescence detection (Fisher, 1996).

Blood glucose was measured using a glucose meter (Glucotrend™). Nitrogen free extractive content was determined as the dry matter content minus protein, ash and fat. For sample analysis parameters, two fish from each replicate were pooled then analysed ($n = 3$ replicates per treatment).

2.4. Tissue histology

Two fish from each tank ($n = 6$ per treatment) were euthanised with a sharp cranial blow at the end of the study and fixed in 10% neutral buffered formalin. Incisions were made in the fish's abdominal wall to allow penetration of the formalin though out the intestinal organs. Following preservation the fish were dissected and samples of their kidney, liver, pyloric caeca and intestine were taken for histological examination. The samples were embedded in paraffin, sectioned and stained with haematoxylin and eosin using standard techniques. The sample sections were examined with a compound microscope (200× and 400× magnification) and two digital images (Olympus DP11) taken of each of the various samples. The slides were examined for lesions. Presence or absence of granular substance and crystalline deposits in the kidney tubules was noted. A subjective score (0 (absent) to 3 (abundant)) was made of the presence of macrophages, cellular atrophy, and the amount of pale golden pigment in tubule epithelial cells.

2.5. Statistical analysis

All figures are mean ± S.E. unless otherwise specified. Effects of diets were examined by ANOVA using the software package Statistica (Statsoft®, Tulsa, OA, USA). Levels of significance were determined using Tukeys HSD test, with critical limits being set at $P < 0.05$. Effects of inclusion level of yellow lupin kernel meal on key

Table 3
Growth, feed utilisation and biochemical parameters of fish from the experimental treatments

	0%	12.5%	25.0%	37.5%	50.0%	Commercial	Pooled SEM
Growth and feed utilisation							
Initial weight (g/fish)	36.3	36.4	35.3	36.2	36.0	35.3	0.14
Final weight (g/fish)	129.2^{a}	126.2^{a}	122.9^{a,b}	120.7^{a,b}	115.1^{c}	124.7^{a}	1.28
Gain (g/fish)	92.9^{a}	89.8^{a}	87.6^{a,b}	84.5^{a,b}	79.1^{c}	89.4^{a}	1.26
DGC (%/d)	4.15^{a}	4.05^{a}	4.03^{a}	3.89^{a,b}	3.71^{b}	4.08^{a}	0.02
FCR (intake/live-weight gain)	0.90^{a}	0.91^{a}	0.90^{a}	1.00^{a,b}	1.05^{b}	0.88^{a}	0.04
Feed intake (g/fish/d)	1.89^{a}	1.78^{a,b}	1.70^{a,b}	1.78^{a,b}	1.87^{a}	1.55^{b}	0.03
Survival (%)	98.3	95.0	96.6	93.3	98.3	91.7	0.93
Biochemical							
Blood glucose (mmol/l)	3.12	3.07	3.05	2.93	3.10	3.50	0.16
Blood tri-iodothyronine (pmol/l)	8.4^{a}	7.6^{a}	4.4^{a,b}	6.2^{a,b}	6.9^{a,b}	2.1^{b}	0.46
Blood thyroxine (pmol/l)	71.9	56.9	4.9	29.6	6.3	4.4	9.54

^{a,b,c}Different superscripts within a row indicate significant differences at $P < 0.05$ where significant treatment effects were observed. DGC: Daily Growth Coefficient. FCR: Food Conversion Ratio.

Table 4
Linear regression relationships between dietary inclusion levels YLKM (×) and weight gain, feed intake and FCR

Parameter	Equation	R^2	p
Feed intake	$y = 1.898 - 0.0003x$	0.0018	0.8798
Weight gain	$y = 93.36 - 0.2631x$	0.5518	0.0015
FCR	$y = 0.8721 + 0.0032x$	0.3737	0.0155

performance parameters were examined by linear regression modeling, also using the software package Statistica.

3. Results

3.1. Growth and feed utilisation

ANOVA analysis of weight gain and growth rates of rainbow trout fed the experimental diets showed a significant negative effect of inclusion of YLKM at the 50% inclusion level: there was no significant effect at lower inclusion levels (Table 3). Linear regression modeling suggested that declines in fish growth were present at each inclusion level (Table 4). A similar significant deterioration in feed conversion ratio (FCR) was also observed. No significant differences in feed intake were seen among the test treatments (Tables 3 and 4).

3.2. Fish metabolites, composition and nutrient retention

The levels of blood glucose in the trout were unaffected by inclusion of YLKM in the diets. Similarly, there were no significant differences or trends in the levels of blood tri-iodothyronine or thyroxine with increasing levels of yellow lupin kernel meal.

Table 5
Nitrogen and energy retention and body composition (g/kg live-weight) for each experimental treatment

	Initial	0%	12.5%	25.0%	37.5%	50.0%	Commercial	Pooled SEM
Nitrogen retention (%)		24.1^{a}	22.6^{a}	23.8^{a}	22.7^{a}	21.0^{a}	29.3^{b}	0.57
Energy retention (%)		36.9^{a}	$36.1^{a,b}$	$34.0^{a,b}$	$31.7^{b,c}$	28.7^{c}	28.9^{c}	0.76
Dry matter	248^{b}	263^{a}	264^{a}	263^{a}	263^{a}	264^{a}	262^{a}	0.14
Crude Protein	140^{a}	129^{b}	130^{b}	125^{b}	126^{b}	126^{b}	125^{b}	1.09
Crude Fat	86^{b}	123^{a}	126^{a}	121^{a}	124^{a}	122^{a}	119^{a}	0.92
Phosphorus	6^{b}	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}	4^{a}	0.04
Calcium	8^{b}	3^{a}	3^{a}	4^{a}	3^{a}	4^{a}	4^{a}	0.08
Ash	29^{b}	21^{a}	22^{a}	21^{a}	21^{a}	23^{a}	21^{a}	0.45
Gross Energy	6.7^{b}	7.9^{a}	8.0^{a}	7.8^{a}	7.9^{a}	7.8^{a}	7.7^{a}	0.36

^{a,b,c} Different superscripts within each row indicate significant differences at $P < 0.05$ where significant treatment effects were observed. N retention calculated as: N gain/N intake × 100. Energy retention calculated on a similar basis (Maynard and Loosli, 1969).

Table 6
Organ somatic indicies (% of live-weight) for each treatment

	0%	12.5%	25.0%	37.5%	50.0%	Pooled SEM
Kidney	1.1	1.1	0.9	0.9	1.0	0.03
Gastrointestinal tract	11.6[a]	11.6[a]	12.3[a,b]	13.1[b]	12.9[b]	0.31
Gill	2.9[a,b]	2.9[a,b]	3.2[a]	2.9[a,b]	3.1[a]	0.07
Liver	1.4[a]	1.6[a,b]	1.6[a,b]	1.4[a]	1.7[b]	0.04
Muscle	56.8[a]	51.7[b]	50.8[b]	51.5[b]	53.8[a,b]	0.86
Bone	13.7[a,b]	12.1[a]	14.5[b]	12.9[a,b]	12.9[a,b]	0.34
Skin	7.1[b]	13.3[a]	8.5[b]	8.4[b]	9.0[b]	0.52

[a,b,c]Different superscripts within each row indicate significant differences at $P < 0.05$ where significant treatment effects were observed.

Composition of rainbow trout fed the experimental diets was not significantly affected by the inclusion of yellow lupin kernel meal (Table 5). However, there were numerous notable significant differences in composition of the fish between those from the end of the experiment compared to those from the start. Clear increases in dry matter, fat and energy content were observed. These were concomitant with decreases in the level of protein, phosphorus, calcium and ash. Nitrogen retention by the fish was unaffected by the inclusion of YLKM in test diets, however, the nitrogen retention by the fish fed the commercial reference diet was significantly higher. In contrast there was a decreasing efficiency in the retention of energy by the fish with increasing inclusion of YLKM in their diet.

3.3. Organ somatic indices and histological evaluation

Several significant differences were noted in the different organ somatic indices among the different treatments (Table 6). Gastrointestinal tract somatic indices (GSI) differed significantly, with those treatments having higher levels of yellow lupin kernel meal also

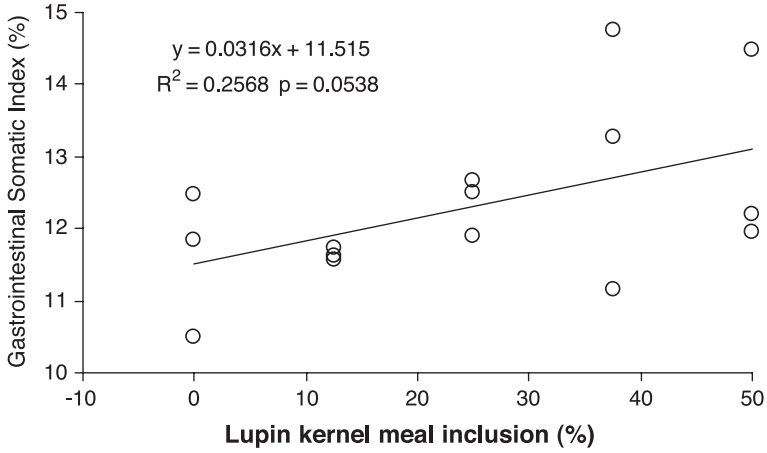

Fig. 1. Relationship between Gastrointestinal Somatic Index (GSI) and yellow lupin kernel meal inclusion level.

having higher GSI values than those of lower inclusion levels. A discernable but non-significant trend ($P=0.054$) was apparent in the relationship between inclusion level and GSI (Fig. 1). Other significant differences were present among the different treatments for other organ somatic indices, though none of these appeared consistent with inclusion levels of yellow lupin kernel meal (Table 6).

Histological examination of distal intestine, liver, kidney and pyloric caeca of fish from the 50% yellow lupin kernel meal and control (0%) treatments revealed few differences. Notable was a significant decrease in the incidence of lipid droplets in the hepatocytes of fish from the 50% YLKM treatment (Fig. 2). An increased prevalence of a granular like substance was observed in the kidney sections as was an increased level of kidney

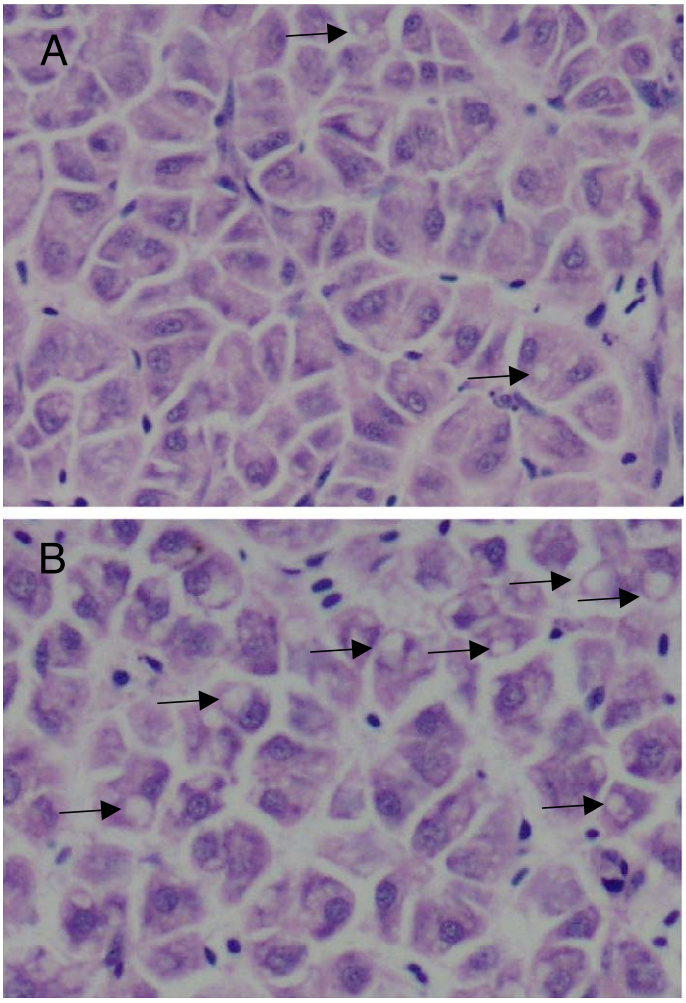

Fig. 2. Hepatocytes of rainbow trout fed either 50% yellow lupin kernel meal (A) or the control (B). Shown are the lipid droplets. Notable is the higher incidence of lipid droplets in the hepatocytes of fish fed the control diet.

macrophages also observed in fish fed the yellow lupin kernel meal. No other significant differences in histological features were noted.

4. Discussion

4.1. Influences of inclusion of yellow lupin kernel meal on fish growth

The results of this study demonstrate that YLKM can be included in the diet of rainbow trout when grown from about 35 to 120 g without significant loss in feed intake, but that there are effects on weight gain or growth rate and feed conversion ratio. These results are similar to those achieved with *L. angustifolius* kernel meal when fed to rainbow trout (Farhangi and Carter, 2001). The main advantage gained in the use of yellow lupin kernel meal over that of *L. angustifolius* kernel meal is the substantially higher protein level of the yellow lupin variety (~ 50% protein cf. ~ 40% protein). This higher protein level allows greater potential in reducing the fish meal component of the diet.

Feed intake was unaffected with increasing inclusion of YLKM. This supports similar observations on other studies on this and other species of lupins (Burel et al., 1998; Glencross et al., 2002). The FCR values obtained for most of the experimental diets in this study were consistent with those obtained for rainbow trout from a commercial extruded diets.

The nitrogen and energy retention values observed in this study show that the biological value of this ingredient is highly consistent with those ingredients it replaced (primarily fish meal). The slight deterioration in energy retention is consistent with the decline in growth, but maintenance of feed intake observed with the higher levels of inclusion.

Consistent with the similar fish size at the end of the experiment, little variation in body composition was observed. The only notable differences were that between the initial stock and those fish at the end of the study. Even fish from the 50% YLKM treatment had a similar composition to the control. This observation is consistent with the change in energy density (primarily fat content) of most fish as they increase in size (Lupatsch et al., 2001).

Although there were no significant changes in any of the Organ Somatic Indices (OSI), a significant effect and discernable linear trend was observed for the influence of YLKM on the size of the gastrointestinal tract. Clearly this observation needs to be more fully investigated with a greater degree of experimental power.

A relationship has been demonstrated in poultry with respect to relative weight of the intestine and the viscosity of feed in the jejunal lumen. Similarly, the inclusion of non-starch polysaccharides (NSP) in diets fed to pigs, rats and poultry was also shown to substantially increase the relative small intestine weight (Simon, 2002). It was suggested that increasing inclusion of NSP increased the rate of cell turnover and as a consequence of the increase digesta viscosity induced GIT hypertrophy (Simon, 2002). It is likely that this hypertrophy of the GIT also increases the relative cost to the GIT protein and energy budget. From the observations of this study, we also believe a similar such trend is apparent with increasing levels of YLKM (which has a high NSP content compared to the control diet ingredients) inclusion. Interestingly, the cost of

protein synthesis in the GIT of fish has been shown to be almost twice that of the muscle, when examined on a whole body basis (Simon, 2002). This cost of protein synthesis is driven largely by the high rate of protein turnover and protein loss in the GIT relative to other tissues.

The decline in growth rate observed with the inclusion of YLKM may be attributable to several factors. It is possible that the proportion of digestible protein and energy derived from the ingredient declines with increasing inclusion level. The digestibility coefficients used to calculate the specifications of the diets were based on those derived from a 30% inclusion level (Glencross and Hawkins, in press). Yellow lupin kernel meal also contains about twice the amount of oligosaccharides as the other domesticated lupins and relatively more so than soybean meal. These compounds have been shown to be problematic in soybean and other lupin meal varieties and as such a possible influence is speculated for yellow lupins (Refstie et al., 1998; Glencross et al., 2003a). At the highest YLKM inclusion levels the amount of phosphorus in the diet is close to the level of requirement (National Research Council (NRC), 1993). It is unknown from this study as to how much of an influence that this may have had on growth performance though it is not considered likely. However, the decrease in levels of dietary phosphorus induced by high inclusion levels of yellow lupin kernel meal may provide opportunities for the use of this ingredient in diets designed to limit phosphorus output by aquaculture.

4.2. Influences of yellow lupin kernel meal on metabolism and tissue histology

Few differences in metabolites compared to the control (0%) treatment were observed, with blood glucose remaining unaffected, as was blood thyroxine (T_4), though notably variation of the later was high similarly, no significant differences in blood tri-iodothyronine (T_3) levels were observed. These results on the influence of a lupin kernel meal on the goitrogenic metabolism of fish contrast those of Burel et al. (1998). However, it should be noted that variability in problems with *L. albus* kernel meals have been noted in fish as well as other species (Gdala et al., 1996; Burel et al., 1998; van Barneveld, 1999).

In contrast to other studies with high levels of soybean meals, little evidence of gastrointestinal enteritis was observed (Refstie et al., 1998; Storebakken et al., 2000). Few histological aberrations between the 50% yellow lupin kernel meal and the control treatments were observed. It would have been of interest to have included a soybean meal treatment as a positive control for enteritic effects. Subtle differences, however, in the level of lipid droplet formation in the liver were observed. The specific implications of this are not fully understood, but may in part be related to the poorer energy retention of the diet also observed at the higher inclusion levels of yellow lupin kernel meal.

4.3. Conclusion

Yellow lupin kernel meal is an excellent ingredient for use in diets for rainbow trout. It has a higher content of digestible protein and energy than many other plant protein resources (Glencross and Hawkins, in press).

However, clear problems have been identified with this feed resource at the highest inclusion level. This may be due to the relatively high content of oligosaccharides. If proven, it would be a relatively easy matter for plant breeders to lower the oligosaccharide content in future cultivars of this species. A prospectively similar problem was identified from soybean meals and *L. angustifolius*, as were ways to address it (Refstie et al., 1998; Glencross et al., 2003a). If it is the total content of NSP causing the problem then further processing to protein concentrates or isolates may resolve the matter.

Future evaluation and development of this grain should focus on several key issues. It would be of value to more clearly define the reasons why a decline in nutritional value is observed at the highest inclusion levels of this grain. Part of this may necessitate establishing the relative metabolic value of this ingredient or its specific protein value (Glencross et al., 2003b, in press). Further gains in the protein content, concomitant with reductions in the NSP content will also increase the value of this ingredient. As such, efforts to develop protein concentrates and isolates would also be clearly useful.

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