



Effects of dietary fishmeal substitution with corn gluten meal and poultry meal on growth rate and flesh characteristics of Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract There is considerable interest in developing diets that maintain growth performance and market appeal for salmon aquaculture while relying less on fishmeal as a major ingredient. Here, we compared growth rate, survival, fat content, tissue colouration and carotenoid levels (astaxanthin) in Chinook salmon (*Oncorhynchus tshawytscha*) fed two diets. The first diet was a typical commercial salmon diet with 59% fishmeal content, while the second diet reduced the fishmeal content to 15% (75% reduction) and substituted 28% corn gluten meal and 16% poultry meal. Over an approximately 14-month growth period, we found no significant difference between fish fed the high fishmeal or low fishmeal diet in either growth rate or survival. Individuals fed the low fishmeal diet did have 25% higher total body fat percentage than those fed the high fishmeal diet. Individuals fed the low fishmeal diet also had flesh that was significantly less red than fish fed the high fishmeal diet. Carotenoid analysis confirmed that the change in tissue colour was the result of reduced astaxanthin levels in salmon fed the low fishmeal diet. Due to the importance of red tissue colour for the market appeal of salmon, the corn gluten and poultry meal diet is not viable for salmon aquaculture in its present formulation, but our results suggest further modifications to the diet that could mitigate this effect.

Keywords Chinook salmon · Aquaculture diets · Poultry meal · Corn gluten meal · Carotenoids · Astaxanthin

Abbreviations

HPLC–MS	High-performance liquid chromatography mass spectrometry
MtBE	Methyl tert-butyl ether
PIT	Passive integrated transponder
YIAL	Yellow Island Aquaculture Limited

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Introduction

Salmonid aquaculture has historically relied on feeds that include fishmeal as the principal ingredient (Gatlin et al. 2007). However, many fisheries that support fishmeal production are in decline, leading to decreased availability and increasing costs of fishmeal and fish oil (Gatlin et al. 2007). There is thus a need to identify alternative protein sources that can serve as a replacement for fishmeal in salmonid feeds. Ultimately, the feasibility of incorporating alternative protein sources into salmonid feeds will depend on the effects of those feeds on key aquaculture performance traits such as survival, growth rate, fat content and tissue colour.

A number of studies have shown that survival is not affected by incorporating alternative protein sources into aquaculture diets. For example, plant-based protein sources had no effect on survival in Atlantic salmon (*Salmo salar*) (Mundheim et al. 2004). Diets that incorporated poultry protein sources also had no effect on survival in rainbow trout (*Oncorhynchus mykiss*), even when fish were experimentally challenged with a pathogen (Sealey et al. 2011). Similarly, diets that replaced fishmeal with a combination of poultry and plant-based ingredients were associated with no change in survival in either Atlantic salmon (Burr et al. 2012) or rainbow trout (Burr et al. 2012; Lu et al. 2015).

Arguably, the most important trait when assessing alternative feeds in aquaculture is growth rate. Feeds that include plant protein sources, such as soybean meal or corn gluten meal, have previously been shown to lead to reduced growth rate in Atlantic salmon and rainbow trout (e.g. Mambrini et al. 1999; Mundheim et al. 2004). Feeds that include poultry protein sources have shown mixed effects on growth; replacing 40–50% of fishmeal with poultry meal had no effect on growth rate in rainbow trout (Steffens 1994; Sealey et al. 2011), but replacing 30% of fishmeal with poultry meal led to significantly lower growth rates in Chinook salmon (*Oncorhynchus tshawytscha*) (Fowler 1991). Combining poultry and plant-based protein sources has shown promising results: Atlantic salmon diets that replaced 50% of fishmeal with soy and poultry meals (Hatlen et al. 2015) or rainbow trout diets that replaced 75% of fishmeal with soy, corn and poultry meals (Lu et al. 2015) led to growth rates equal to the control diets. Based on these results, salmonid diets that include a mixture of plant and poultry proteins sources appear to have the potential to maintain normal growth rates in aquaculture.

Replacing fishmeal with alternative protein sources can result in altered body composition, specifically fat content, which influences tissue texture, flavour and subsequently market appeal (Gatlin et al. 2007; Bjerkeng et al. 1997). Plant protein sources have a low crude lipid content, which must be supplemented, commonly using fish oil, to create a balanced fatty acid profile (Gatlin et al. 2007; Parés-Sierra et al. 2014). In contrast, poultry meal has a high crude fat content, and its inclusion in alternative aquaculture diets at levels as low as 25% has led to increased body fat in Atlantic salmon and rainbow trout (Sealey et al. 2011; Steffens 1994). The effect on body fat content of diets that use a combination of plant and animal protein sources have not yet been assessed.

A major concern in diet formulation for salmonids is the maintenance of red flesh colouration. Astaxanthin is the primary carotenoid responsible for this red colouration and is also a major precursor to vitamin A (Anderson 2001; Saez et al. 2016). In previous studies of rainbow trout, diets that included plant protein sources, mainly corn gluten meal, were associated with reduced tissue colouration relative to fishmeal diets (Saez et al. 2014, 2016). This difference may occur because corn gluten meal contains high levels of yellow xanthophylls (lutein and zeaxanthin; 150–500 mg/kg) (Park et al. 1997; Moros et al. 2002), which do not impart the same red flesh colouration and could limit astaxanthin uptake due to passive interference during digestion (Furr and Clark 1997; Olsen and Baker 2006). The effects of diets containing poultry meal on salmonid flesh colouration have not been assessed, but minimal effect on tissue colouration is predicted because poultry meal contains few carotenoids that could limit astaxanthin uptake due to passive interference (Moreno et al. 2016). The effects of diets containing both corn gluten meal and poultry meal on flesh colouration are uncertain.

Currently, there are no studies assessing the efficacy of a combination protein source diet for Chinook salmon aquaculture. In this study, we examine the effects of using a combination of corn gluten meal and poultry meal to replace 75% of the fishmeal in an aquaculture diet fed to Chinook salmon. Specifically, we analyse survival, growth rate and tissue quality (total fat percentage and colouration).



Materials and methods

Experimental fish

Experiments were conducted at Yellow Island Aquaculture Ltd. (YIAL), which is a Chinook salmon farm located on Quadra Island, British Columbia. In fall 2013, milt was collected from ten male Chinook salmon from each of eight locations in British Columbia ($n = 80$ total males). The males were collected at Chilliwack River, Puntledge River, Nitinat River, Big Qualicum River, Robertson Creek, Capilano River, Quinsam River and YIAL. Eggs were collected from 17 female Chinook salmon from YIAL and pooled. The eggs were then divided into groups of approximately equal numbers, with each group fertilized with the milt of a different male. This design resulted in a total of ten families for each of the eight lines (i.e. paternal locations). The fertilized eggs were reared in Heath incubation trays separated by family until emergence, after which the families from each line were pooled and then moved to 190 L cylindrical tanks (8 tanks per line). Water was continuously aerated, and temperature was maintained at 7–9 °C by a flow-through system. Fish were kept on a 12 h light:12 h dark photoperiod and fed to satiation twice daily using Chinook grower feed (Taplow Feeds Inc., Vancouver, British Columbia).

Experimental diets

Two diets were created in collaboration with Taplow Feeds. The control diet was a high fishmeal diet that is the standard Chinook grower feed produced by Taplow Feeds. The experimental diet was a low fishmeal diet designed to match the control diet apart from replacing 75% of the fishmeal with poultry meal and corn gluten meal (Table 1). Astaxanthin from the algae *Haematococcus pluvialis* was added to both diets at a concentration of 60 ppm as in the Chinook grower feed produced by Taplow Feeds. The low fishmeal diet also removed 25% of the fish oil and increased wheat content by 25%. To obtain a crude analysis of diet composition, a sample of each diet was ground to a fine powder and analysed in a Spectrastar 2400 NIR Spectrometer (Unity Scientific Inc.) The crude analysis showed that fat, protein and fibre content of both diets were similar (Table 1). Vitamin A and vitamin E contents were higher and vitamin B content was lower in the high fishmeal diet compared to the low fishmeal diet (Table 1).

Table 1 Ingredients and proximate composition of the high fishmeal and low fishmeal diets

	High fishmeal diet	Low fishmeal diet
<i>Ingredients</i>		
Fishmeal (%)	59	15
Fish oil (%)	20	15
Wheat (%)	20	25
Poultry meal (%)	0	16
Corn gluten meal (%)	0	28
Vitamin premix (%)	1	1
Astaxanthin (PPM)	60	60
<i>Proximate composition</i>		
Protein (%)	40	40
Fat (%)	24	25
Fibre (%)	3	2
Vitamin A (IU/kg)	8000	3000
Vitamin B (IU/kg)	2000	3000
Vitamin E (IU/kg)	200	135



Growth and survival

In August 2014, fish were PIT tagged to identify individuals. From September 7 to 14, 2014, the tanks were transitioned from freshwater to saltwater. Interval 1 growth rate measurements began on November 24 and 25, 2014. Fish were anesthetized using a clove oil bath, the PIT tag was read and then total body length and body mass measurements were recorded. After measurements were completed, all fish were moved into two saltwater net pens with 184 fish in the first pen and 196 fish in the second. Both pens remained on the Chinook grower feed (Taplow Feeds Inc.) and were fed twice daily. On December 15, 2014, the second net pen was switched to the low fishmeal diet and these individuals remained on this diet for the duration of the study. On June 25 and 26, 2015, fish were moved from the net pens to the 190 L tanks in the hatchery, at which point total body length and body mass measurements were repeated for the end of interval 1. Fish were held in the hatchery for 4 weeks for a separate study of metabolic rates, during which time they continued to be fed the same diet but growth rate was not calculated. Total body length and body mass measurements were taken on July 26, 2015 to start interval 2 and fish were then moved to the net pens, switching the pens of the fish receiving the high and low fishmeal diets. On March 17 and 18, 2016 the final total body length and body mass measurements were taken for the end of interval 2 and fish were euthanized with an overdose of clove oil for tissue analysis. Mortalities were collected three times a week for the duration of the study. Mortality within each diet treatment was calculated for interval 1 and 2, and the hatchery interval from June 2015 to July 2015, based on the number of surviving fish.

Tissue analysis

After euthanization, full body fillets were removed, filleting over the rib bones to ensure removal of the membrane. Total body fat percentage was measured using a Distell fish fat meter (FFM 692; Distell, Scotland, UK) as the average of four readings on the skin side of each fillet, along the lateral line. Tissue colour analysis was performed using the DSM SalmoFanTM (© 2011 Koninklijke DSM N.V.). The colour was analysed using the 20–30 colour index (20 being low red values and 30 being high red values). Fillets were placed skin side down, on a white non-reflective baseboard and observed under indirect sunlight. The fan was closed, then fanned directly above the centre of the fillet in three sections (anterior, dorsal and posterior), and the number corresponding to the most similar colour chip was recorded. This process was repeated for the second fillet from the same fish. Two observers performed independent colour readings for each fish. These colour readings were averaged to obtain whole fillet colouration. Finally, a sample of tissue was taken from the right fillet of each fish, directly in front of the dorsal fin, and immediately frozen for subsequent carotenoid analysis.

Carotenoid analysis

Carotenoid content was measured in both diets and in a subset of fish from each diet treatment. Carotenoids were extracted after Garner et al. (2010), as described below. All solvents were cooled on ice prior to extraction to prevent retinoid and carotenoid degradation. Three replicates were analysed for each of the high fishmeal and low fishmeal feeds. A total of 37 individuals were analysed for the low fishmeal diet and 36 individuals for the high fishmeal diet. Tissue samples were thawed and homogenized, and then retinoic acid was included as an internal standard to correct for extraction efficiency. The tissue samples were extracted three times in acetone and concentrated, then extracted six times with methyl tert-butyl ether (MtBE) and concentrated, then sealed in an amber glass vial.

HPLC–MS analysis was carried out on an Agilent 1260 HPLC coupled with a 6230 TOF–MS (Agilent technologies, USA). Shortly after extraction, samples (25 μ L) were injected onto a Poroshell 120 EC-C18 column (3.0 \times 50 mm, 2.7 μ m, Agilent technologies, USA) and eluted with a solvent gradient as follows: an isocratic flow of solvent “A” (methanol–water, 8:1) was maintained at 100% from time 0–4 min, followed by a linear gradient to 100% Solvent “B” (methanol–hexanes, 4:1) over 2.5 min followed by an isocratic flow at 100% for 10 min before the mobile phase was switched over to 100% A and maintained for an additional 7 min for equilibration before the next injection. The HPLC flow rate was 0.5 mL/min and the column temperature was maintained at 25 °C. Retinoic acid and astaxanthin were detected at 325 nm and 480 nm, respectively, prior to infusion into the TOF. For LC–MS measurements, the LC was coupled to the TOF–MS



with an atmospheric pressure chemical ionization (APCI) interface, operating in positive ionization mode. Nitrogen was used as the drying gas at a flow rate of 5 L/min, and was maintained at 350 °C with a nebulizer pressure of 60 psi. The Corona needle was operated at 4 μ A (5235 V). The capillary was operated at 6.1 μ A with a cone voltage of 3500 V. The vaporizer chamber temperature was set to 350 °C, and dry gas temperature was held at 350 °C. Astaxanthin in sample extracts was identified and compared using the chromatographic retention times and full-scan mass spectra derived from an astaxanthin reference standard (Sigma 0982). Retinoic acid was included in sample extracts as an internal standard, and was identified and compared alongside a reference standard (Sigma R2625). Both standards were prepared and diluted to 0.01 mg/mL and run alongside the samples under the same conditions. Retinoic acid ($C_{20}H_{28}O_2$, exact mass 300.4351) reference standard was found at 301.2171 m/z ($[M + H]^+$), while the astaxanthin ($C_{40}H_{52}O_4$, exact mass 596.3865) reference standard was found at 597.3847 m/z ($[M + H]^+$). Astaxanthin levels in the samples were then calculated as the area of the astaxanthin absorbance peak divided by the mass of the sample and corrected using the area of the internal standard (retinoic acid) peak.

Statistical analysis

Growth rate for each interval was calculated as $GR = (\ln(W_1) - \ln(W_0)/T) \times 100$, where W_0 is the individual's initial body mass, W_1 is the individual's final body mass, and T is the number of days between body mass measurements. Growth rate, SalmoFan colour values, carotenoid concentrations and tissue fat percentages were each analysed using an ANOVA with diet (high fishmeal and low fishmeal) as a fixed factor. Line was included as a random factor in the ANOVAs. Survivorship was analysed using a Fisher's exact test to compare the number of survivors and mortalities between the two diet treatments within each sampling period. Pearson's correlation was used to compare the colour card values with corresponding astaxanthin tissue values. All statistical analyses were performed using R (V.0.99.484, RStudio, Inc.; www.rstudio.com) or JMP (v4.0.4, SAS Institute Inc., Cary, NC, U.S.A.).

Results

At the start of the study, neither total body length nor body mass differed between the fish assigned to the two diet treatments (Table 2). At the following measurement point (June 2015), individuals on the high fishmeal diet were 3% longer than individuals on the low fishmeal diet ($F_{1,308} = 4.56$, $p = 0.035$; Table 2). There was no significant difference in body length for the remaining measurement points in the study (Table 2). Body mass did not differ between diet treatments during the entirety of the study (all $p > 0.05$) (Table 2). There was no significant difference in growth rate between fish from the two diet treatments during the first interval

Table 2 Body mass and total body length of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fishmeal or low fishmeal diets at four sampling points

	November 2014	June 2015	July 2015	March 2016
<i>Body mass (g)</i>				
High fishmeal	27.3 \pm 5.9	184.6 \pm 49.8	184.3 \pm 54.0	498.5 \pm 118.0
Low fishmeal	27.5 \pm 7.0	172.9 \pm 50.9	190.2 \pm 62.7	467.4 \pm 131.2
Test statistics	$F_{1,366} = 1.05$ $p = 0.30$	$F_{1,309} = 1.14$ $p = 0.28$	$F_{1,189} = 0.11$ $p = 0.74$	$F_{1,99} = 3.01$ $p = 0.08$
<i>Body length (mm)</i>				
High fishmeal	140 \pm 9	262 \pm 27	268 \pm 24	355 \pm 34
Low fishmeal	140 \pm 10	255 \pm 30	261 \pm 27	341 \pm 50
Test statistics	$F_{1,366} = 0.05$ $p = 0.82$	$F_{1,309} = 4.56$ $p = 0.035$	$F_{1,189} = 3.01$ $p = 0.09$	$F_{1,99} = 2.70$ $p = 0.12$

Test statistics (F statistic, degrees of freedom, p value) are included for the ANOVAs comparing body mass and body length between diets at each measurement point. Means \pm SD are presented

($F_{1,311} = 3.62$, $p = 0.058$; Fig. 1a) or the second interval ($F_{1,91} = 1.48$, $p = 0.23$; Fig. 1b). There was no significant difference in survival during any of the measurement intervals (all $p > 0.05$) (Table 3).

The diets had a significant effect on multiple aspects of salmon tissue quality (tissue fat and tissue colouration). Individuals fed the low fishmeal diet had 25% higher total body fat percentage than those fed the high fishmeal diet ($F_{1,91} = 4.58$, $p = 0.035$; Fig. 2). Individuals on the low fishmeal diet also had lighter coloured flesh than those on the high fishmeal diet ($F_{1,91} = 33.51$, $p < 0.001$; Fig. 3a). Carotenoid analysis was consistent with the tissue colour results; astaxanthin levels in salmon fed the low fishmeal diet were significantly lower than in salmon fed the high fishmeal diet ($F_{1,64} = 10.98$, $p = 0.002$; Fig. 3b). As expected, there was a significant positive correlation between red colouration and astaxanthin levels in the flesh ($F_{1,70} = 53.76$, $p < 0.001$; $r^2 = 0.43$). LC–MS analysis of carotenoid content in feed pellets showed no significant difference in astaxanthin levels between the high and low fishmeal diets (Mean \pm SD; high fishmeal diet = 0.93 ± 0.24 absorbance/mg feed; low fishmeal diet = 1.57 ± 0.62 absorbance/mg feed; $F_{1,4} = 2.80$, $p = 0.17$).

Discussion

Aquaculture diets that replace fishmeal with plant or animal meal could influence the health and survival of fishes if they do not provide an adequate source of all essential nutrients. In previous studies, alternative diets that replaced fishmeal with a combination of poultry and plant-based ingredients were associated with no change in survival (Burr et al. 2012; Lu et al. 2015). In our study, Chinook salmon were fed a diet that replaced 75% of fishmeal with a combination of corn gluten meal and poultry meal, and we similarly found no difference in survival between diets. Alternative aquaculture diets likely have not been found to affect survival because these diets are specifically formulated to provide all essential nutrients. Indeed, the alternative diet in our study had the same composition of protein, fat, and fibre as the high fishmeal feed, which is formulated based on the needs of salmonids (National Research Council 1993). Overall, reduced survival does not appear to be a barrier to the inclusion of plant or animal meal in alternative aquaculture diets for salmon.

Aquaculture diets that include plant or animal meal can also affect growth rate. Our study found no difference in growth rates in Chinook salmon fed the alternative diet versus the conventional diet. These results are consistent with previous studies that found that diets containing a combination of poultry meal and

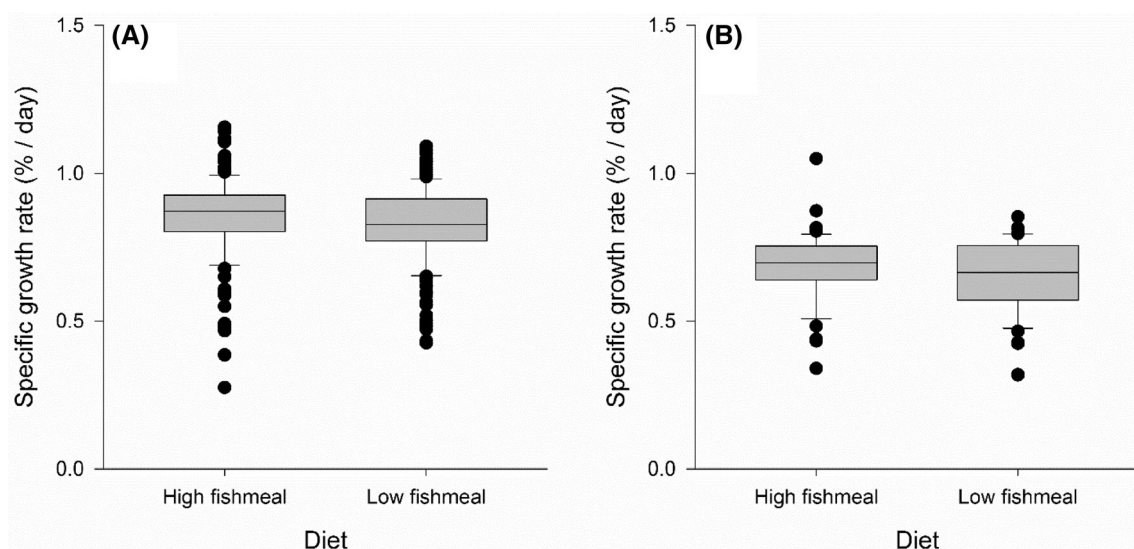
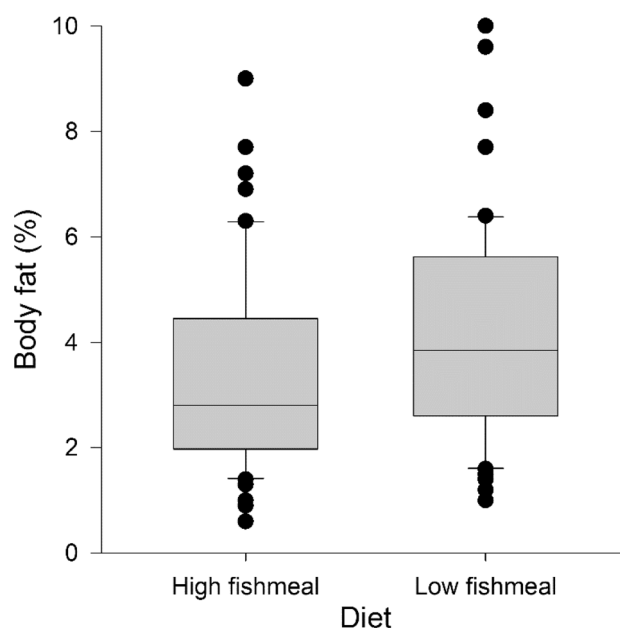


Fig. 1 Specific growth rate (% body mass/day) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either a high fishmeal or low fishmeal diet. Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the 10th and 90th percentile. Individuals outside of this range are indicated with a black dot. **a** Interval 1, which occurred from November 2014 to June 2015, and **b** interval 2 which occurred from July 2015 to March 2016. Growth rate did not differ significantly between diets for either interval

Table 3 Survivorship of Chinook salmon (*Oncorhynchus tshawytscha*) fed either the high fishmeal or low fishmeal diet during three intervals. Significance is based on a Fisher's exact test comparing survivors and mortalities between diets at each measurement time

	Diet	Total	Survivors	Mortalities	% Survival	Significance
November 2014–June 2015	High fishmeal	192	154	38	80.2	0.89
	Low fishmeal	185	150	35	81.0	
June 2015–July 2015	High fishmeal	154	82	72	52.9	0.08
	Low fishmeal	150	65	85	42.9	
July 2015–March 2016	High fishmeal	82	50	32	60.5	0.11
	Low fishmeal	65	48	17	73.4	
Total	High fishmeal	192	50	142	26.0	1.00
	Low fishmeal	185	48	136	25.9	

**Fig. 2** Total body fat (% body composition) of Chinook salmon (*Oncorhynchus tshawytscha*) fed either a high fishmeal or low fishmeal diet. Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the 10th and 90th percentile. Individuals outside of this range are indicated with a black dot. Fish fed the low fishmeal diet had significantly higher body fat than those fed the high fishmeal diet

other plant protein sources result in growth rates comparable to standard fishmeal diets (e.g. Burr et al. 2012; Hatlen et al. 2015). The success of these diets that combine poultry meal and plant protein sources is likely because combinations of protein meals produce a more balanced amino acid composition and increased digestible protein:energy ratio than either plant or animal protein meal only. Indeed, plant protein sources generally have lower levels of lysine, methionine, arginine, and tryptophan compared to fishmeal, whereas poultry meal has a similar amino acid composition to fishmeal (Mente et al. 2003; National Research Council 2011). Together, these data suggest that poultry meal in combination with a plant-based meal can be incorporated at high levels into alternative aquaculture diets with no adverse effect on growth rate.

Plant-based meal and poultry meal differ from fishmeal in fat content and fatty acid composition, which could influence body fat in fishes. We found that fish fed a diet that included poultry and corn gluten meal had 25% higher total body fat than fish fed the high fishmeal diet. This difference in body fat occurred despite the similar fat content of the two diets (High fishmeal = 24%, Low fishmeal = 25%). However, the diets differed in fatty acid composition: both poultry meal and corn gluten meal are low in omega-3 fatty acids, poultry meal



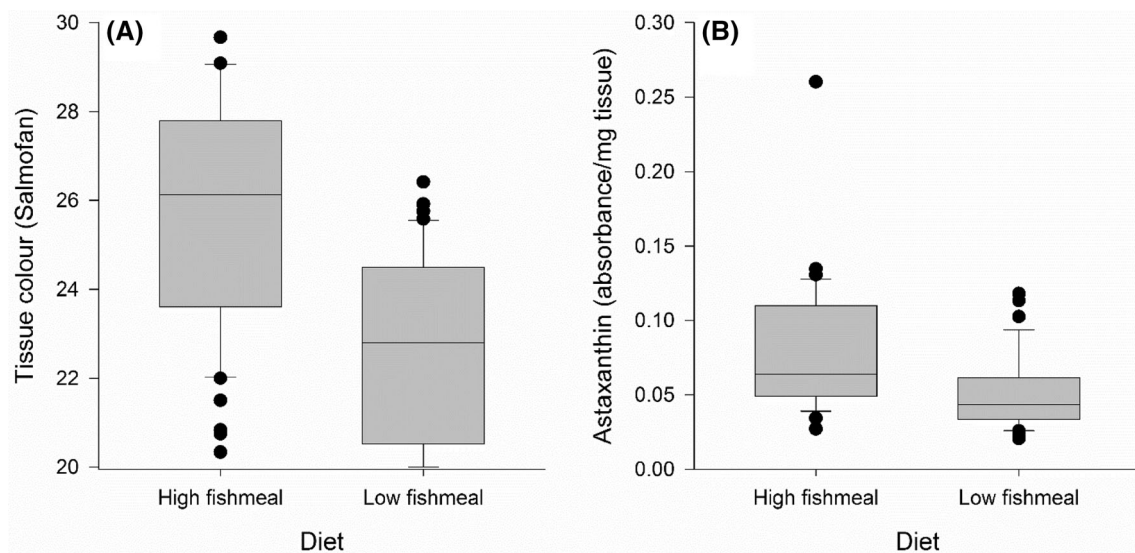


Fig. 3 Tissue colour and astaxanthin levels for Chinook salmon (*Oncorhynchus tshawytscha*) fed either a high fishmeal or low fishmeal diet. Boxes show the 25th and 75th percentile with a line indicating the median. Whiskers show the 10th and 90th percentile. Individuals outside of this range are indicated with a black dot. **a** Tissue colour based on the SalmoFan colour card measurements, which use a scale of 20 (least red) to 30 (most red). **b** Astaxanthin levels as the absorbance/mg tissue after correcting for sample extraction efficiency using an internal standard. Both tissue colour and astaxanthin levels were significantly different between diets

is rich in saturated and monounsaturated fatty acids, whereas fishmeal is rich in omega-3 and polyunsaturated fatty acids (National Research Council 2011). Salmon require high levels of polyunsaturated fatty acids in their diets and low levels of saturated fatty acids (Parés-Sierra et al. 2014; Sargent et al. 1999). Saturated fatty acids that are not required for other functions may be more likely to accumulate in tissues such as muscle. Differences in fatty acid composition between the diets may have led to increased fat storage in the fish fed the diet containing the poultry meal and corn gluten meal substitution. Regardless, the low fishmeal diet did not lead to a reduction in total body fat.

In salmonids, diets containing corn gluten meal have been associated with reduced concentrations of astaxanthin and the associated red tissue colouration (Saez et al. 2014, 2016). Consistent with these findings, in our study the individuals fed the alternative diet that contained corn gluten meal had a significant reduction in tissue redness and tissue astaxanthin levels relative to the fish fed the high fishmeal diet. Astaxanthin was added to both diets in equal concentrations and HPLC analysis showed no difference in astaxanthin levels between the diets, so a difference in diet-based astaxanthin levels cannot explain the deficiency. Instead, the difference in tissue colouration may occur because of dilution from novel xanthophylls present in the alternative feed. Similar to astaxanthin, these novel xanthophylls are absorbed in the gut and deposited in the flesh, but these xanthophylls do not impart the same distinctive red colour (Furr and Clark 1997; Olsen and Baker 2006). Based on previously reported concentrations of xanthophylls (lutein, zeaxanthin) in corn gluten meal (150–500 mg/kg; Moros et al. 2002; Park et al. 1997) and a 28% contribution of corn gluten meal to the low fishmeal diet, our low fishmeal diet likely contained these xanthophylls at concentrations that were similar to astaxanthin (estimated 40–140 mg/kg for the xanthophylls versus 60 mg/kg for the astaxanthin). Passive interference by other xanthophylls may have contributed to an overall dilution of astaxanthin levels in our study.

Another explanation for decreased tissue colouration could be that the two diets led to differences in astaxanthin utilization. Up to half of the astaxanthin absorbed by salmon can be metabolized to Vitamin A (Torrissen et al. 1989), especially when dietary levels of vitamin A are low (Goodwin 1986; Matsuno 1991). Our alternative diet had 60% less vitamin A (3000 IU/kg) compared to the high fishmeal diet (8000 IU/kg). Dietary requirements for vitamin A in *Oncorhynchus* species are reported to range from about 2500 to 7000 IU/kg (De Silva and Anderson 1994). It is conceivable that the alternative diet provided insufficient Vitamin A, leading to greater conversion of astaxanthin to vitamin A and consequently reduced astaxanthin



concentrations in the muscle tissue. Overall, our study adds to the growing evidence that the inclusion of corn gluten meal in salmon diets is associated with reduced astaxanthin levels in the flesh, albeit the specific mechanism leading to this effect is not yet resolved.

Due to the importance of tissue colouration in marketability of salmon, a corn gluten and poultry meal diet combination cannot be used in its present formulation in salmon aquaculture. Corn gluten meal is a cost-effective and readily available ingredient, but appears to be the cause of reduced tissue astaxanthin concentrations and colouration. Given the potential contribution of astaxanthin utilization for vitamin A synthesis, further study is warranted to determine if the addition of greater concentrations of vitamin A to the alternative diet can mitigate the effect on colour. A side from this adverse effect, our study adds to the growing evidence that poultry meal, in combination with another plant protein source, is a viable alternative to high concentrations of fishmeal in salmonid diets.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable national and institutional guidelines for the care and use of animals were followed by the authors.

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