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Online estimation of lipids in fish by means of ultrasound

The estimation of the fish composition is of interest in modern recirculation aquaculture systems during mast for reacting possibly on changes in composition with an adequate feeding. For an online estimation of lipids ultrasound can be used because velocity of ultrasound propagation is influenced by the fat content. In the following described project the correlation between the signal shape and fat content is checked additionally. For that purpose a measuring system was developed especially which measures the thickness, temperature and transmitted ultrasound signal of a fish sample placed on the sensor. The realised investigations show good results if the transmitted signal shape is used for predicting the fat content. The prediction error is less than 1 % fat in fresh mass.

Keywords

Ultrasound, fat, recirculation aquaculture system

Abstract

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The fat content of fish is an important quality feature and can be determined invasively by chemical methods after slaughter or with random testing during mast. A non-invasive estimation of fat content in fish is mostly based on the indirect measurement of water-content and utilization of the water-fat-correlation [1]. For example, the handheld “Distell Fish Fatmeter” [2] uses microwave radiation which is sensitive to the water content.

The benefit of using ultrasound for fat-estimation is its very good propagation in water. So it is not obligate to remove the fish as a sample from the tank as it is necessary for microwave or near-infrared measurements. Because the ultrasonic velocity is water-dependent, it can also be used for the water-fat correlation [3]: In pure fish oil at 12° C the velocity is 1500 m/s, in pure water 1450 m/s. Because of this difference, it appears possible in principle, to quantify a mixture of fat, water and protein - as in fish occurs - at a known temperature. The relationship of the proportions of fatty oil l, water w and protein p to the respective ultrasonic velocities c is approximately described by the following equation [4]:

$$\frac{1}{c_{fish}^2} = \frac{l}{c_{lipid}^2} + \frac{p}{c_{protein}^2} + \frac{w}{c_{water}^2} \qquad \text{(Gl. 1)}$$

This article will present results and possibilities for estimating the fat content of turbots from recirculation systems by ultrasonic measurements without sample preparation after slaughter.

Materials and methods

For the present measurements of the ultrasonic velocity lateral to the body of the fish a special measuring system has been developed which measures the thickness and temperature of fish and sends a short (7 ns) 5 MHz ultrasonic pulse simultaneously through the sample and records the transmitted signal. The investigated sample volume has a diameter of 12.7 mm and a variable height s (fish thickness) and is cylindrical due to the used ultrasound transducers. The ultrasonic velocity v = s/t can be calculated from the propagation distance s and the required time t. But also the transmitted waveform - the so-called transient – is recorded for the further signal analysis. The “real” fat content of the same sample is determined afterwards with a standard wet-chemical method (e. g. [5]) as a reference.

As the ultrasonic transmission is measured, the signal only passes the sample prior data acquisition once and a possible existing dispersion (change of the waveform with the propagation length) is minimized. **Figure 1** shows the typical ultrasonic signals for a few selected fish samples. The signals were placed with their first minimum of each transmission to the time “zero” in order to compare their remaining zero crossings chronologically. There is a good coincidence in time of the ultrasonic signals from fish samples of different thickness (**Figure 1**). Therefore, it is assumed that the fish thickness does not affect the waveform with respect to the frequency.

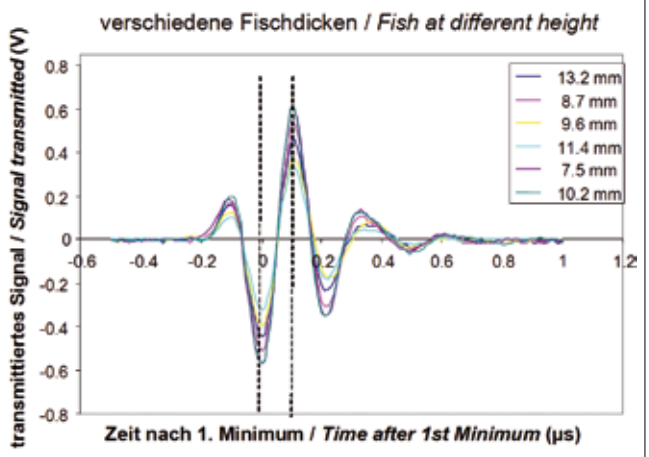
The complete developed measuring system consists of the following components (**Figure 2**):

- ultrasonic transmitter and receiver (Olympus Panametrics 5800, 100 µJ) with 5 MHz heads (Immersion transducer V326-SU) with 12.7 mm diameter;
- micrometer screw (Mitutoyo 164-163 with 1 micron accuracy) for determining the sample thickness;
- temperature sensor PT100;

- digital oscilloscope (Agilent MSO7054A 500 MHz) for digitizing the ultrasonic signals, sampling interval 0.25 ns;
- laptop with MatLab R2010a for controlling and documentation.

The propagation duration of the ultrasonic signals is determined with the control software MatLab R2010a by finding the time of the first characteristic minimum of the ultrasonic wave by means of numerical approximation of a 7th order polynomial. The transients from each ultrasonic measurement are modified and saved in 150 data points averaged over 10 ns around the previously determined first minimum for the subsequent multivariate analysis. This post processing is realised with the chemometric software "The Unscrambler 9.6". With this shifted timeline the actual propagation time, and thus the respective propagation length which is dependant on the different fish thickness, does not affect the evaluation of the signal. The system-related offset of the ultrasonic velocity measurement - the recorded time from transmission until the detection of the pulse is greater than 0 µs at 0 mm sample thickness - was taken into account by the measurement of multiple spacers of known thickness and ultrasonic velocities and was compensated.

Fig. 1



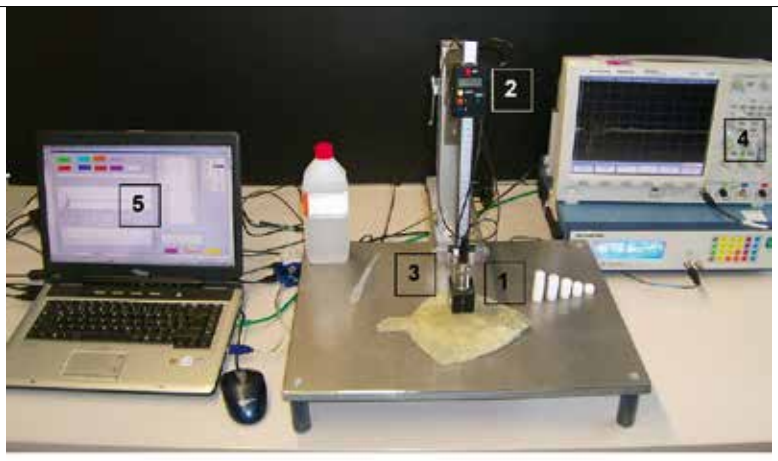
Ultrasound signals from different fish with shifted timeline

Experimental procedere and selected results

In principle, first a calibration of the system was conducted on single fish pieces and finally this model was validated on whole fish. By the use of small samples with a diameter of 12.7 mm it is possible to determine the material properties exactly of the measured sample. Then this calibration was applied to the whole fish by measuring at several locations and averaging the calculated fat levels. These were set in relation to the reference fat analysis of a composite sample, conducted over 3 fish.

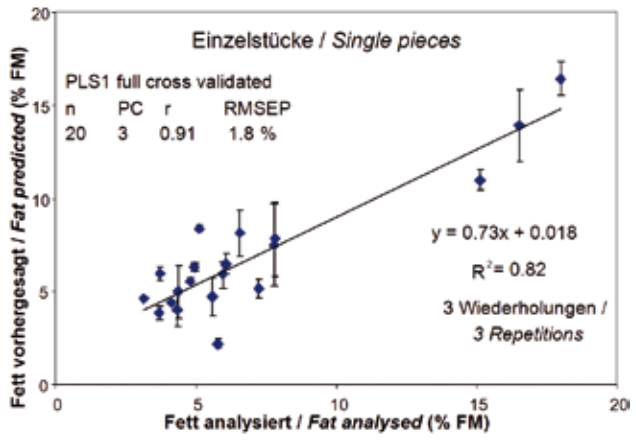
20 turbot of different sizes (0.1 -1.2 kg) were used to calibrate the measurement system. Measurements were performed in triplicate at different fish locations and subsequently these patches were cut exactly and the fat content was estimated by means of dry matter determination. Own experiments before showed a correlation of 0.91 between dry matter and fat content with the functional relationship fat [% FM] = 0.7 × dry matter − 12 % FM (similar to [1]). This "detour" has been chosen because the direct wet chemical determination of the fat content of these small, approximately 1 g samples, causes high estimation errors [5]. The assignment of the obtained fat contents with a span of 3 to 18 % fat in the fresh material to the ultrasonic velocities provided satisfactory results with a correlation of 0.91. In addition, a multivariate regression model using a partial least squares regression (PLS1) with 3 principle components was created from the transients. The cross-validation parameters (full cross validation) are shown in **Figure 3**. A small scatter appears in the predicted levels of fat due to the repeated measurements but these variations do not exceed the prediction accuracy of the model. The crucial point in the chosen approach of the calibration on single pieces is that heterogeneity within a fish is taken into account, so that samples with extreme levels of fat, such as the samples with up to 18 % fat in the fresh mass (**Figure 3**), is incorporated in the model with its associated ultrasonic signals. Upon a calibration of the

Fig. 2



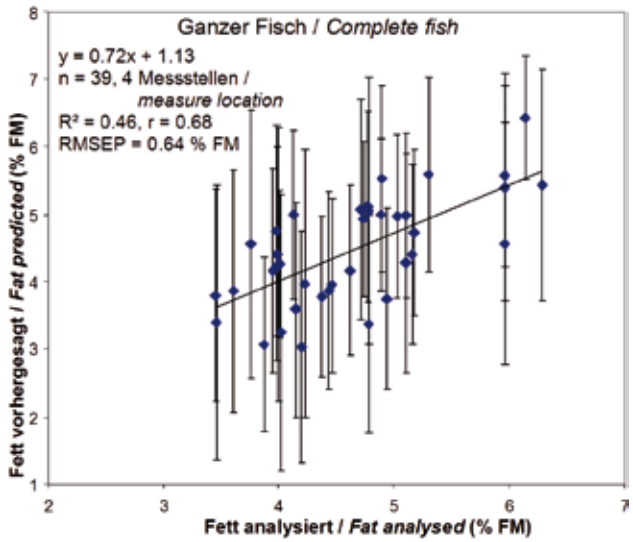
Ultrasound measure system: 1 ultrasound transducer, 2 micrometer calliper, 3 temperature sensor, 4 digital oscilloscope, 5 control software (Foto: Thiessen)

Fig. 3



Fat content of single pieces predicted with the transients versus the analysed fat content (calculated from the dry matter content). FM: fresh mass, PC: number of principle components, RMSEP: root mean square error of prediction

Fig. 4



Fat content predicted with the transients versus the analysed fat content of the whole fish. FM: fresh mass, RMSEP: root mean square error of prediction

fat content by means of a composite sample a level of fat would be assigned to the respective ultrasonic measurement which is not the same fat content of the patch, on which the ultrasonic measurement actually took place.

Using the before created multivariate regression model, the fat content of individual measurement locations was predicted and averaged over the whole fish. For that validation, turbots were used from a feeding trial in which a whole-body fat analysis was conducted finally. The fish were kept by a controlled heating in water to 25 °C after killing before the ultrasonic measurement. The sample temperature during the measurement was recorded additionally. There were 3 ultrasound measurements at 4 different positions on the fish body (2 on the back, 2 in the middle) of 3 individual fish from a total of 39 basins (i. e. 117 fish). The fat analysis was conducted after a freeze-drying of a composite sample of 3 fish from each basin, so a total of 39 fat analysis values was the result. **Figure 4** shows the dependence of the predicted fat content (averaged over the measurement locations of the 3 fish from the composite sample) to the analyzed reference fat content. The high variation of the predicted fat content is due to the heterogeneity of the distribution of fat within the fish. The mean predicted values, which were indeed determined by wet-chemical analysis as a mean of the fish, are overall in good agreement with the reference values. The rather high offset of about 1.1 % fat in the fresh mass is probably due to the fact that the single pieces for calibration derived from a different stock than the complete fish for the validation model.

Conclusions

The accuracy of the ultrasonic method appears quite low with a prediction error of 0.64 % fat in the fresh material with a range of about 3–7 %. However, it has to be put into relation with the reference method, which showed an error of about 0.6 % fat in the fresh material estimated with the triple determination in this experiment. In general the turbot with its low fat content does not seem to be a suitable candidate for this method. Studies on fish with higher absolute fat content and thus a larger range are still pending. Nevertheless, the ultrasonic method is suitable for non-invasive estimation the fat content of fish and probably can be adapted to the measurement of free-swimming fish, because ultrasound in the water requires no direct contact of the sensor to the sample.

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