ISSN 1130–061X
CODEN RIBAEG

UDK 597.553.2.05
Original scientific paper

EVALUATION OF IMAGE ANALYSIS AS A METHOD FOR EXAMINING CARCASS COMPOSITION OF RAINBOW TROUT

B. Rønsholdt, H. Nielsen, J. Færgemand, E. McLean

Summary

Two year groups (1+ and 2+; n = 10/group) of rainbow trout (*Oncorhynchus mykiss*) were sampled from a Danish marine fish farm. Fish were selected to span the same weight range (1+: 2643 — 3875 g; 2+: 2651 — 3912 g). During production (April to November) fish were fed identical commercial diets. Visual fat depots of faster and slower growing animals were examined by image analysis of directly scanned cutlets. No significant ($P > 0.05$) correlation between meat percentage and fish size (length, weight, condition factor, dressed weight) was found, and *t*–tests failed to expose any difference in fat: meat ratio between 1+ and 2+ yr old rainbow trout. However, a significant difference ($P < 0.05$) between variances for the two groups was observed, being approximately four–fold larger for 1+ yr animals compared to 2+ yr. This finding indicates that selection for faster growth results in less uniform products from a compositional perspective. Where specific product specifications should be met, this might complicate production management.

Key words: pigmentation, fat: meat ratio, product quality, growth rate

INTRODUCTION

The three major constituents of food are protein, carbohydrate and fat of which the latter represents the most controversial in Western society. Depending upon origin and class, dietary lipids are known to cause obesity and

B. Rønsholdt, and E. McLean, Aalborg University, Department of Civil Engineering, Laboratory for Aquatic Biotechnology, Sohngaardsholmsvej 57, 9000 Aalborg, Denmark.

H. Nielsen, Aalborg University, Institute of Electronic Systems, Laboratory of Image Analysis, Fredrik Bajersvej 7, 9220 Aalborg Ø, Denmark.

J. Færgemand, Bureau Veritas BVQI Denmark, Oldenborggade 1B, 7000 Fredericia, Denmark

*corresponding author: tel. +45 9635 9411; fax. + 45 9814 2554; e–mail: i5@civil.auc.dk

coronary heart disease in humans (Leaf and King, 1998). At the same time however, food fats are attributed with health benefits and praised for their contribution to culinary sensation. The monitoring and control of lipid accumulation in terrestrial meats has become of increasing interest to the meat industry due to the concerns expressed by a sophisticated and health–conscious marketplace. The meat industry has responded to this predicament by establishing various meat inspection procedures that consider different types of fat deposition *viz.* fat depots (*i. e.,* subcutaneous fat) and fat layers in the meat structure (marbling). The formation of fat depots in meat products may be considered detrimental since production losses are experienced during slaughtering. Extensive fat depoting may also result in a reduction in value of processed meats. Marbling however, contributes to meat flavour and juiciness such that a major objective of the industry has been to achieve optimum processing configurations for final products. This includes production of meat cuts that contain adequate amounts of fat to avoid drying and firming following cooking, while limiting fat content to minimise cooking loss. Similar considerations apply to the fish industry, especially for salmon and trout products.

Fat content and distribution represent important quality traits in salmonids (Gjedrem, 1997) that may be influenced, both beneficially and negatively, by dietary manipulation (Rasmussen *et al.,* 1999). Contemporary aquafeeds tend towards incerased lipid incorporation. The utilisation of such feeds during production has been associated with declining product quality in various species (Bell *et al.,* 1998; Eckhoff *et al.*, 1998; Nortvedt and Tuene, 1998), with fish generally expressing higher fat content in all tissues. To the smoking industry muscle fat storage has importance since it contributes to the development of the sepcific smoked taste, while reducing the risk of product dryness. Visible fat depots may cause losses for the smoked salmon industry since in some countries, fat layers are removed prior to smoking. Some producers even remove major parts of the dark muscle as a consequence of customer specifications. In salmonids fat layers are concentrated in the belly lining as subcutaneous fat, particularly along the sideline, and in the dorsal vertical septum. Very fatty fish may even exhibit fat depots along the vertebral column. These fat layers are visibly distinguishable from the meat, whereas fat stored in the muscle remains indiscernible. Histological analyses (Færgemand, 1993) have shown that fat cells are found in greater numbers in the connective tissue of myofibrillar junctions.

In the meat industry, video image analysis techiques have been developed and are employed commercially to estimate fat/lean ratio (Newman, 1987) conformation, fatness, and fat colour (beef carcass classification: Borggaard *et al.,* 1996). These techniques have been used to evaluate carcass composition (bone, meat, fat) (beef: Karnuah *et al.*, 1994), fat layer size (pork: Newman, 1984a; Branscheid *et al.*, 1995); and marbling (pork: Newman 1984b; Scholz *et al.*, 1995; beef: Kuchida *et al.,* 1992; Albrect *et al.*, 1996). The purpose of the present study was to develop an image analysis technique by which the effect of growth rate on fat to meat ratio could be monitored in rainbow trout. The technique was applied to animals exhibiting different growth rates to examine whether fat deposition was affected by growth velocity. In addition, the developed method was employed to examine the impact of growth rate upon pigmentation.

MATERIALS AND METHODS

Raw material

Ten 1+ yr and ten 2+ yr rainbow trout, all female and deriving from the same hatchery, were sampled from a marine fish farm (Samsø Laks, Denmark). Fish were sampled to span the same weight range in the two groups (1+: 1643 — 3975 g; 2+: 2651 — 3912 g). Individual lengths and weights were recorded. During on–growth from April until November, fish were fed identical commercial diets (Ecolife 18, Biomar A/S). Animals were bled on location, transported on ice to the laboratory, and subsequently frozen (–18°C) until analysis.

Image analysis

A 2–cm thick cutlet was taken immediately in front of the dorsal fin from each fish in the two groups. After thawing, entrails were removed and put aside. Each cutlet was placed in a transparent polyethylene bag and scanned directly using a desk–top colour scanner (HP–ScanJet 2c) with a spatial resolution of 75 points per inch equivalent to a pixel size of 0.3x0.3 mm and with 256 levels of each of the colours: red, green and blue. The pictures were stored in TIFF format using 24 bits per pixel (8 bits for each of the colours: red, green and blue). The scanner was calibrated using a standard colour map (art. no. 6817, JOBO). Lightness and contrast from this calibration was maintained during sample scanning.

Each picture was separated in its blue (B), green (G) and red (R) image. Background was isolated from fish and shadows using a threshold value of 103 in the blue picture (B), and meat, shadows and kidney tissue were separated from the rest in the green picture (G) using a threshold value of 60. Combining R and reverse B resulted in fish without kidney tissue and shadows but with noise from the thickness of the sample. This noise was removed manually in each sample using a drawing facility in the software. The mask (M) now produced defines the fish in the picture. Adding G and M gave the area which was outlined as meat (K) whereas adding M and reverse K gave the area outlining the fat tissue (F).

Measurements and Chemical analyses

All entrails were weighed, and dressed weight (g fish weight — g weight of entrails) and dress out percentage (g dressed weight x 100%/g fish weight) were calculated. In addition, condition factor (K) was calculated as weight (g x 100/length/cm^3). Fillet chemical analyses included dry matter content, determined by oven drying (105°C, 20–24h). Oil content was determined using chloroform: methanol extraction (Bligh and Dyer, 1959). Pigments were extracted twice using the Bligh and Dyer procedure. A standard curve was established using pure astaxanthin (Hoffman la Roche, Copenhagen) dissolved in chloroform. Absorbance was measured at 492 nm in a Shimadzu DB–150 spectrophotometer.

Statistical analysis

Correlation analysis between weight and percentage meat was performed. Student's *t*–test was applied to distinguish between year–groups and F–test to difference in variance. The level of significance was chosen at 5%. All analyses were carried out using the Analysis ToolPack in Microsoft® Excel97.

RESULTS AND DISCUSSION

Examination of meat percentage and fish size (length, weight, condition factor, dressed weight) revealed no correlation ($P > 0.05$; Table 1) and *t*–tests failed to expose any difference in fat: meat ratio between 1+ and 2+ yr old rainbow trout. However, a significan difference ($P < 0.05$) between variances for the two groups was observed, being approximately four–fold larger for 1 + yr animals compared to 2+ yr. Thus, under commercial conditions, faster growing fish were found to exhibit increased variation in groos body composition. This finding has several possible negative consequences from breeding and production perspectives. Many reports relating to breeding programmes for salmonids highlight selection for growth rate and enhanced food conversion efficiency (*e. g.,* Gjedrem, 1997; Jonasson and Gjedrem, 1997; O'Flynn *et al*., 1999). If the findings from the present study were to be reproduced in selectively bred fish, then faster growth rates might result in production of a less uniform product. The repercussion of this to the fish farmer would be increased effort expended during production management, and hence elevated costs *viz.* increased inspection and control, if certain product specifications are to be met.

The correlation matrix, presented in Table 1, illustrates clear correlation between weight and dressed weight, length and weight and dry matter and oil. Since oil comprises a certain component of dry matter content, any correlation with oil will also result in similar correlation with dry matter.

Table 1. Correlation matrix for measured variables. N = 20 fish.
Tablica 1. Matrica međusobnih odnosa mjernih varijabli N = 20 riba

	Lenght (cm)	Weight (g)	K (g)	Dressed weight (%)	Dress out yield (pixels)	Area (%)	Fat tissue (%)	Meat tissue (%)	Dry matter	Oil (%)	Pigment (mg/kg)
Length	1										
Weight	0,73**	1									
K	n.s.	0,57**	1								
Dressed weight	0,69**	0,99**	0,60**	1							
Dress out yield	n.s.	n.s.	n.s.	n.s.	1						
Area	n.s.	0,76**	0,68**	0,82**	0,54*	1					
Fat tissue	n.s.	n.s.	n.s.	n.s.	0,51*	0,67**	1				
Meat tissue	n.s.	n.s.	n.s.	n.s.	–0,49*	–0,68**	–0,99**	1			
Dry matter	n.s.	n.s.	n.s.	n.s.	0,46*	0,59**	0,68**	–0,66**	1		
Oil	n.s.	n.s.	n.s.	n.s.	n.s.	0,57**	0,75**	–0,73**	0,92**	1	
Pigment	n.s.	n.s.	0,74**	n.s.	n.s.	0,55*	n.s.	n.s.	0,50*	n.s.	1

** significant at 1% level; * significant at 5% level; n. s. not significant.
** značajno na 1% razini; * značajno na 5% razini; n.s. nijeznačajno

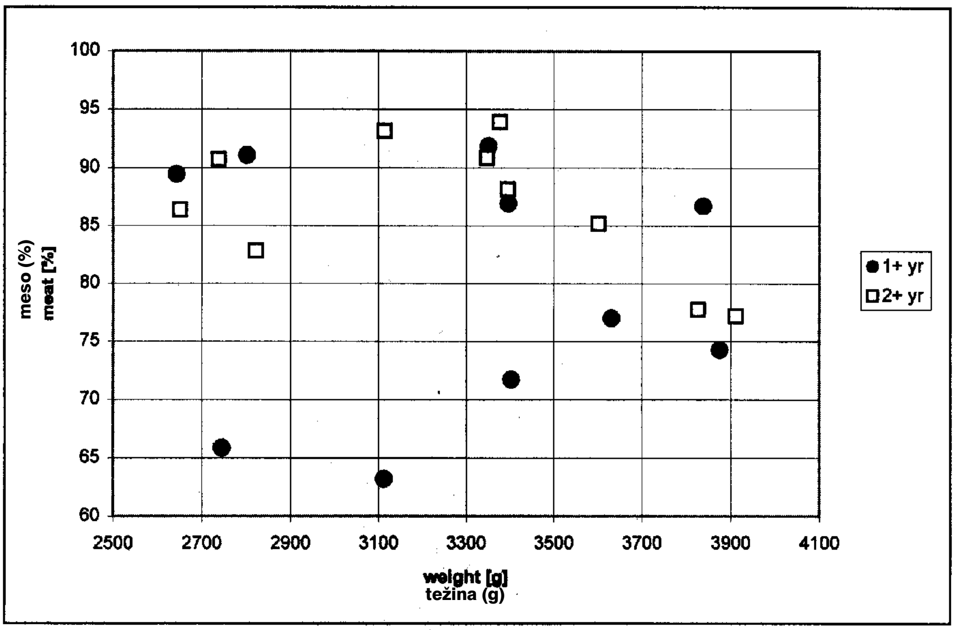

Figure 1. Percentage of meat tissue versus whole body weight (g) for 1+ and 2+ rainbow trout.

Slika 1. Postotak mesa u odnosu na ukupnu težinu tijela (g) kod 1+ i 2+ kalifornijskih pastrva

Inverse correlation between fat % and meat % was also observed, but this only reflected the contrary nature in the relative proportions of easch tissue.

Dress out yield varied independent of weight, length, dressed weight, and condition factor indicating allometric growth, which supports the fact that visceral index increases as the fish develop gonads (Weatherleay and Gill, 1987). Moreover, the lack of growth rate effect upon dress out yield supports this assumption. Moderate correlation, although significant, was observed between dress out yield and area, fat tissue, meat tissue (negative correlation), and dry matter (but not oil) respectively. This complies with findings of other (Wathne, 1995; Rasmussen *et al.*, 1999) that high–energy diets cause an increase in fat deposition and, when not deposited in viscera, contributes to the dress out yield. Cutlet area was observed to change independently of fish length, thus illustrating that alterations to various body compartments result due to growth in animal thickness, with larger areas being associated with higher condtition factors (Table 1).

Pigmentation was significantly correlated with condition factor ($P < 0.01$; Table 1), but less so with area and dry matter ($P < 0.05$; Table 1). Partial correlation analysis revealed that for fixed area and dry matter, pigmentation and condition factor remained significantly correlated. A higher condition factor implies a thicker fish, which probably results due to increased lipid accumulation. In theory, enhanced pigment uptake would be expected ion animals expressing higher condition factors due to the fact that astaxanthin is fat soluble. However, no significant correlation ($P > 0.05$; Table 1) was observed between pigmentation and oil content although this result conforms to the findings of others (Henmi *et al.*, 1989; Henmi and Hata, 1990) and provides support for the contention that carotenoids bind to hydrophobic cavities in the actomyosin of the fish muscle (*idem*).

Although no age–related differences were apparent for any of the measured variables (length, weight, dressed weight, dress yield, area of cutlet, condition factor, oil, dry matter, fat percentage, meat percentage, and pigment) the correlation matrix did reveal interesting relationships. However, since the size of the data material employed was comparatively small, the present findings should only be considered as preliminary in nature. Nevertheless, the study, provides suggestions for designs of further experiments exploring these relationships and indicate that image analysis may prove useful as a tool in such studies.

Sažetak

PROCJENA ANALIZE PASTRVA KAO METODE ZA ODREĐIVANJE KEMIJSKOG SASTAVA TIJELA KALIFORNIJSKIH PASTRVA

B. Rønsholdt, H. Nielsen, J. Færgemand, E. McLean

Dvije grupe (1+ i 2+; n=10/grupi) kalifornijskih pastrva *(Oncorhynchus mykiss)* potjecale su s morskog uzgajališta u Danskoj. Riba je selektirana tako da je bila podjednake težine (1+:2643–3875 g; 2+:2651–3912 g). Za vrijeme proizvodnje (travanj do studeni) riba je hranjena jednakom komercijalnom hranom. Vizualno nakupljena mast brže ili sporije rastućih riba bila je ispitana s pomoću prikaza izravnih skaniranih poprečnih presjeka tijela riba. Nije ustanovljen značajan (P>0,05) odnos između postotka mesa i veličine ribe (dužine, težine, kondicijskog faktora), a t–testovi nisu pokazali razliku u odnosu mast:meso između 1+ i 2+ starih kalifornijskih pastrva. No, zapažena je značajna razlika (P<0,05) između varijanci za dvije grupe, koja je bila približno 4 puta veća za 1+ ribe u usporedbi s 2+ starim ribama.

Ključne riječi: pigmentacija, odnos mast:meso, kvaliteta proizvoda, prirast

*

B. Rønsholdt, and E. McLean,Aalborg University, Department of Civil Engineering, Laboratory for Aquatic Biotechnology, Sohngaardsholmsvej 57, 9000 Aalborg, Denmark.

H. Nielsen, Aalborg University, Institute of Electronic Systems, Laboratory of Image Analysis, Fredrik Bajersvej 7, 9220 Aalborg Ø, Denmark.

J. Færgemand, Bureau Veritas BVQI Denmark, Oldenborggade 1B, 7000 Fredericia, Denmark

*corresponding author: tel. +45 9635 9411; fax. + 45 9814 2554; e–mail: i5@civil.auc.dk

REFERENCES

Albrecht, E., Wegner, J., Ender, K. (1996): Eine neue Methode zur objektiven Bewertung der Marmorierung von Rindfleisch. *Fleischwirtshaft* 76, 95–98.

Bell, J. G., McEvoy, J., Sargent, J. R. (1998): Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar). Journal of Agriculture and Food Chemistry* 46, 119–127.

Bligh, E. D., Dyer, W. J. (1959): A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**, 911–917.

Borggaard, C., Madsen, N. T., Thodberg, H. H. (1996): In–Line Image Analysis in the Slaughter Industry, Illustrated by Beef Carcass Classification. *Meat Science* 43, 151–163.

Branscheid, W., Dobrowolski, A., Hoereth, R. (1995): Die Video Image–Analyse. Methode zur online–Erfassung des Teilstueckwertes von Schweineschlachtkoepern. *Fleischwirtschaft* 75, 636–671.

Eckhoff, K. M., Aidos, I., Hemre, G. I., Lie, O. (1998): Collagen content in farmed Atlantic salmon (*Salmo salar,* L.) and subsequent changes in solubility during storage on ice. *Food Chemistry* 62, 197–200.

Gjedrem, T. (1997): Selective breeding to improve aquaculture production. *World Aquaculture* 28, 33–45.

Færergemand, J. (1992): Kvalitetdesign of ofdrætsfisk. Ph. D. thesis. Dansk Ørredfoder A/S Bande, Denmark, 206 pp.

Henmi, H., Hata, M., Hata, M. (1989): Astaxanthin and/or canthaxanthin–actomyosin complex in salmon muscle. *Nippon Suisan Gakkaishi* 55, 1583–1589.

Henmi, H., Hata, M. (1990): Combination of astaxanthin and canthaxanthin with fish muscle actomyosins associated with their surface hydrophobicity. *Nippon Gakkaishi* 56, 1821–1823.

Jonasson, J., Gjedrem, T. (1997). Genetic correlation for body weight of Atlantic salmon grilse between fish in sea ranching and land–based farming. *Aquaculture* 156, 203–212.

Karnuah A. B., Moriya K., Sasaki, Y. (1994): Computer image analysis information extracted from beef carcass cross section and its pression. *Animal Science and Technology* 65, 515–524.

Kuchida, K., Yamaki, K., Yamagishi, T., Mizuma, Y. (1992): Evaluation of meat quality in japanese beef cattle by computer image analysis. *Animal Science and Technology* 63, 121–127.

Leaf, A., King, J. X. (1998): Omega 3 fatty acids and cardiovascular disease. *World Review of Nutrition and Diet* **83**, 24–37.

Newman, P. B. (1984a): The use of video image analysis for quantitative measurement of visible fat and lean in meat. I. Boneless fresch and cured meats. *Meat Science* 10, 87–100.

Newman, P. B. (1984b): The use of video image analysis for quantitative measurement of fatness in meat. II. Comparison of VIA, visual assessment and total chemical fat estimation in a commercial environment. *Meat Science* 10, 161–166.

Newman, P. B (1987): The use of video image analysis for quantitative measurement of visible fat and lean in meat. III. Lipid content variation in commercial processing beef and its prediction by image analysis. *Meat Science* 19, 129–137.

Nortvedt, R, Tuene, S. (1998): Body composition and sensory assessment of three weight groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed three pellet sizes and three dietary fat levels. *Aquaculture* 161, 295–313.

O'Flynn, F. M, Bailey, J. K., Friars,. G. W. (1999): Responses to two generations of index selection in Atlantic salmon (*Salmo salar). Aquaculture* 173, 143–147.

Rasmussen R. S., Ostenfeld; T. H., Røensholdt, B., McLean, E. (2000): Manipulation of end product quality in rainbow trout with finishing feeds. *Aquaculture Nutrition*, in 6, (1), 17–24.

Sholz, A., Paulke, T., Eger, H. (1995): Determining the degree of marbling in the pig. Use of computer–supported video picture analysis. *Fleischwirtshaft* 75, (11), 1322–1324.

Wathne, E. (1995): Strategies for directing slaughter quality of farmed Atlantic salmon (*Salmo salar)* with emphasis on diet composition and fat deposition. Ph. D. Thesis. Agricultural University of Norway. pp. 1–230.

Weatherlery, A. H., Gill, H. S. (1987): The Biology of Fish Growth, London: Academic Press Inc. 443pp.

Received 15th August, 1999
Accepted 20th October, 1999