Colouring of Atlantic salmon with natural pigments

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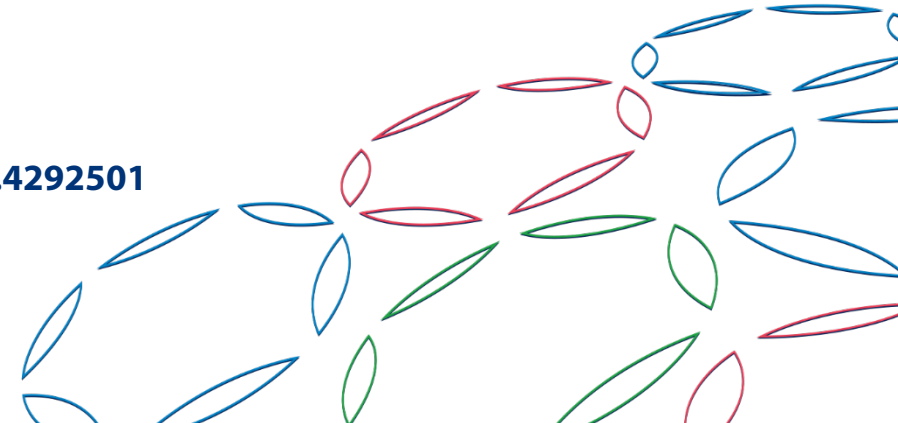
Skýrsla Matís 21-20
Nóvember 2020
ISSN 1670-7192
DOI 10.5281/zenodo.4292501



Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	**Colouring of Atlantic salmon with natural pigments**		
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Skýrsla / Report no.	21-20	*Útgáfudagur / Date:*	November 2020
Verknr. / Project no.	2004 2360		
Styrktaraðilar /Funding:	AVS Research Fund		
Ágrip	Könnuð voru áhrif mismunandi litarefna og styrks litarefna í fóðri fyrir Atlantshafslax á holdlit, eftir eldi í 438 daga í tilraunakerjum. Litarefnið Panaferd AX, sem er unnið úr bakteríunni Paracoccus carotinifaciens, var blandað í mis miklum styrk í tilraunafóður og og áhrifin metin. Til viðmiðunar var notað efnagerða litarefnið Lucantin® Pink, sem algengt er fóðri fyrir eldislax. Upphaflega átti einnig að prófa litarefnið Aquasta® en það hvarf af markaði og því var það eingöngu notað í fyrsta hluta (af þremur) tilraunarinnar. Í seinni hluta tilraunarinnar var því eingöngu samanburður á hold-litun með Panaferd AX og Lucantin Pink. Í upphafi rannsóknarinnar var kannað hversu mikið tapaðist af litarefni við framleiðslu tilraunafóðursins. Athugunin sýndi að við notkun á Panaferd AX varðveittist aðeins 76,9% af íblönduðu astaxanthini en 100+% þegar Lucantin Pink var notað. Því var brugðið á það ráð að bæta 30% meira litarefni í fóðrið umfram það sem ráðgert var í upphaflega tilraunaplaninu. Einnig var framleitt fóður án litarefnaíblöndunar og því blandað við tilraunafóðrið. Þannig samræmdist gefið magn litarefnis í hverja meðferð upphaflegri áætlun. Vöxtur, fóðurtaka, fóðurnýting og lifun var eðlileg í öllum hópum. Áhrif mismunandi litarefnainnihalds í fóðri á holdlit voru mæld með DSM Salmofan®, Konica Minolta CR-400 Chroma Meter og með efnagreiningu á astaxanthini og litarefnum sem þekkt eru í Panaferd. Niðurstöðurnar sýnda lítinn mun á milli meðferða, í holdlit mælt með Salmofan og Konica Minolta Chromo Meter. Sýnilegur holdlitur var vel ásættanlegur skv. Salmofan mati, yfir 26 við lok tilraunarinnar. Lítið samhengi var milli efnagreinds styrks litarefna og sýnilegs litar. Niðurstaða: allar prófaðar litunaraðferðirnar og bæði litarefnin sem rannsökuð voru gáfu svipaðan og ásættanlegan holdlit í laxflökum.		
Lykilorð á íslensku:	*Holdlitun; Lax, Náttúrlegt litarefni*		

Summary in English:	Different colourant and colourant inclusions in diets for Atlantic salmon and its effect on flesh colour was tested. Experimental diets of similar nutritional composition, but different colourant sources and inclusions were produced in commercial fish feed mill (Laxa ltd.). Panaferd-AX colourant was compared to standard colouring regime using Lucantin® Pink (30/60/50mg/kg) as a control. Atlantic salmon were fed these diets in a growth trial in experimental tanks in saline water for 438 days. The original plan was to test the colourant Aquasta® in addition but since its production was halted, it was only used in the first phase of the experiment. The continuing experiment compared Panaferd in different inclusion and Lucantin® Pink. Initially The recovery of the colorants in the experimental feed production was tested. Using Panaferd, the recovery of astaxanthin was only 76,9% after feed processing and extrution compared to 100+% recovery using Lucantin. Therefore the experimental diets were added plus 30% colourantsto ensure astaxanthin level according to feed analyses. To adjust planned colourant consumption the feeding was mixed with unpigmented basal diet. The growth, feed intake and feed conversion were similar between groups, and no mortalities were related to treatments during the experimental period. The flesh colour was measured by DSM Salmofan®, Konica Minolta CR-400 Chroma Meter and by chemical analyses of astaxanthin and sum of carotenoids. Only small differences were found in visual filet pigmentation, measured by Salmofan and Konica Minolta Chromo Meter, between the different colouring regimes. The filet colour was considered acceptable in all treatment groups. The correlation between chemically analysed content of carotenoids, including astaxanthin, and visual colour score of the filets was poor. In conclusion: all tested regimes and carotenoid content in the diets for Atlantic salmon resulted in acceptable fillet colour.
English keywords:	*Colouring of flesh; Atlantic Salmon, Natural colorants*

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Introduction

The characteristic pink colour of the flesh is one of the most important quality criteria for salmonid fishes (Sigurgísladóttir et al., 1997) and determines consumer acceptance. Salmonids, like other animals, are unable to biosynthesize carotenoids. In salmon aquaculture the diets are supplemented with carotenoids to obtain flesh colour typical for the species and to meet their requirements.

Chemically synthesized and formulated astaxanthin (typically containing 10% astaxanthin) is currently the most widely used colorant in salmonid fish farming. In recent years, new products are based on biological manufacture of astaxanthin and are available in the market. One such product is Ecotone™ (ADM, Decatur IL, USA) made from the red yeast Phaffia rhodozyma (or Xanthophyllomyces dendrorhous) and is registered for use in the EU. This product is accepted as a colorant in organic production of farmed fish in all European countries.

Two other new products defined as organic colorants have been approved by EU. Those are Aquasta® (Igene Biotechnology, Inc., Columbia, USA) made from the yeast Phaffia rhodozyma and Panaferd- AX (JX Nippon Oil &Energy Corporation (NOE), Tokio, Japan) containing carotenoids from the bacterium Paracoccus carotinifaciens. Ecotone™ is however not available in the market but seems to be replaced by Aquasta®. The dominating carotenoid in Aquasta is Astaxanthin while Panaferd additionally contains significant amounts of the carotenoids Adonirubin, Cantaxanthin and other carotenoids that can add to the reddish/ pink colour of salmonides. In addition to the effect on flesh colour the carotenoids are also important antioxidants and might therefore positively affect the shelf life of the fish, post mortem.

To our knowledge, very limited informations are available on the efficiency of the new biological colorants compared to the synthetic colorants such as Lucantin® Pink, 10%. In a recent experiment synthetic formulated astaxantin (Lucantin® Pink, 10% astaxanthin; BASF, Germany) and Ecotone™ in two diets was compared in a feeding trial with Atlantic salmon (Bjekeng et al., 2007). Astaxanthin from P. rhodozyma was considerably more efficient for muscle pigmentation than Lucantin® Pink. Astaxanthin from P. rhodozyma cells(Ecotone™) had 86% higher retention than the synthetic astaxanthin in the control treatment (Lucantin® Pink). The difference can be explained by higher apparent digestibility coefficient of astaxanthin originated from the red yeast (64-68%) compared to 38-42% in the salmon fed the synthetic control (Bjerkeng et al., 2007). Practical production scale trials

on Atlantic salmon and rainbow trout have also shown positive effect of Ecotone™ on flesh colouring (information from ADM).

In Icelandic salmon culture the experience of using Panaferd as a colorant in salmon diets has apparently resulted in less coloring of salmonides compared to use Aquasta in the diets. However, no direct comparison has been made on these two sources of colorants to verify this conclusion.

The aim of the experiment was to compare the colouring effect different commercial colorants, Lucantin® Pink, Panaferd- AX and Aquasta®,as well as different schemes for administration in Atlantic salmon. Original plan was to compare the three different pigment sources in different regimes as shown in Table 2. However, during the course of the trial Aquasta was not obtainable on the market so the research plan had to be revised to the plan shown in Table 3.

Materials and Methods

Diets

The basis formulation of the diets was reflecting the commercial salmon diets produced by Laxa feedmill Ltd, Akureyri, Iceland (Table 1). Formulation of all diets was restricted to fulfil all nutritional requirement of the fish in terms of crude protein and amino acids, using tabulated values for the amino acids in the raw materials. Moisture content in the diets was in the range of 5 – 7% . The lipid content was 23 – 25% in the DM. Diets of different pellet size were formulated isonitrogenous and isoenergetic, in terms of gross energy (GE). The average estimated gross energy content was 25 MJ/ kg in the DM.

Table 1. Basal ingredient composition of the experimental diets

	Eco 3.0	Eco 4.0	Eco 6.0
Ingredients %:			
Fish meal	40,9	29,8	32,5
Corngluten meal	15,0	20,0	10,0
Fish oil	14,3	17,0	20,7
Wheat	10,0	10,2	10,0
Wheatgluten meal	8,7	1,9	4,0
Soya Hipro	4,5	10,0	7,0
Rapeseed oil	4,3	4,8	6,9
Rapeseed meal	0,0	4,0	6,8
Laxa Fish feed premix	1,0	1,0	1,0
MonoCalsium Phosphate	0,8	1,0	0,8
Calc nutrient composition %:			
Crude protein	49,0	42,0	38,0
Crude lipid	23,0	26,0	32,0
Crude ash	7,5	7,3	6,9
Starch	9,9	10,4	9,1
NFE	13,2	17,0	14,5
Lysine	3,5	2,9	3,0
Methionine	1,2	1,1	1,0
Cystine	0,7	0,6	0,5

Plan of coloration

Originally the plan was to compare three different pigment sources in different regimes as shown in Table 2. However, during the course of the trial, Aquasta was not obtainable in the market so the research plan had to be revised to the plan shown in Table 3. The shift of plan was introduced when the fish was on average 480 grams.

Table 2. Original test plan of colourant inclusion and regimes in diets of different pellet size (mm in diameter).

Group	Fish size Colorant	100 – 200g 3mm	200 – 500g 4mm	600 - 2000g 6/9mm
Feed 1	Lucanthin Pink 10%	30	60	50
Feed 2	Panferd	30	60	50
Feed 3	Aquasta	30	60	50
Feed 4	Aquasta	70	50	30
Feed 5	Aquasta	5	70	30

Table 3. Revised test plan of colourant inclusion in experimental diets of different pellet size (mm in diameter).

3 mm feed		4 mm feed		6 mm feed	
Colorant	Inclusion mg per kg	Colorant	Inclusion mg per kg	Colorant	Inclusion mg per kg
Lucanthin Pink 10%	30	Lucanthin Pink 10%	60	Lucanthin Pink 10%	50
Panaferd	30	Panaferd	60	Panaferd	50
Aquasta	30	Panaferd	30	Panaferd	50
Aquasta	70	Panaferd	50	Panaferd	30
Aquasta	5	Panaferd	70	Panaferd	30

After the shift in pellet size from 3 to 4mm (April 2016) the fish was fed according to the plan shown in Table 3.

Stability of Astaxanthin

After production of the first experimental (3 mm) all diets were analysed for Astaxanthin and other carotenoid content at Nofima Biolab, Bergen, Norway. The comparison of added colourants doses and analysed colourant content showed considerable deviation of astaxanthin during the processing of the diets. To confirm these results another set of 4 mm diets with different inclusions of Astaxanthin were produced and analysed. The results of the analyses are shown in Table 4.

Table 4. Recovery of Astaxanthin after feed production (Extrusion)

	3 mm diet			4mm diet		
Colorant	Asta added mg/kg feed	Asta analysed mg/kg feed	analysed % of added	Asta added mg/kg feed	Asta analysed mg/kg feed	analysed % of added
Lucanthin Pink 10%	30	38	126,7	60	62	103,3
Panaferd	30	22,7	75,7	60	46,8	78,0
Aquasta	30	29	96,7	60	50	83,3
Aquasta	70	49	70,0	50	39	78,0
Aquasta	5	15	300,0	70	55	78,6

Experimental fish, set-up and conditions

The trial was run at experimental facilities of Holar University College in Verið Sauðárkrókur, Iceland. The experimental fish was provided from commercial salmon smolt producer (Islandsbleikja –Núpum), already smoltified and vaccinated, at the average size of 45g at arrival. The fish was from the commercial salmon stock bread by Stofnfiskur ltd/Benchmark. The fish were acclimatized for few weeks (in seawater, T = 8-9°C), and fed commercial feed during that period. At the beginning of the trial a bulked group of fishes were sorted by hand, for homogenizing the fish size in the experimental tanks, 50 fishes in each tank at an average weight in the range of 72-77g (± 10,2-11,7g) respectively. . The fish got 20 days of recovery after this handling until the feeding of the experimental diets was launched (5/10 2015).

The trial was set up as shown in Table 2 and 3 with three replicate tanks per treatment (diet). The experiment was initially carried out in 800 liters rectangular tanks (fish size: 75 – 600 gr) and then moved to circular tanks of 1600 litres (fish size 600– 1600 gr.) in four identical system lines. The average temperature and was 8.4°C in the smaller tanks but 10,8° the bigger tanks. The temperature profile during the trial is shown in Figure 1. The average salinity was 26,2 ppt in the smaller tanks and slightly less in the bigger tanks (23,4 ppt) (Figure 2). Oxygen saturation at the outlet of the tanks was kept above 94% throughout the experiment. The fish was kept at constant light, (24:0) with a light intensity above 50 lux at water surface. The experiment lasted from the 16th of September 2015 to the 11th of December 2016.

The fish, in each tank, was fed to satiation (+10 – 15% over feeding) 6 days a week using automatic continuous 24hrs. feeding. Leftover feed was collected in a pellet trap at the tank outlet. Feed consumption was estimated by counting the number of uneaten pellets once or two times per day and subtracting average weight (as dry feed) from the amount of feed given. The weight (g) and length (cm) of the fish was measured four times during the trial period in addition to the initial- and final weighing. The fish were starved for two days prior measuring day. Mortalities were measured (g and cm) and registered throughout the trial period.

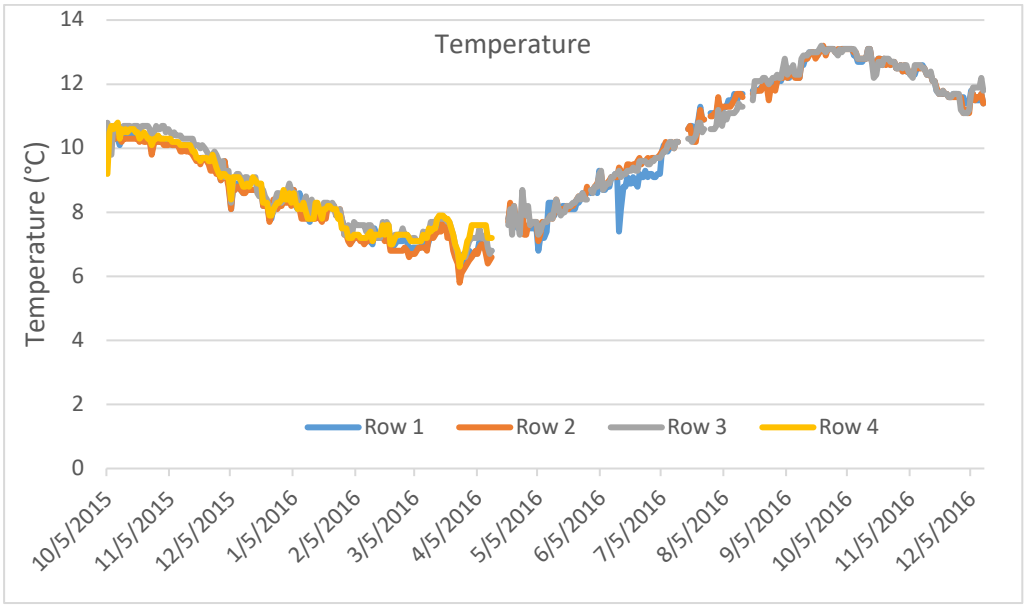

Figure 1. Temperature in the experimental tanks during the trial period. The fish was transferred to the bigger tanks in the middle of April.

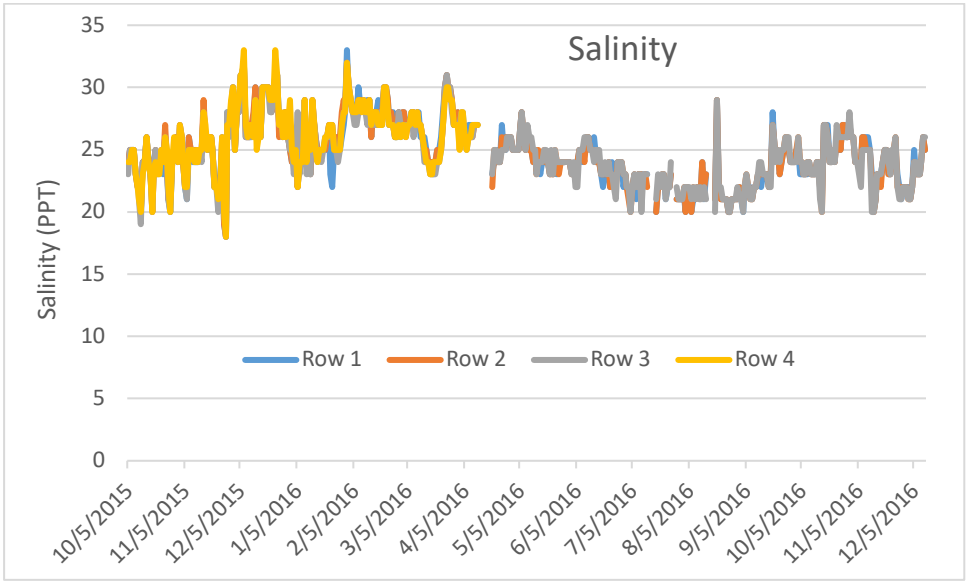

Figure 2. Salinity in the experimental tanks during the trial period. The fish was transferred to the bigger tanks in the middle of April.

Analyses of nutrient content in filet and colouring evaluation

At two occasions, when fish weighed around 500 grams (April 2016) and at the end of the trial, samples of fish (5 fish per tank) were taken for analyses of nutrient composition and colour measurements of the flesh.

Visual colour was estimated, in 15 filets per treatment, using DSM Salmofan®.

The same 15 fillets from each treatment were also analysed by Konica Minolta CR-400 Chroma Meter for the following:

L = difference in lightness and darkness (+ = lighter, - = darker)
a* = red and green (+ = redder, - = greener)
b* = yellow and blue (+ = yellower, - = bluer)

Filets from all the fish (and diets) from each group were pooled, deep frozen at – 80°C and sent for chemical analyses at Nofima Biolab, Bergen, Norway for chemical determination of carotenoids. The carotenoids analysed in addition to Astaxanthin are: β- Caritene, Echinonine, 3-Hydroxyechinenone, Cantaxanthine, Adonirubin, Asteroidenone and Adonixanthin.

Calculations

Specific growth rate (SGR) % day-1 was calculated as:

$$\mathbf{SGR} = \mathbf{100} \times \frac{\ln(w_2) - \ln(w_1)}{feeding\ days}$$

Where W1 is the initial weight and W2 is the final weight.

Thermal growth coefficient was calculated as:

TGC: Thermal growth coefficient: TGC = 1000*($Wf^{1/3}$ – $Wi^{1/3}$)/ day degrees,
where Wf is the final weight and Wi is the initial weight.

The daily feed intake (DFI %) was calculated as:
DFI% = feed eaten /average BM/feeding days %

The feed conversion ratio (FCR) for each tank was calculated as:

$$\mathbf{FCR} = \frac{Feed\ consumed}{increase\ in\ biomass}$$

Results

Intermediate sampling of fish (after the first two growth periods)

In April 2016 15 fish, with average weight 486 grams from each treatment were sacrificed for analytical purposes.

Growth

At this point the fish had grown from 74 grams to 486 grams in 196 days (73+ 123 days), feeding on the experimental diets 3mm pellets according to the originally planned colour regime (Table 2). The average SGR and TGC are shown in Table 5. and the growth curve in Figure 3.

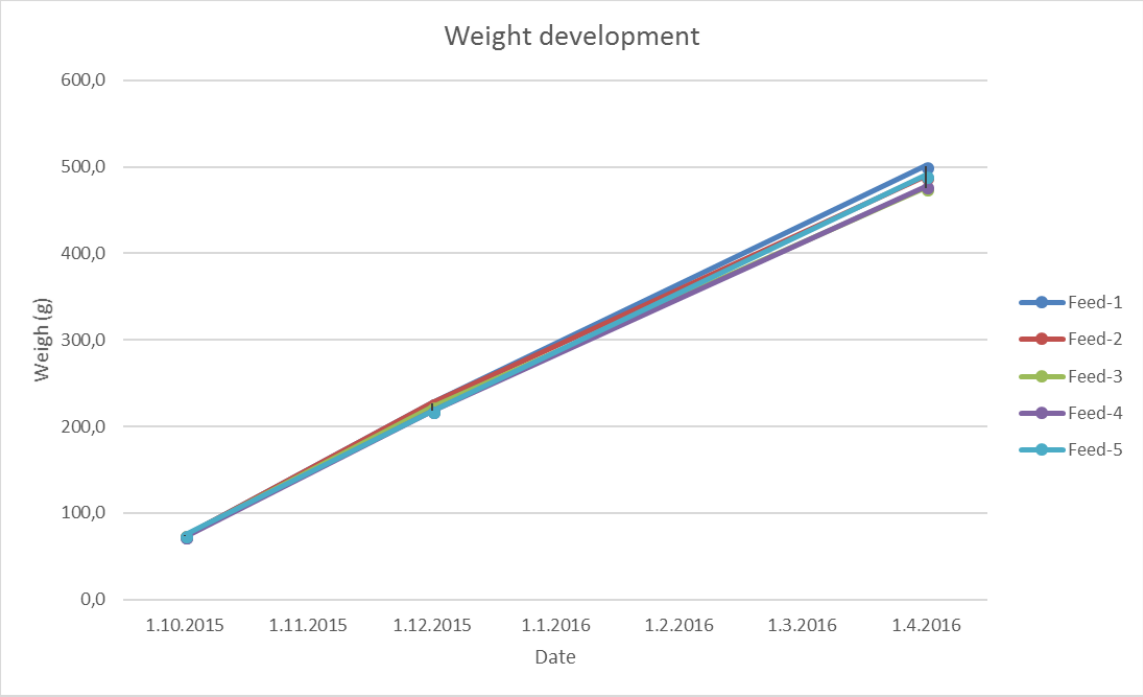

Figure 3. Weight development until immediate sampling

Table 5. Average SGR and TGC for the two first intervals of the experiment

	Average SGR		Average TGC	
Treatment	period-1 (73 days)	period-2 (123 days)	period-1 (73 days)	period-2 (123 days)
Feed-1	1,55	0,64	2,62	1,99
Feed-2	1,53	0,63	2,59	1,93
Feed-3	1,50	0,62	2,55	1,89
Feed-4	1,50	0,64	2,53	1,95
Feed-5	1,46	0,66	2,46	2,02

There is a substantial drop in SGR and TGC from period 1 to period 2 as seen from Table 5. This drop can partly be explained by fluctuations in water temperature during this interval of the experiment (See Figure 1.) as the temperature falls from around 10°C to about 7°C during these 196 days.

Nutrient composition in fillets

As seen in Figures 4. and 5. there appears to be some variation in the protein and lipid content in the filet between the different trial groups. It is hard to find any reason for this variation as the basal diet formulation was similar in all diets except for the type and concentration of colourants.

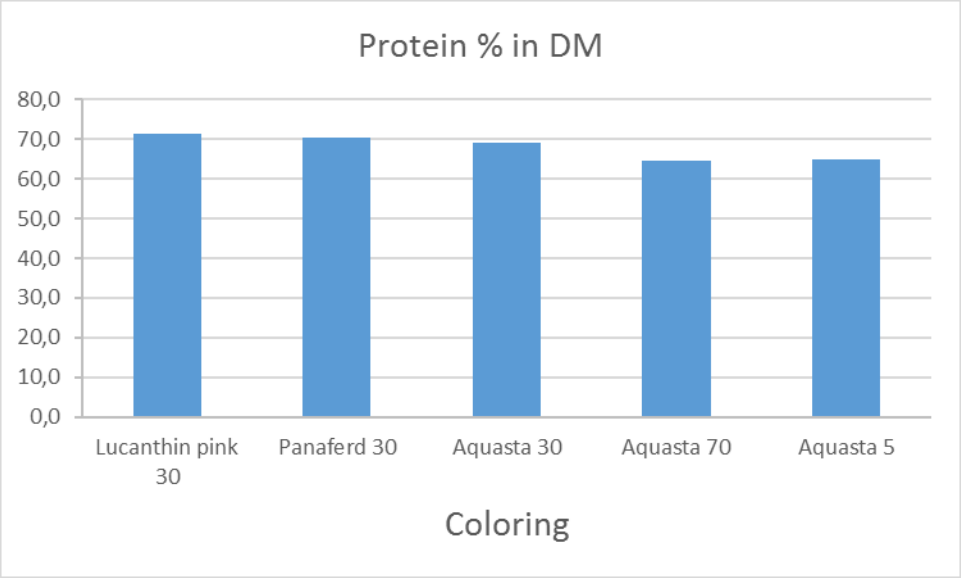

Figure 4. Protein content in fillets (% in DM)

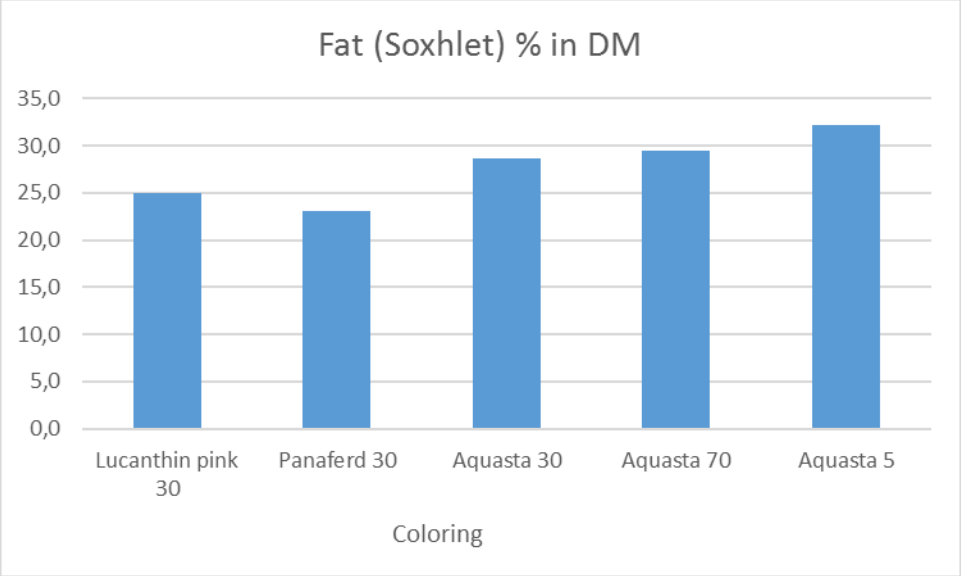

Figure 5. Lipid content in fillets (% in DM)

Colour measurements in filets

Visual colour was estimated, in 15 filets per treatment, using DSM Salmofan™, Konica Minolta CR-400 Chroma Meter as well as by chemical analyses of Astaxanthin in the filets.

As seen in figure 6. the visual colour of the filets in different treatments is similar.

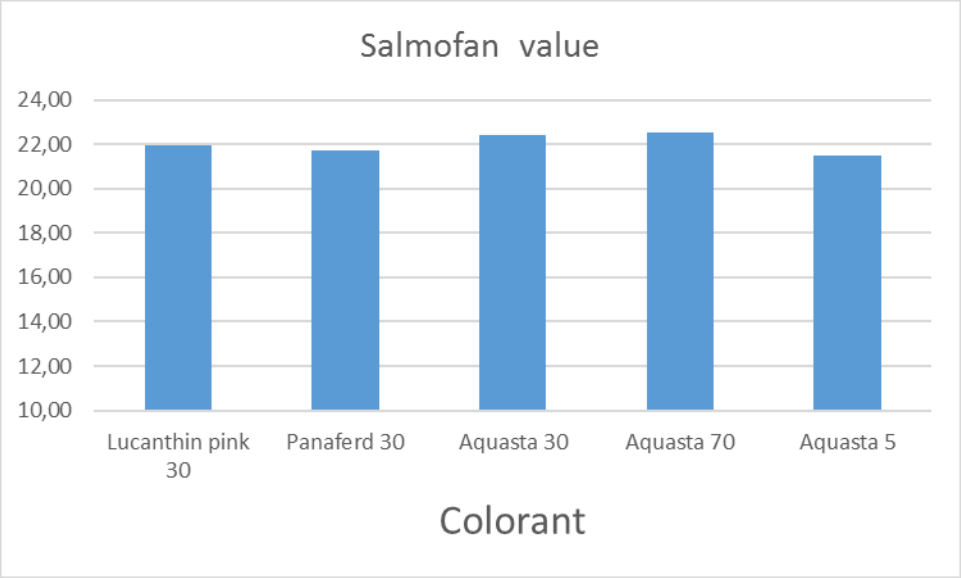

Figure 6. Salmofan value score of salmon fillets, after feeding with different colorants and different astaxanthin content in diets.

The results of Konica Minolta CR-400 Chroma Meter readings are shown in figures 7 – 9. showing the effect of treatments on colour intensity (L), redness (a*) and yellow ness (b*).

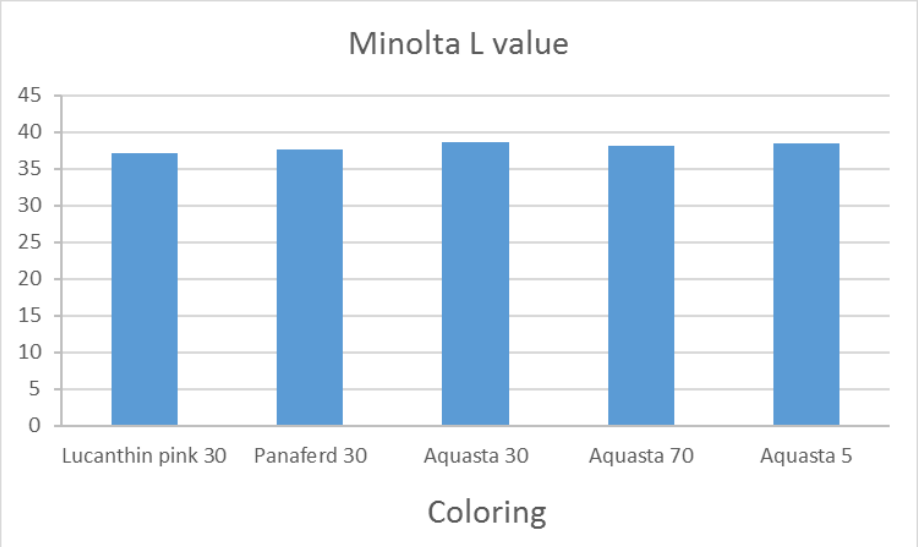

Figure 7. Analysed Konica Minolta CR-400 Chroma Meter L values in fillets of salmon fed different colorants and different astaxanthin content in diets.

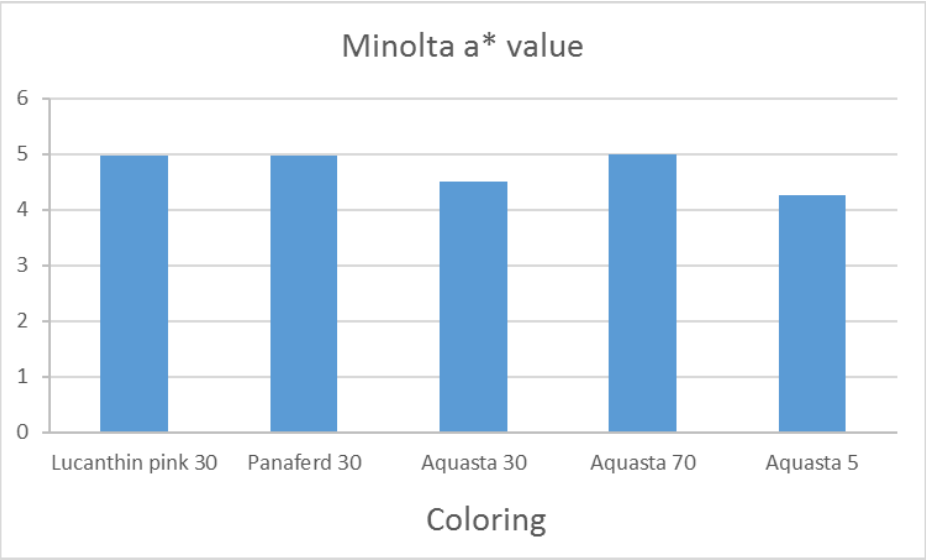

Figure 8. Analysed Konica Minolta CR-400 Chroma Meter a* values in fillets of salmon fed different colorants and different astaxanthin content in diets.

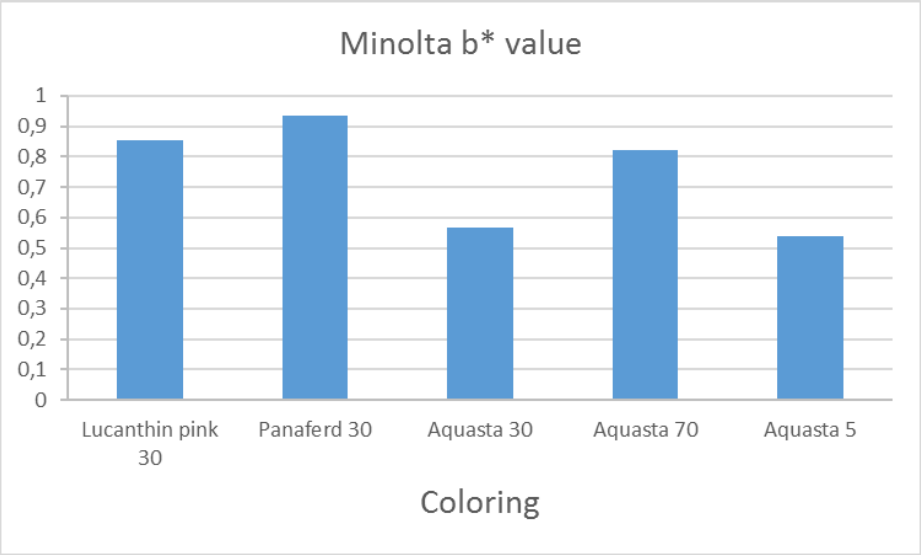

Figure 9. Analysed Konica Minolta CR-400 Chroma Meter b values in fillets of salmon fed different colorants and different astaxanthin content in diets. Numbers refer to mg/kg.*

There seem to be effects of both type of colourant and amount of colorant on the values obtained in the chromameter measurements. Particularly in the Minolta b* value the lowest score is in the groups fed by Aquasta colourant, indicating that Aquasta has less effect of the yellowish colour tone in the fillets compared to Lucanthin and Panaferd

Pooled samples of fillets from each treatment groups were chemically analysed for Astaxanthin content in deboned and skinned fillets. The results are shown in Figure 10.

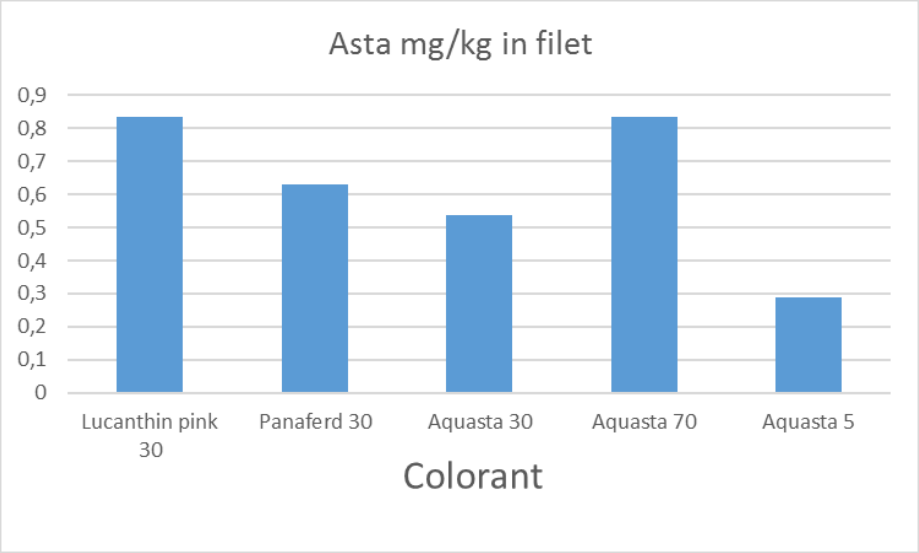

Figure 10. Chemically analysed content of Astaxanthin in filets

The chemical Astaxanthin analysis shows much higher variation than detected in the colour evaluation done with the chromameter and visual Salmofan score analyses. For example, is more than double astaxanthin content in analysed in the Aquasta 70 group compared to Aquasta 5 group but it does not seem to have any particular visual effects when the fillets are compared and scored with the Salmofan scoring test. The chemical analysis seems to have more consistency relation with the Minolta b* value, except for the Panaferd group.

Results from final sampling

Synonyms of Dietary treatments in final sampling:

Feed-1 = Lucanthin 30/60/50
Feed-2 = Panaferd 30/60/50
Feed-3 = Aquasta/ Pana./Pana. 70/50/30
Feed-4 = Aquasta/ Pana./Pana. 30/30/50
Feed-5 = Aquasta/ Pana./Pana. 5/70/30

Growth

Figure 11. shows the weight development of the fish during the 438 days' experimental period. Over all the growth in the experiment can be characterized as normal except for the variability between treatments. Rearing of experimental fishes in small tanks, in gradually increasing density are not absolutely optimal growth conditions, although the oxygen level was kept high throughout the culturing period. The decrease in growth rate after transfer to bigger tanks is most probably linked to the handling and environmental shift as well as decrease in water temperature. A steeper growth curve towards the end of the experiment indicate recovery as well as stimulating growth following increased water temperature. At the final measuring day it is a significant difference in final weight between groups.

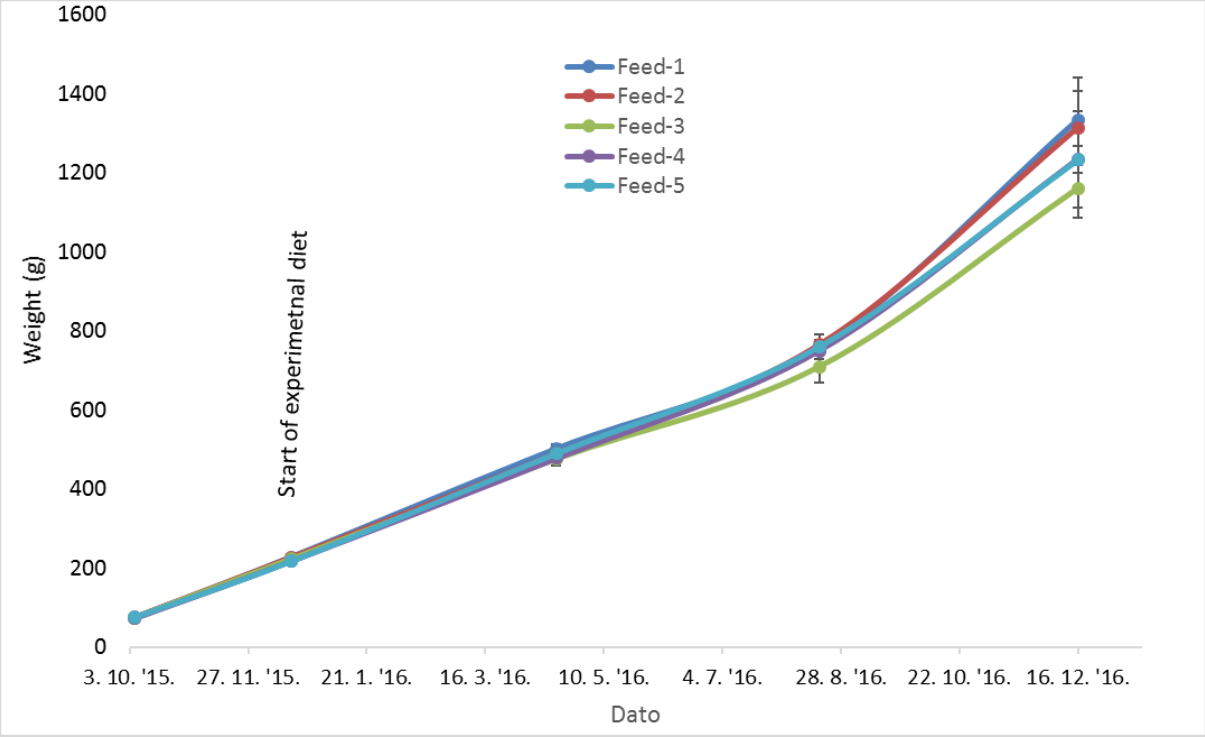

Figure 11. Weight development of Atlantic salmon fed diets containing different colourants and colourant inclusions during the whole trial period. The fish were transported to bigger tanks early in April ´16.

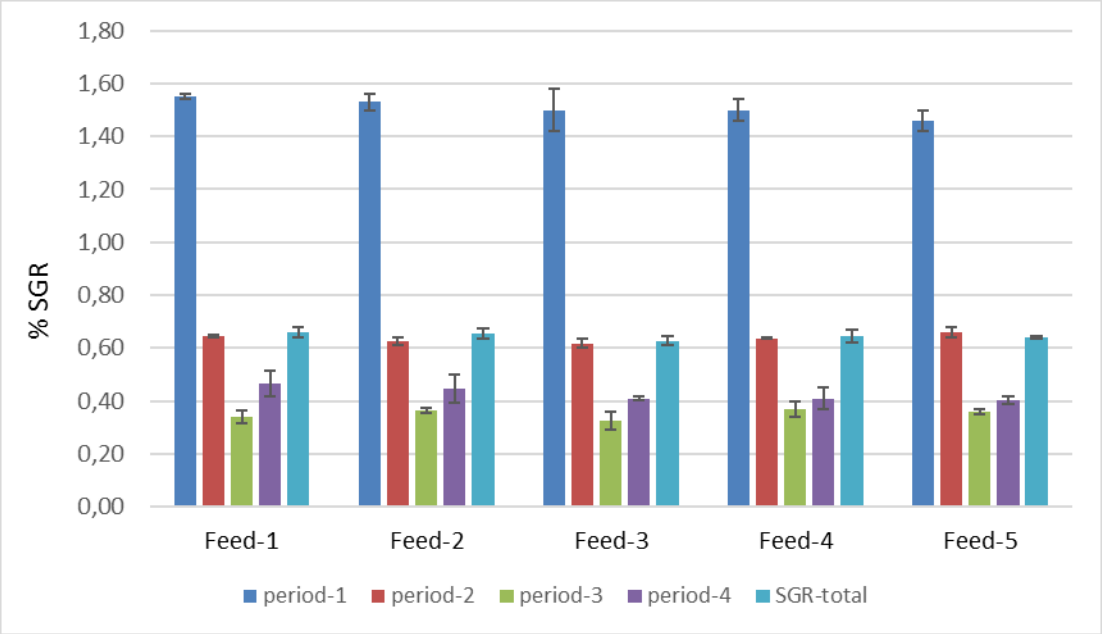

Figure 12. Specific growth rate (%SGR±SEM) of Atlantic salmon in the measuring periods and total average SGR during the 438 day growth period (n=3). The fish was fed with different colourants and colourant content in diets.

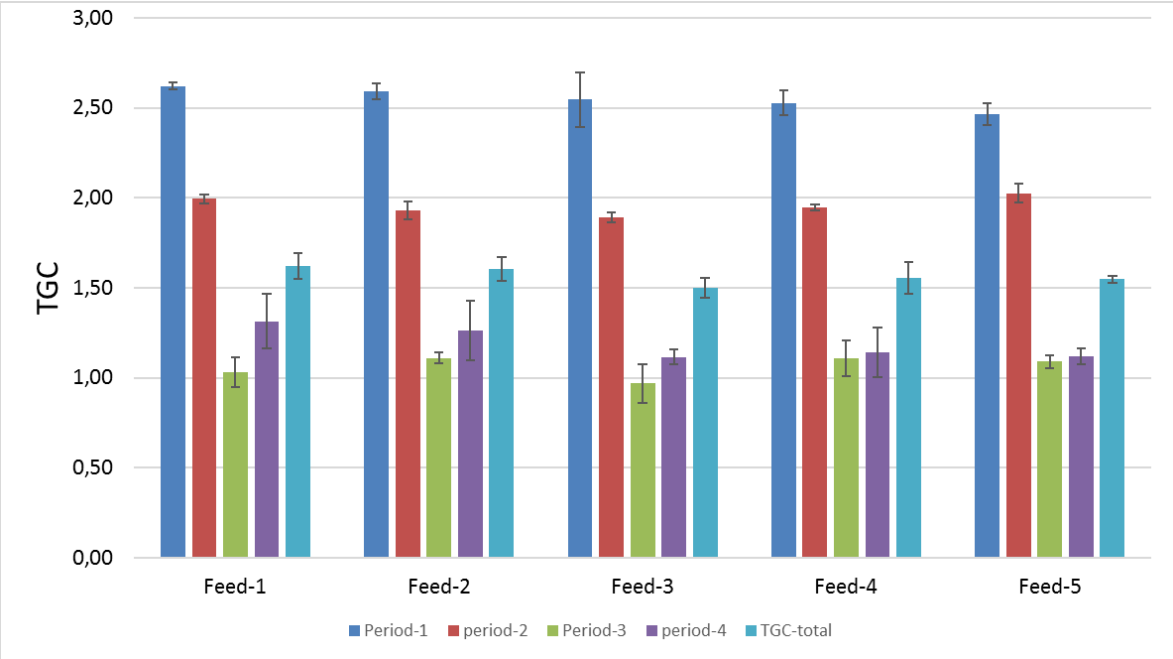

Figure 13. Thermal growth coefficient (TGC3 ± SEM) of Atlantic salmon in the measuring periods and total TGC during the 438 day growth period (n=3). The fish was fed with different colourants and colourant content in diets.

Figures 12. and 13. shows decreasing growth rate (%SGR) in relation to water temperature as well as gradual decrease as the fish grows bigger. In the final period the growth rate is stimulated by increased temperature as well as probable acclimatization to bigger tanks and more favourable culture conditions. The drop in TGC the first periods is indicating semi- optimal culture conditions but at the final growth period the TGC has started to rise again. Overall TGC close to 1,5 seems to indicate some restricted growth, in terms of the fish size.

Feed intake and FCR

Figure 14. shows certain variation between treatments in the daily feed intake DFI%, measured as feed eaten as percentage of average body weight per day, when calculated thorough out the whole trial period. The only difference in nutrient composition between treatments was the inclusion of colourants that is just a minor part of the formulation. A similar variation was found in FCR (figure 15).

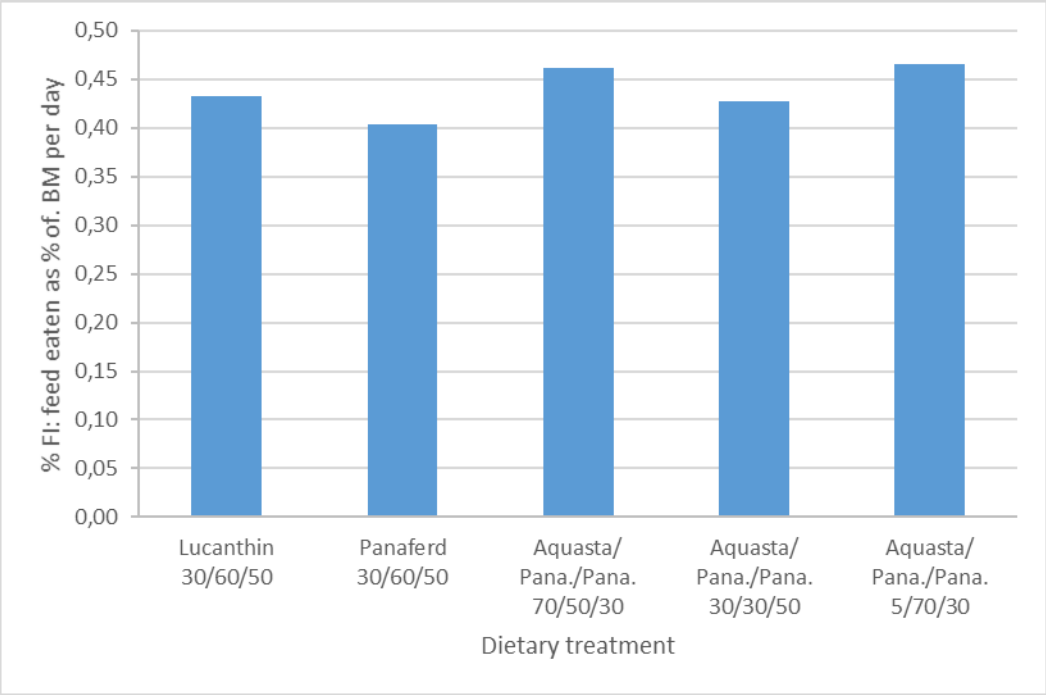

Figure 14. Average daily feed intake as a ratio of fish weight (DFI%) in the trial groups during the overall experiment. (n=3)

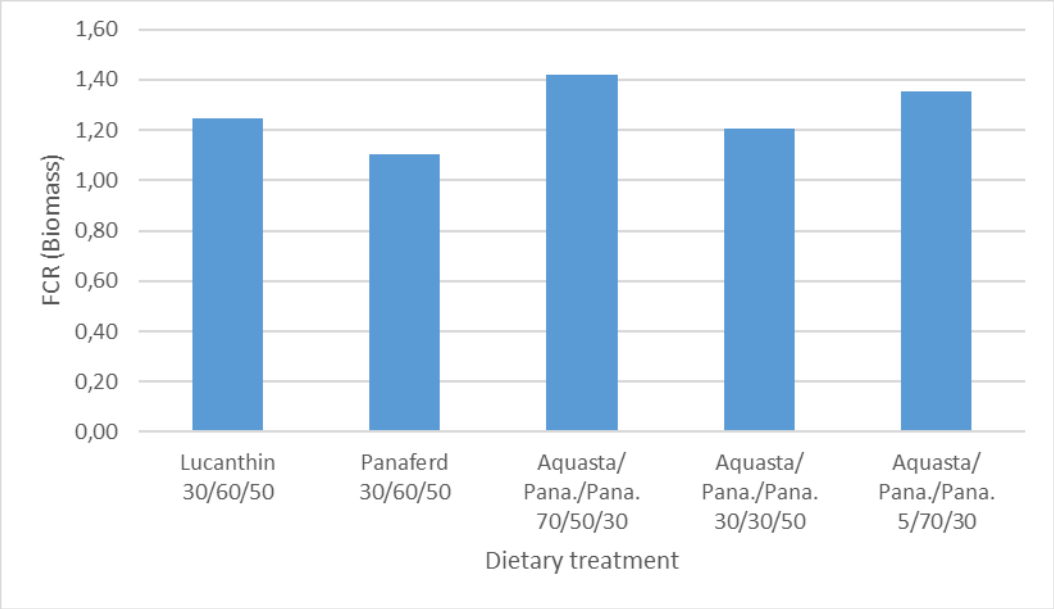

Figure 15. Average feed conversion ratio (FCR) during the overall experiment, for all the treatment groups. (n=3)

Intake of Astaxanthin

In relation of different inclusion of colourants in the diets and variations in amount of feed consumed, it is interesting to estimate / calculate consumed the intake of astaxanthin in different groups. When compared to the weight increase during the growth period the real colourant consumption is quite variable between groups (fig. 16). Although consumed astaxanthin consumption difference is more than 50% it is remarkable this difference is not detected in the comparison of visual fillet colour, according to Salmofan score (fig.18). Similarly the estimate of consumed astaxanthin is not a reflection of analysed astaxanthin content in the flesh (fig.20).

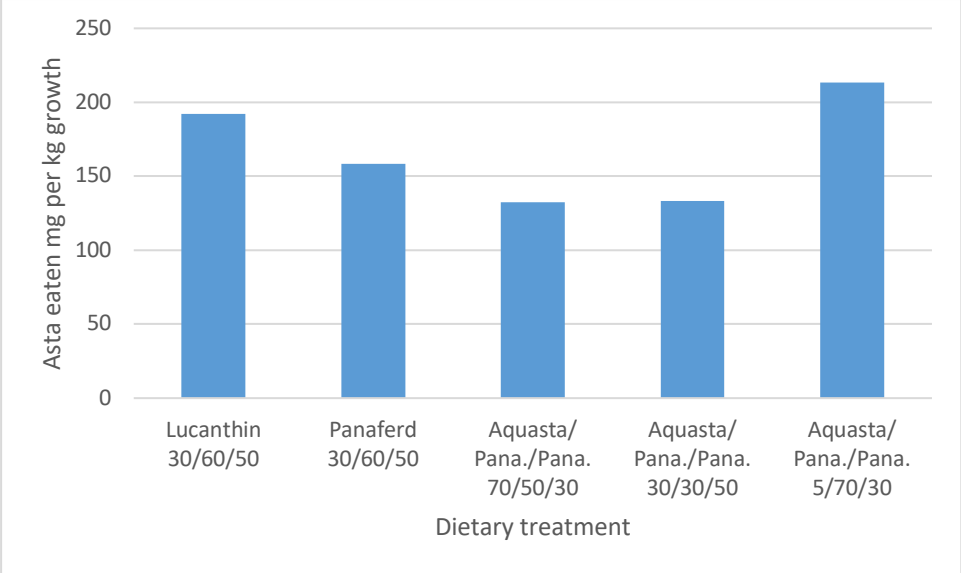

Figure 16. Average intake of Astaxanthin per kilogram of weight increase in the trial groups.

Nutrient composition of Fillets

The dry matter content and dry matter protein and lipid content was measured in the fillets and appeared to be was similar in all groups. The dry matter content was in the range 29,4 – 30.2%, the crude protein content around 68% and lipid content around 30% (fig. 17). Only minor differences were detected between treatment groups.

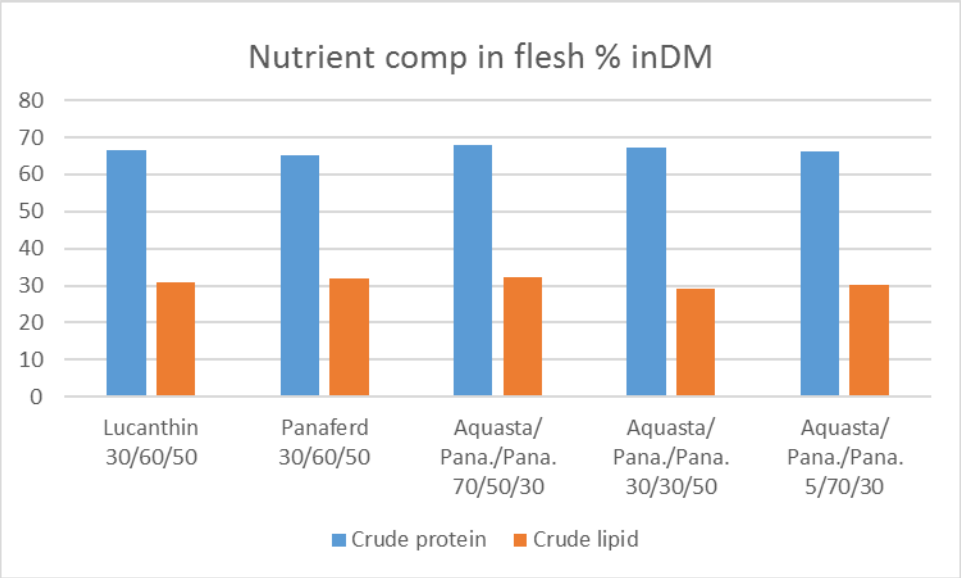

Figure 17. Dry matter protein and lipid content in filets at the final sampling, for the treatment groups.

Colour measurements in filets.

Flesh colour in the final sampling was measured similarly as in the intermediate sampling, i.e. using DSM Salmofan™, Konica Minolta CR-400 Chroma Meter and by chemical analyses of Astaxanthin in addition to other carotenoids contributing to pink flesh colour in the fillets.

The visual colour was evaluated with Salmofan™, detected in constant lighted white background by two personnel. The results of the Salmofan™ (figure 18) indicate in general an acceptable visual colour of the fillets with reasonable variation between treatments (values from 28 - 29).

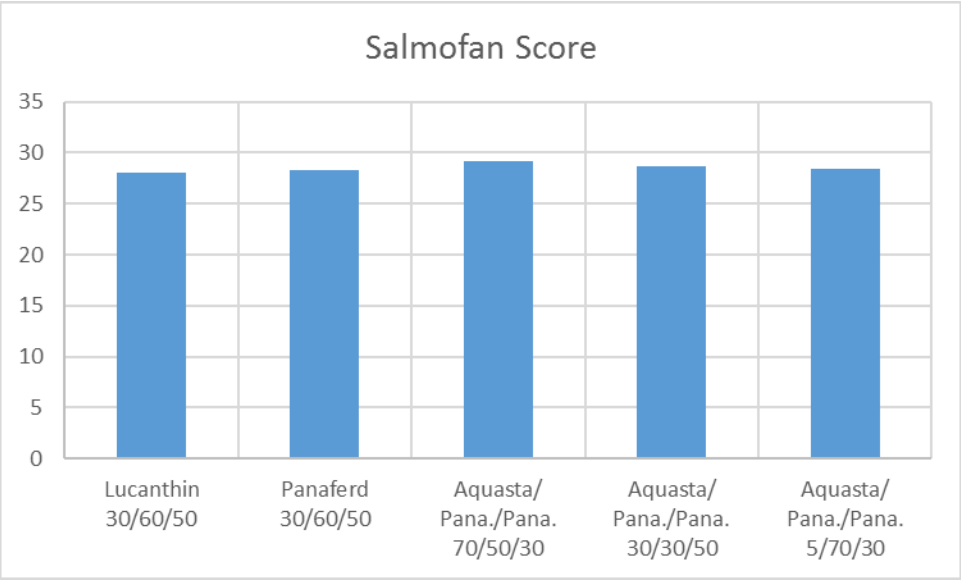

Figure 18. Colour score, indicating visible flesh color measured by Salmofan™ lineal, on all treatment groups. Samples are pooled groups for each treatment (n= 5 x 3).

The colour measurements with the Konica Minolta CR-400 Chroma Meter don't show much variation in the L values (38 – 40) but it appear to be more effect of treatment on the a* and b* values (9,3 – 7,6 and 8,5 – 6,7 respectively) (figure 19).

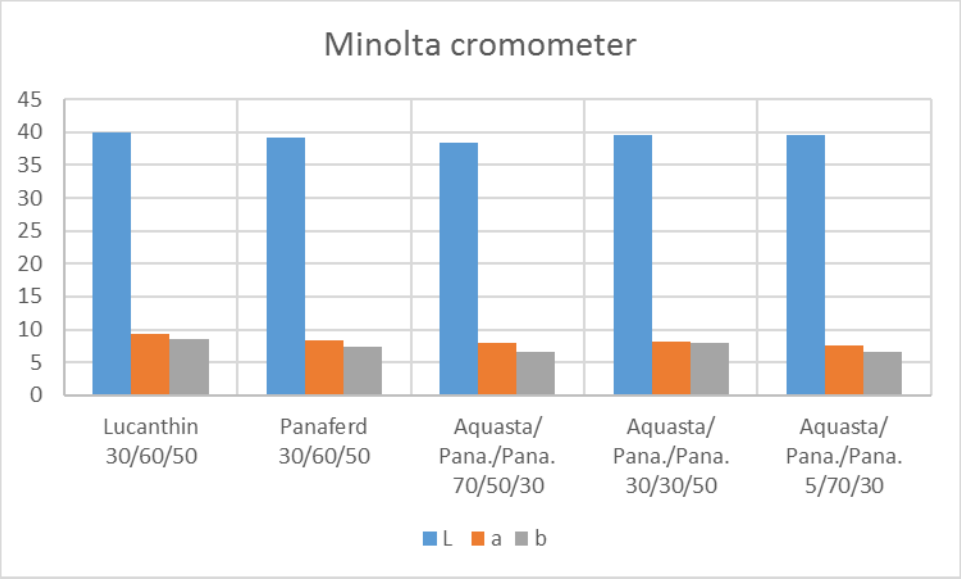

Figure 19. L, a and b* values of Konica Minolta CR-400 Chroma Meter readings of visible filet colour.*

Figure 20. shows the chemically analysed content of Axtaxanthin and total carotenoids in homogenised sample of deboned and skinless filets. The Astaxanthin content is lower in the filets of fish fed the organic colorants. There appears to be limited effects of colouring scheme (different inclusion) on the analysed values of Astaxanthin. The group getting feed with Lucanthin inclusion is primarily fed with astaxanthin as a colorant, but the colourants provided in the other groups contain a mixture of

carotenoids although the astaxanthin is in high proportion. This difference in carotenoid content in the diets is not reflected in the fillet colour measured with Salmofan or Minolta (fig. 18 & 19).

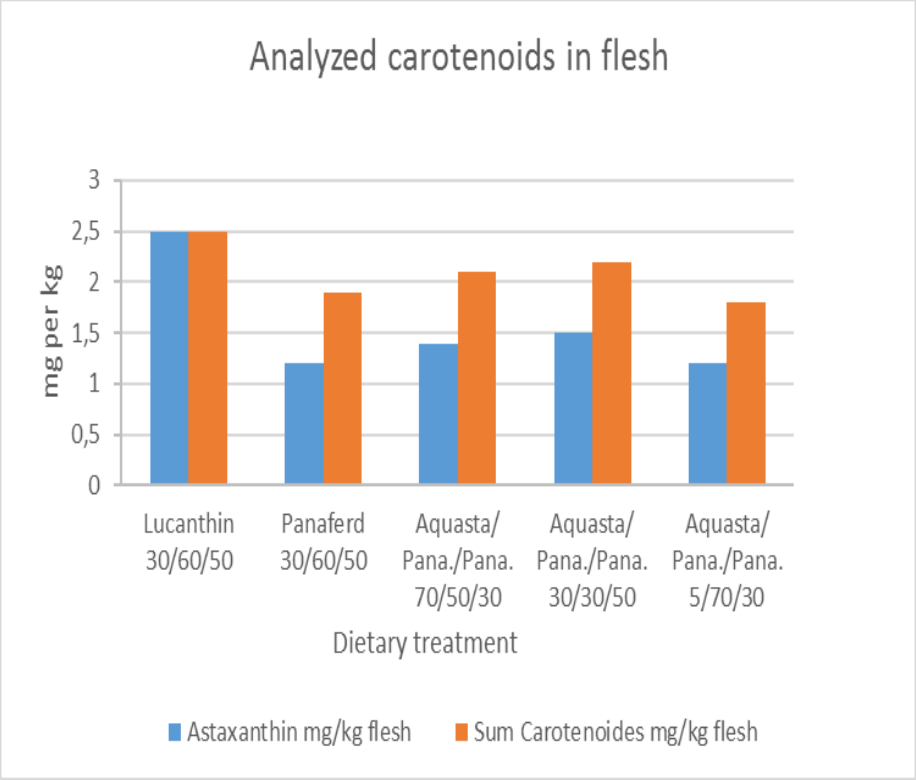

Figure 20. Chemically analysed content of Astaxanthin and sum of carotenoids in fish flesh

Correlation of Astaxanthin consumed and analysed colorants in fillets.

Due to variation in feed intake the overall intake of Astaxanthin during the experimental period varies between treatments (figure 16.). The relationship between ingested Astaxanthin and total carotenoids and Astaxanthin analysed in the filet is shown in figure 21. and figure 22 respectively. As seen in the figures the relation seems to be week, if any, between Astaxanthin intake and sum of colorants in the filet. However, the relationship is somewhat stronger between Astaxanthin intake and analysed Astaxanthin in filet (figure 22).

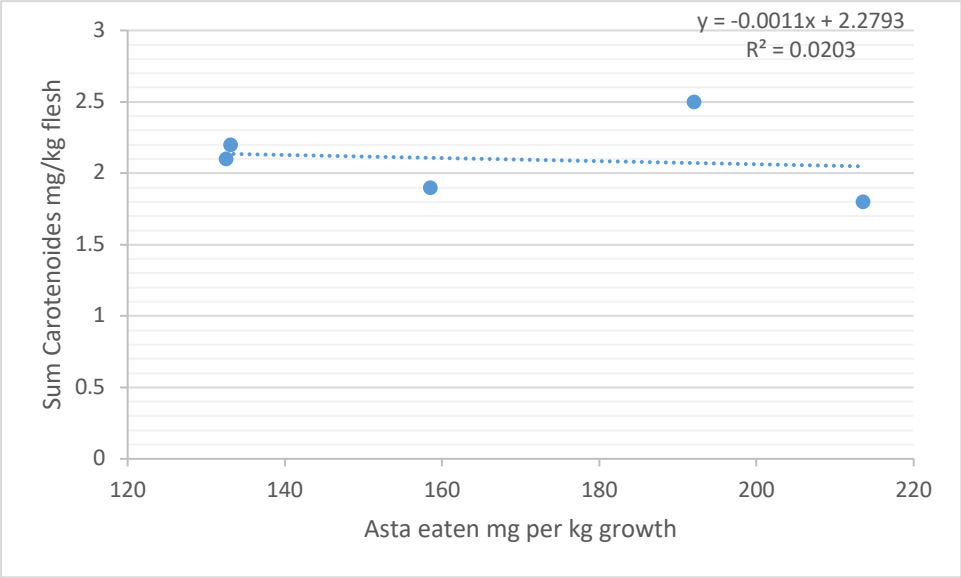

Figure 21. Relation between Astaxanthin consumed (mg/kg growth) and sum of Carotenoids in fillet (mg/kg flesh)

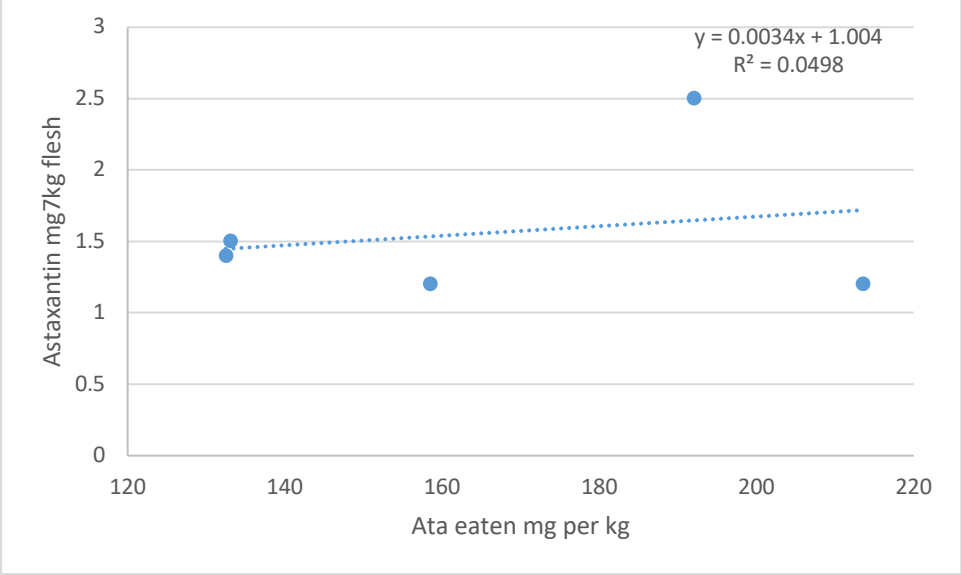

Figure 22. Relation between Astaxanthin eaten (mg/kg) and Astaxanthin in filet (mg/kg flesh)

Discussions

The original plan was to test the effect of dietary inclusion of colourants and three different colouring regimes on fillet colour in Atlantic salmon. The plan was to compare the effect of organic colourant Aquasta®, made from the yeast *Phaffia rhodozyma* (or *Xanthophyllomyces dendrorhous*), the organic colorant Panaferd-AX (JX Nippon Oil &Energy Corporation (NOE), Tokio, Japan), made from the bacterium *Paracoccus carotinifaciens*, and inorganic colorant (Lucantin® Pink, 10% astaxanthin; BASF,Germany) as control . However, during the trial Aquasta was not obtainable so the research plan

needed to be revised and ended up as comparison between different colouring regimes using the organic Panaferd-AX with Lucantin® Pink, 10% astaxanthin as control.

Stability of colorants

The diets were all dosed colorants according to the original plan (See Table 2) based on the producer's declaration for content of Astaxanthin and earlier experience from Laxa Feedmill Ltd on expected production losses of Astaxanthin (10%).The extrusion process involves water, heat, pressure, and mechanical stress, all of which can impact on carotenoid stability. The inclusion level and different types of carotene sources may affect the final carotene level in the feed pellets. Therefore, an evaluation post production is important, also as a quality assurance for the feed production companies. The analysed carotene content in the diet is although not a consistent indicator on its functionality for colouring the fish flesh.

Analyses of the diets showed marked differences in the recovery of Astaxanthin using different colorants in the diets. The recovery of Lucantin® Pink, 10% was above 100% while the recovery of Panaferd-AX was in average 76,9% (75,7-78,0%) and of Aquasta® was in average 81,3% (70,0-96,7%). Analyses of the basal diet (without addition of any colorant) showed content of 6 mg per kg (originated from the fish meal and fish oil).

As a result of variable recovery of colourants in the feed processing, all the experimental diets for were re-produced, containing surplus of Astaxanthin according to the experimental plan. Additionally, a "blanc" diet was produced without any colourant inclusion, but still containing astaxanthin from the fishmeal and fish oil sources (6mg/kg). The experimental diets were then mixed with the "blanc" diet to ensure Astaxanthin content in the fed according to plan.

The findings in this experiment on losses during the feed production, it is necessary to overdose both Panaferd-AX and Aquasta by 15 to 20% due to losses during the extrusion process of the feed pellets.

Growth

All the diets, within each pellet size, in this experiment were produced according to exactly the same basic formulation. The inclusion of colorant is the only variable in the feed (0,03 – 0,96% of raw materials in the formulation). The diets were fed to satiation in triplicate (10 – 15 percent over-feeding). However, the results from the experiment show some difference in growth between treatments. This difference cannot be explained by the trial variables, i.e. the nutrient composition of the diets. Increased growth towards the end of the experiment might be that the fish was moved from 600 litre tanks to 1600 litre tanks in period 3 and also that the water temperature was raising towards the end of the experiment (from 8°C to 13°C).

The protein and lipid content in the filets of the fish was normal with only minor variability between treatments.

Feed and colorant intake

The daily feed intake (DFI%) varied, from 0,4 to 0,47% of life weight, between treatments and similar variation is also seen in FCR. These results are strange as the only variable in the diet formulation is different addition of colorants, which has no logical connection to variation in feed intake. However, this variability in feed intake influences the intake of astaxanthin per kg of growth in the experiment (Figure 16). As seen from the figure there a considerable variation in intake of astaxanthin per kilogram growth.

Flesh colour

The flesh colour was measured at two intervals, on day 196 in the experiment, at average weight of 486 grams and at the end of the experiment on day 438 at average weight of 1254 grams. Three different methods were applied to evaluate the flesh colour: DSM Salmofan®, Konica Minolta CR-400 Chroma Meter and chemical analyses of Astaxanthin both at day 196 (1stsampling) and at day 438 (2nd sampling) and in addition sum of carotenoids in the 2nd sampling.

Intermediate sampling

At the first sampling there was a limited variation between treatments in DSM Salmofan® score. The average colour score was 22 with a variability from 21,5 and 22,5 between treatments. The highest value was registered in fish feed with 70 mg Aquasta and the lowest value was in the fish fed feed with 5 mg Aquasta while the other treatment was intermediate.

The Konica Minolta CR-400 Chroma Meter readings at the 1st sampling did vary somewhat more in the a* and b* values than the L value. The average value of the L reading was 38 with a minimum reading of 37 and a maximum reading of 39. The average a* value was 4,7 with minimum value of 4.3 and max value of 5,0. The b* value had an average of 0,74 and max and min values of 0,93 and 0,54 respectively. The variability in Minolta values was somewhat more pronounced than that of the Salmofan values in particular in the b* value. The lowest L value was in the group that had been fed the diet with 30 mg Lucanthin pink, but the highest value was found in the groups that had been fed with 30 mg Aquasta. The highest a* value was found in fillets of fish that had been fed diet containing Panaferd 30 but the lowest value after feeding with Aquasta 5. At this point the b* values, group into three groups, with fish fed Aquasta 5 and Aquasta 30 at the lower end (0,54 and 0,57 respecticely), fish fed Panaferd 30 and Lucanthin pink 30 at the high end (0,93 and 0,85 respectively) with the fish fed Aquasta 70 having a value of 0,82. It is therefore hard to see any trend in the Minolta values regarding type of colorant or dosing of colorants.

At this point only content of Astaxanthin (Asta) was analysed chemically in the fillets of the sampled fish. The results of the analyses showed equal content of Asta after feeding with 30 mg luanthin pink and 70 mg Aquaasta (0.84 mg per kg filet), in fish fed diet with 30 mg Panaferd contained 0,63 mg Asta per kg filet, similar to the fish that was fed with a diet containg 30 mg Asta (0,54). Interestingly there seems to be a strong linear relationship between amount of Asta content from Aquasta in the feed and Asta in fillet.

Final sampling

The DSM Salmofan® score at the end of the experiment was on average 28,5 with max and min values of 29,2 and 28,1 respectively, a slightly lower variability than at the 1st sampling. There are no visual effect of treatments on the Salmofan score regardless of difference in in astaxanthin consumption. That is indicating that the visual colour in the filets is saturated. All the treatments resulted in a Salmofan score higher than 26 which is regarded as normal flesh colour of salmon at commercial size.

At the final sampling The colour measurements with Konica Minolta CR-400 Chroma Meter gave an average L value of 39,3 with maximum of 39,9 and minimum of 38,3. These values are in the same range as the values found in the 1st sampling regarding the intensity of the flesh colour. The a* value was on average 8,3 with maximum of 9,3 and minimum of 7,6 at the final sampling. Indicating an increased reddish colour in the fillet compared to the values found at the 1st sampling as the values are higher. The values of the b* values had an average of 7,4 with maximum and minimum of 8,5 and 6,7 respectively. These values considerably higher than at the 1st sampling indicating a more yellow ton in the colour. The highest values in all three parameters are found in fish that got feed coloured with Lucanthin Pink.

At the final sampling the filets were analysed for Astaxanthin and filets of fish that was fed diets with Panaferd was in addition analysed for β Carotene, Echinenone, 3-Hydroxyechinenone, Canthaxanthin, Adonirubin, Asteroidenone and Adonixanthin. These carotenoids can all contribute to the red/pink colour in Atlantic salmon. The average content of Astaxanthin in fillets at the end of the experiment was 1,6 mg per kg filet, maximum 2,5 mg per kg and minimum 1,2 mg per kg fillet. The highest content was found in fillets of fish that had been fed Lucantin pink 30/60/50 as colorant while the lowest amount of astaxanthin was found in the Panaferd 30/60/50 group and that had been fed with Aquasta/Panaferd/Panaferd 5/70/30. Analyses of the sum of the carotenes found in Panaferd, including astaxanthin, in the fish fillets showed an average of 2,1 mg per kg fillet with a maximum and minimum of 2,5 and 1,8 mg per kg respectively. The highest value was found in the fish that had been fed Lucantin pink 30/60/50 as colorant but the lowest value in the fish that had been fed according to the Aquasta/Panaferd/Panaferd 5/70/30 regime.

In general, the correlation between the different assessments of the flesh colour is poor and moderate variability between the different treatments.

All the diets were formulated according to their content of astaxanthin so the content of other carotenoids should add to the red/pink colour as can be seen in the Salmofan score. However, there is no effect of the carotenoids found in the Panaferd groups on the different measurements in the Konica Minolta CR-400 Chroma Meter as the treatment with only Lucantin pink showed the highest values in all the tree parameters.

Effect of the variability of astaxanthin eaten, given as mg per kg weight gain on visual colour measured by the Salmofan score is moderate and the same goes for the measurements in the Konica Minolta CR-400 Chroma Meter. The same is true for the analysed Astaxanthin and sum of carotenoids in the fillet.

Conclusion

At the start of the experiment the recovery of the colorants after feed production was tested. That test showed that the recovery of Panaferd was only 76,9 percent of the colorant added.

There were only small differences found in visual fillet pigmentation (Salmofan score) between the different colouring regimes and the filet colour was acceptable.

There was also poor correlation between chemically analysed content of the different carotenoids, including astaxanthin, and visual colour of the fillets.

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