UNIVERSITÀ DEGLI STUDI DI UDINE

Molecular based identification of insect ingredients in animal feeds

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Innovative Ingredients

EU **Regulation 2017/893** authorized the use of seven breeding insect species in feed for aquaculture animals.

- ✓*Hermetia illucens*

- ✓*Tenebrio molitor*

- ✓*Alphitobius diaperinus*

- ✓*Musca domestica*

- ✓*Acheta domesticus*

- ✓*Gryllus assimilis*

- ✓*Gryllodes sigillatus*

Species for aqua-feed purposes

✓*Hermetia illucens* 

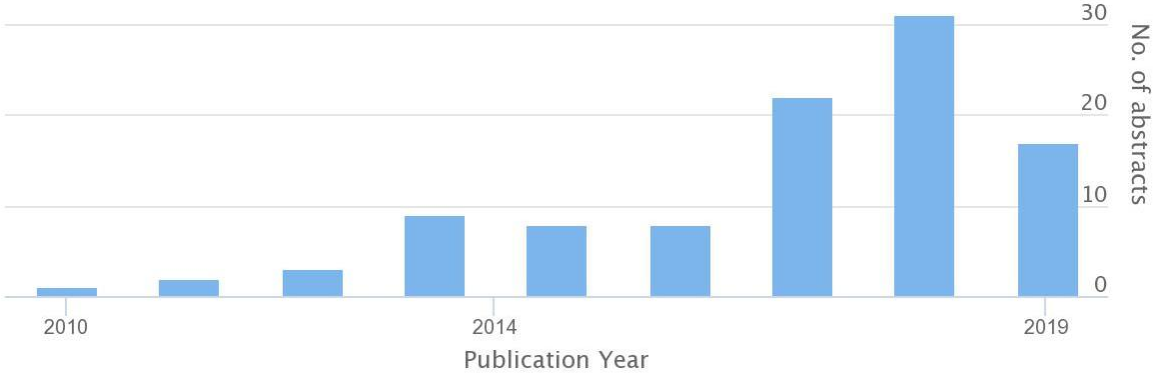
✓*Tenebrio molitor* 

Tenebrio molitor (TM) and *Hermetia illucens (HI)* are the most attractive species that could be suitably used in aquafeeds for their availability and market price.

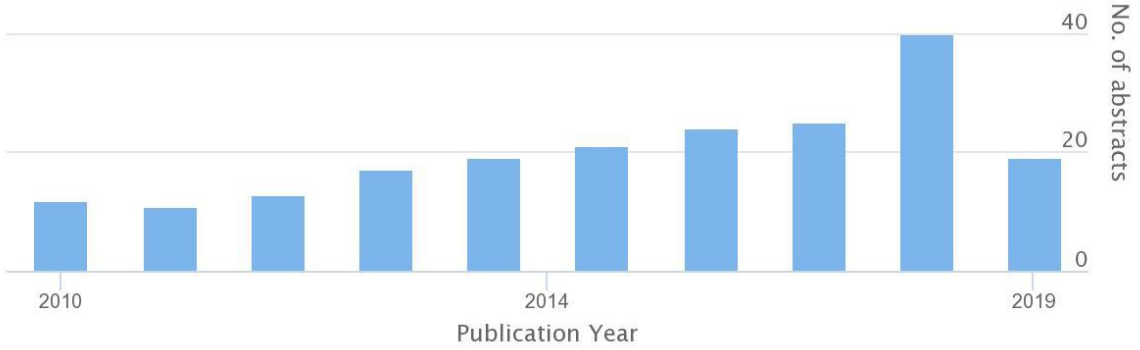
Publications

These innovative ingredients have been largely tested to evaluate their effects and properties

Fish



Poultry



(CabDirect access on 19/08/2019)

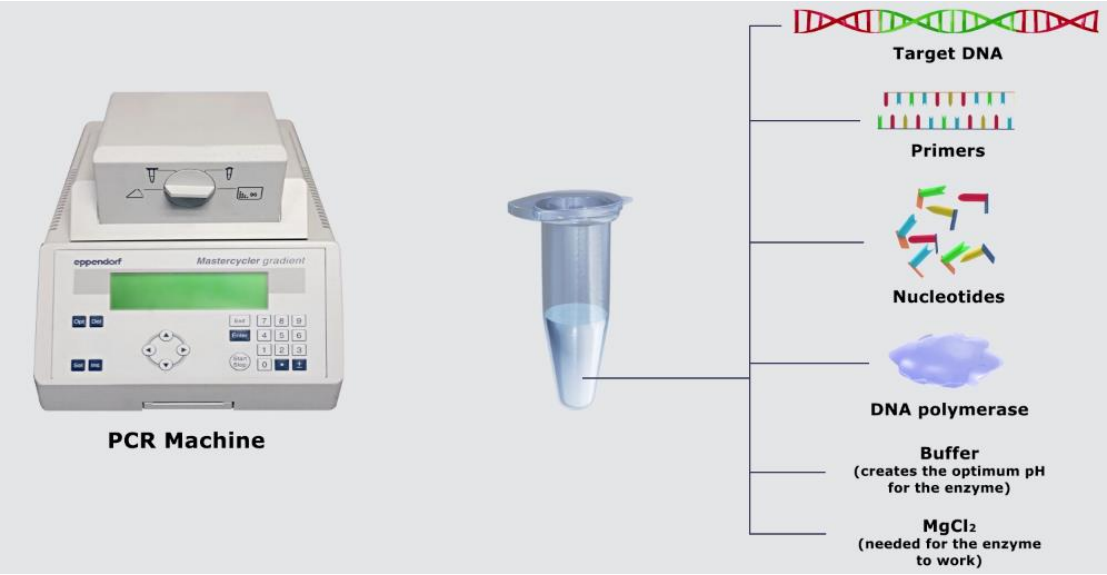
Feed/Food control

The development of assays is needed for

- **labeling** of products containing insects
- identification of **fraud** and adulteration in industrial insect products

Use of the qPCR

The qPCR is still the election method for the detection of contaminants and to check for the presence or absence of a target in a biological matrix.



Species specific primers were designed for the detection of the 7 allowed insect species.

Primers specificity

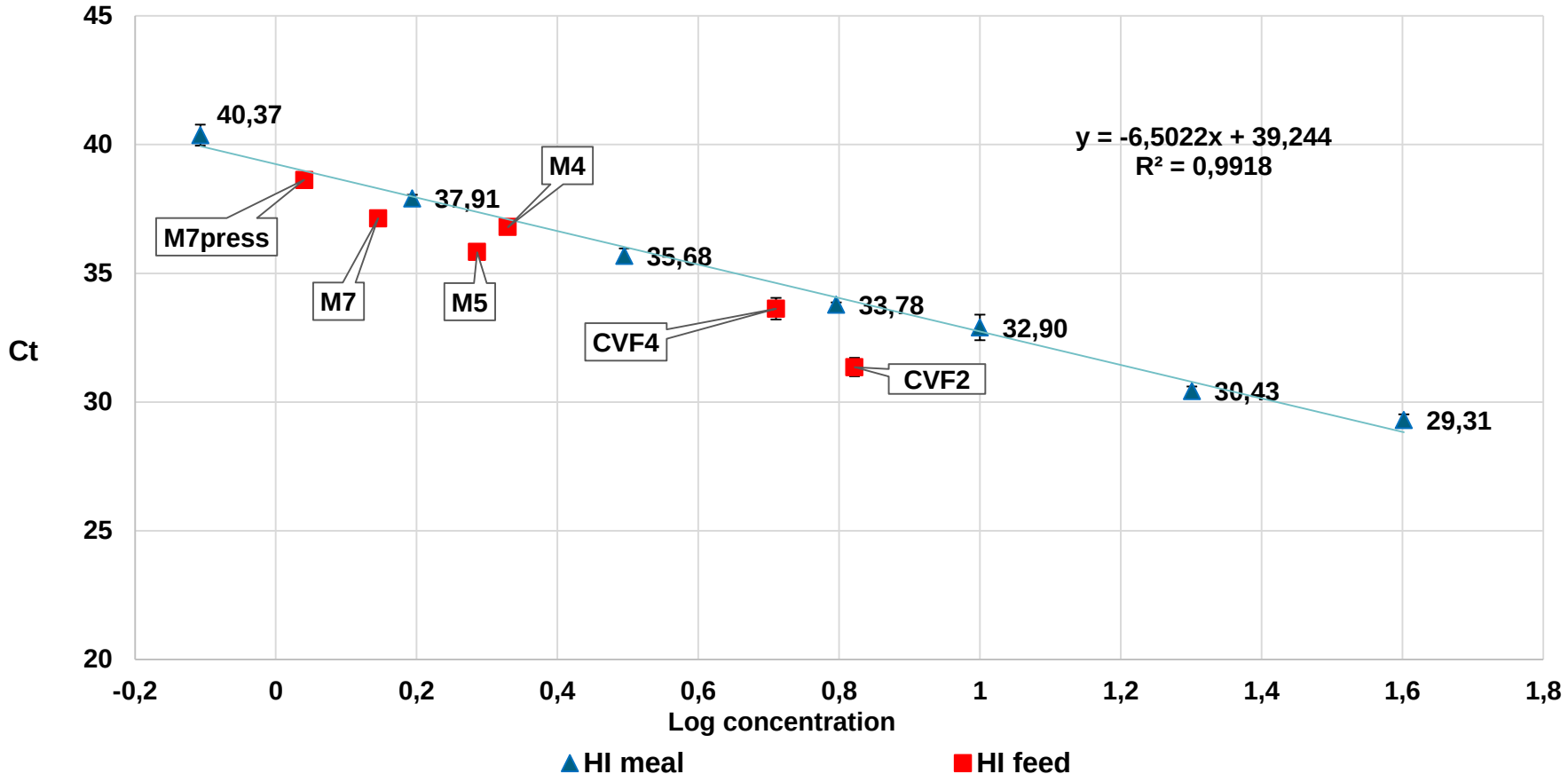
Primers / DNA	Hermetia illucens	Tenebrio molitor	Musca domestica	Gryllodes sigillatus	Gryllus assimilis	Alphitobius diaperinus	Acheta domesticus
Hermetia illucens	**23,2 ***	N/A	N/A	N/A	N/A	N/A	N/A
Tenebrio molitor	N/A	**20,2***	N/A	N/A	N/A	N/A	N/A
Musca domestica	N/A	N/A	**24,1***	N/A	N/A	N/A	**38,7^**
Gryllodes sigillatus	N/A	N/A	N/A	**18,9***	N/A	N/A	N/A
Gryllus assimilis	N/A	N/A	N/A	N/A	**21,1***	N/A	N/A
Alphitobius diaperinus	N/A	N/A	N/A	N/A	N/A	**26,5***	N/A
Acheta domesticus	N/A	N/A	N/A	N/A	N/A	N/A	**19,2***

⇐ Perfection doesn't exist

Cross reactivity table

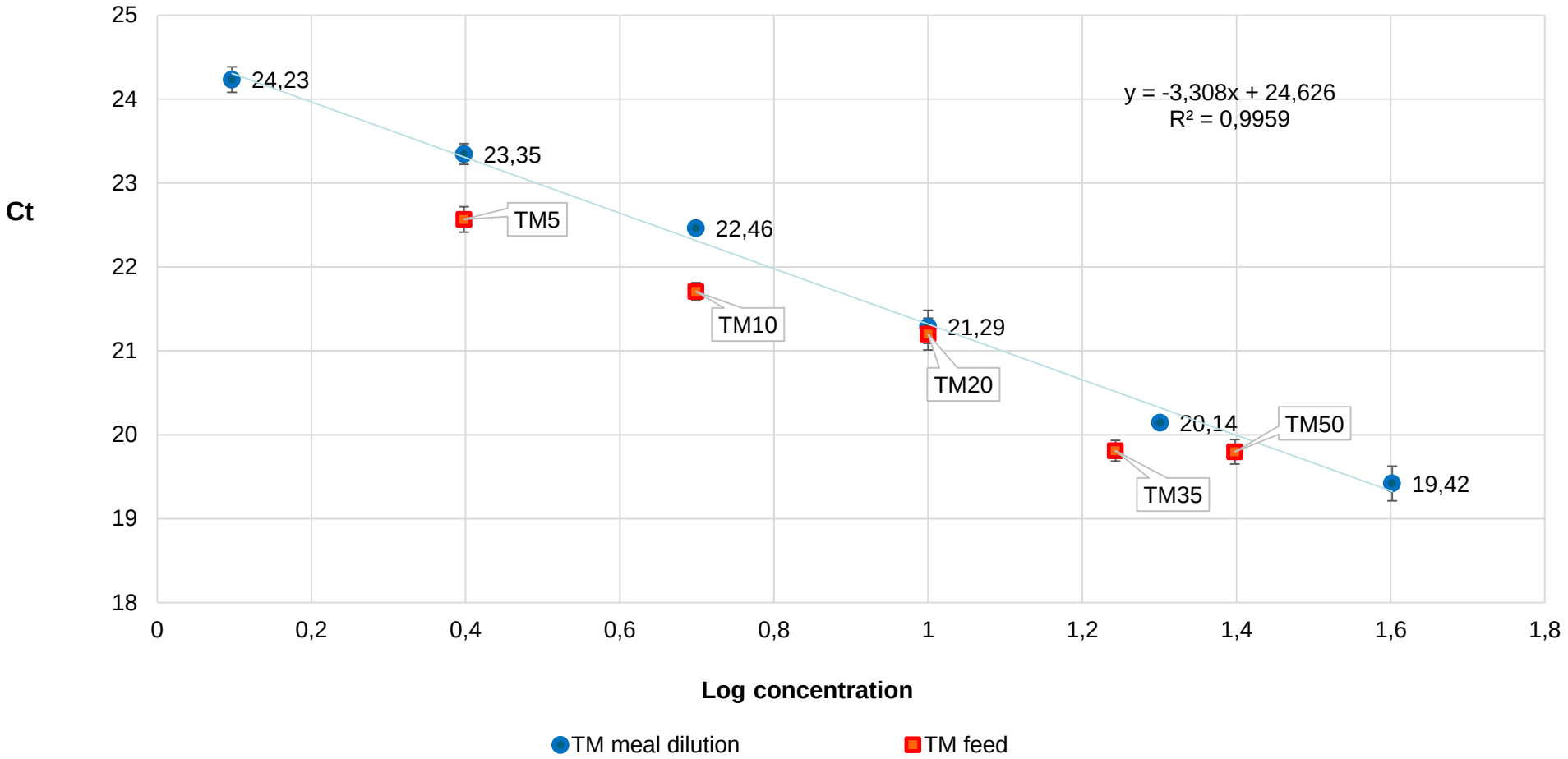
Test with feed matrix - HI

Six different diets (▮) including graded levels of *H. illucens* meal (0,5 – 5,0 ng/ul) and a negative control provided from CycleFarms (F) were tested. Results were compared with pure HI meal (▲) dilution curve.



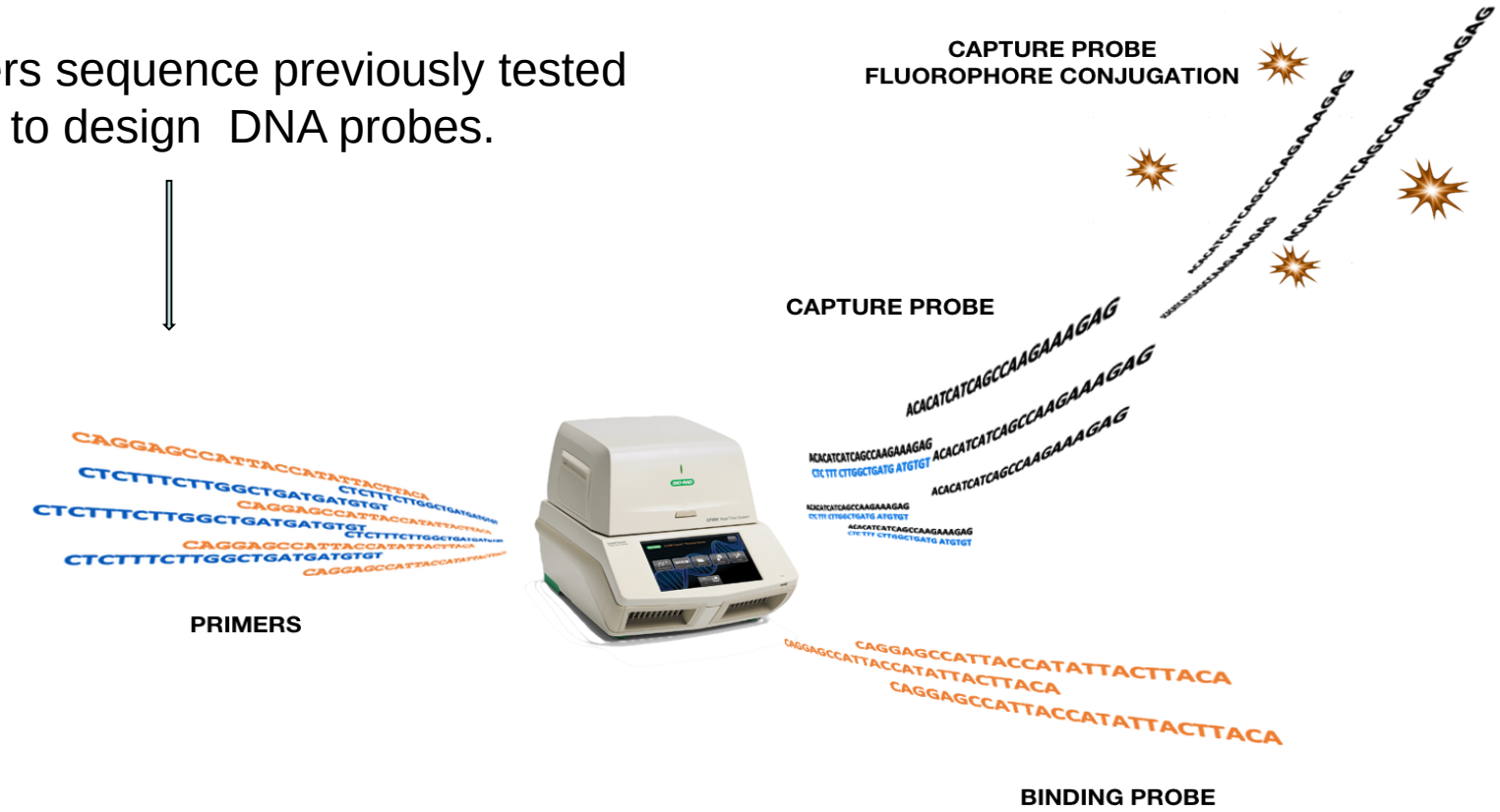
Test with feed matrix - TM

Five different experimental diets including graded levels of *T. molitor* meal (0,5 – 5,0 ng/ul) (■) and a negative (TM0) control were produced and tested. Results were compared with TM meal (●) dilution curve.



From qPCR to Bio-Sensor

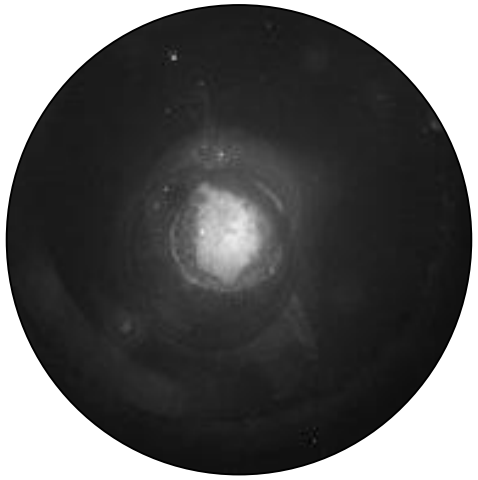
PCR Primers sequence previously tested were used to design DNA probes.



Bio-Sensor assay

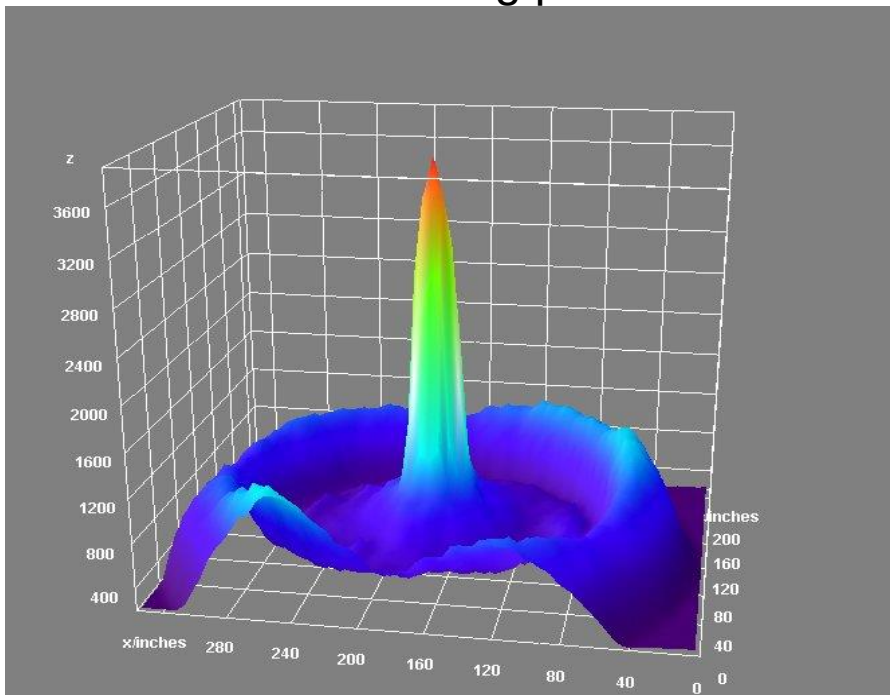
The probes have been applied to develop an innovative Point Of Care (POC) method of detection based on fluorescence excitation by Oled. This technology use a disposable cartridge as Bio-Sensor

Detection with Oled based Bio-Sensor



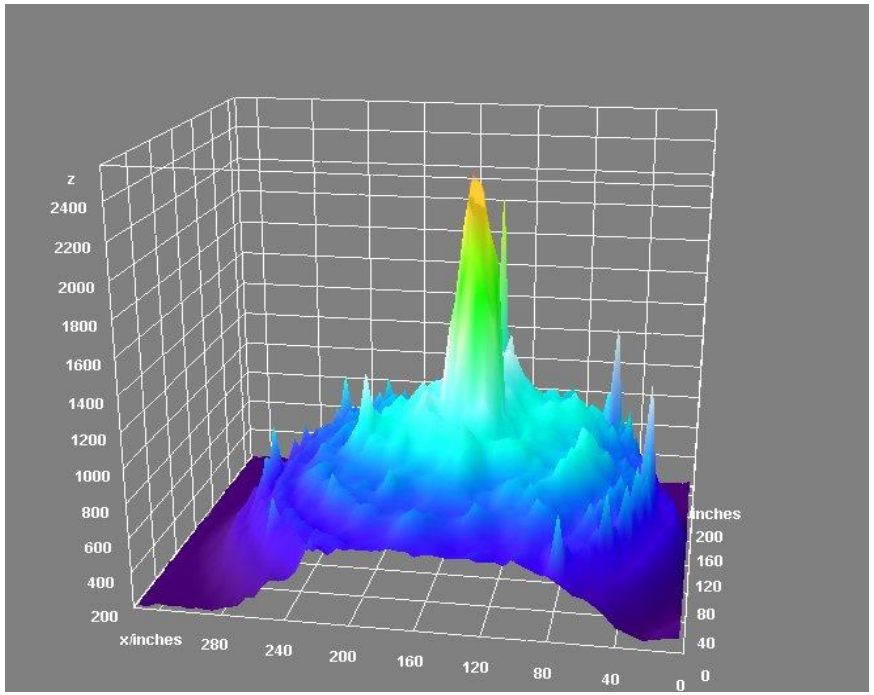
A relationship between the fluorescent signal and the DNA target concentration was observed.

25 ng/µl



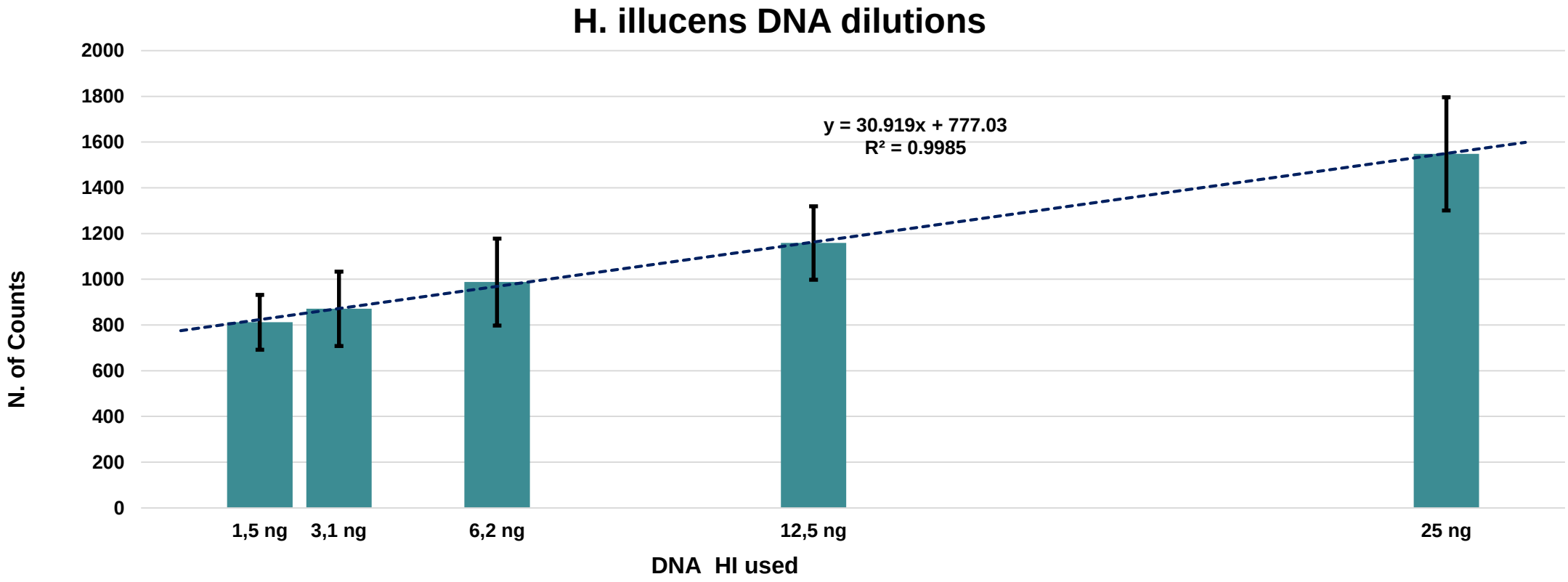
Or-el d.o.o.
Organska elektronika

3,1 ng/µl



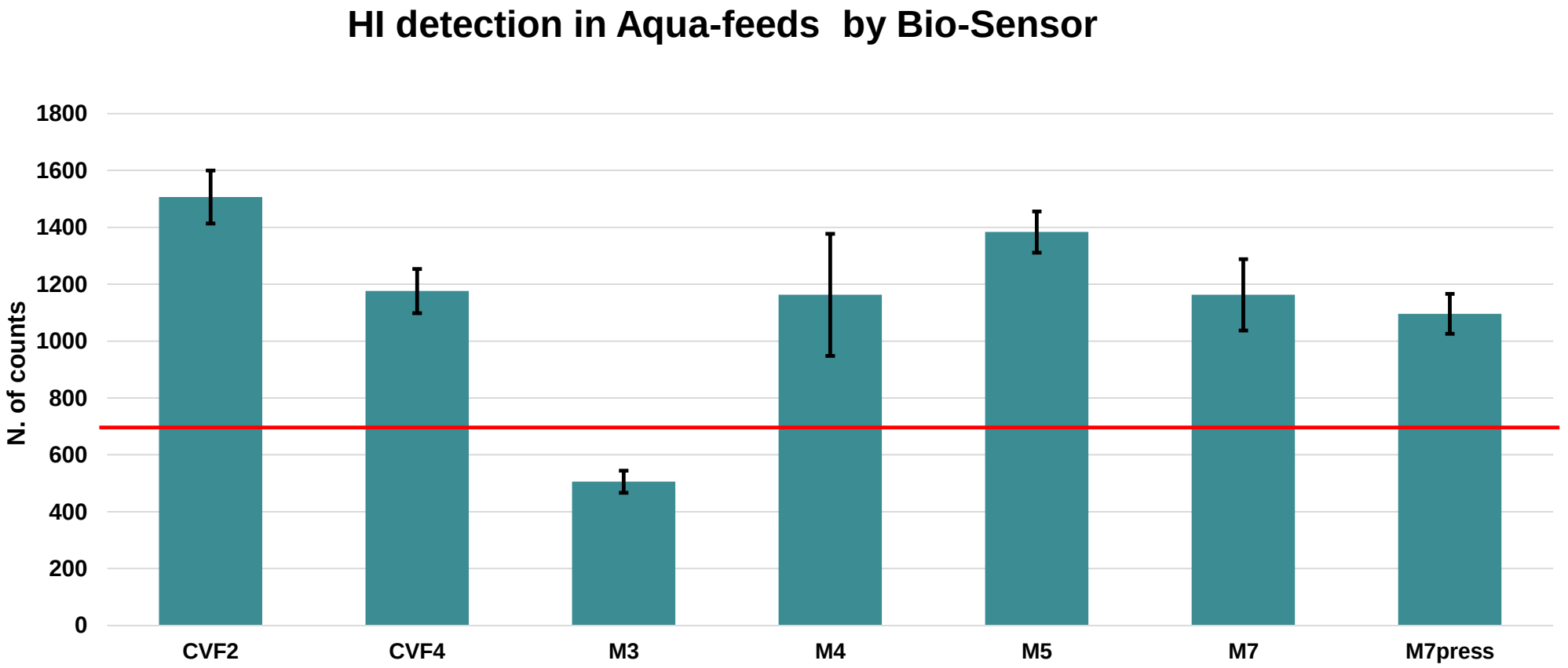
Bio-Sensor Data

- The images acquired by the Bio-Sensor were analysed with a Photo images program.
- Measured fluorescence intensity is proportional to the number of counts per pixel with a grey scale range from **0** (complete black) to **4095** (white/saturation)
- The signal is the average of the number of counts for each pixel in the area considered



Test with feed matrix - HI

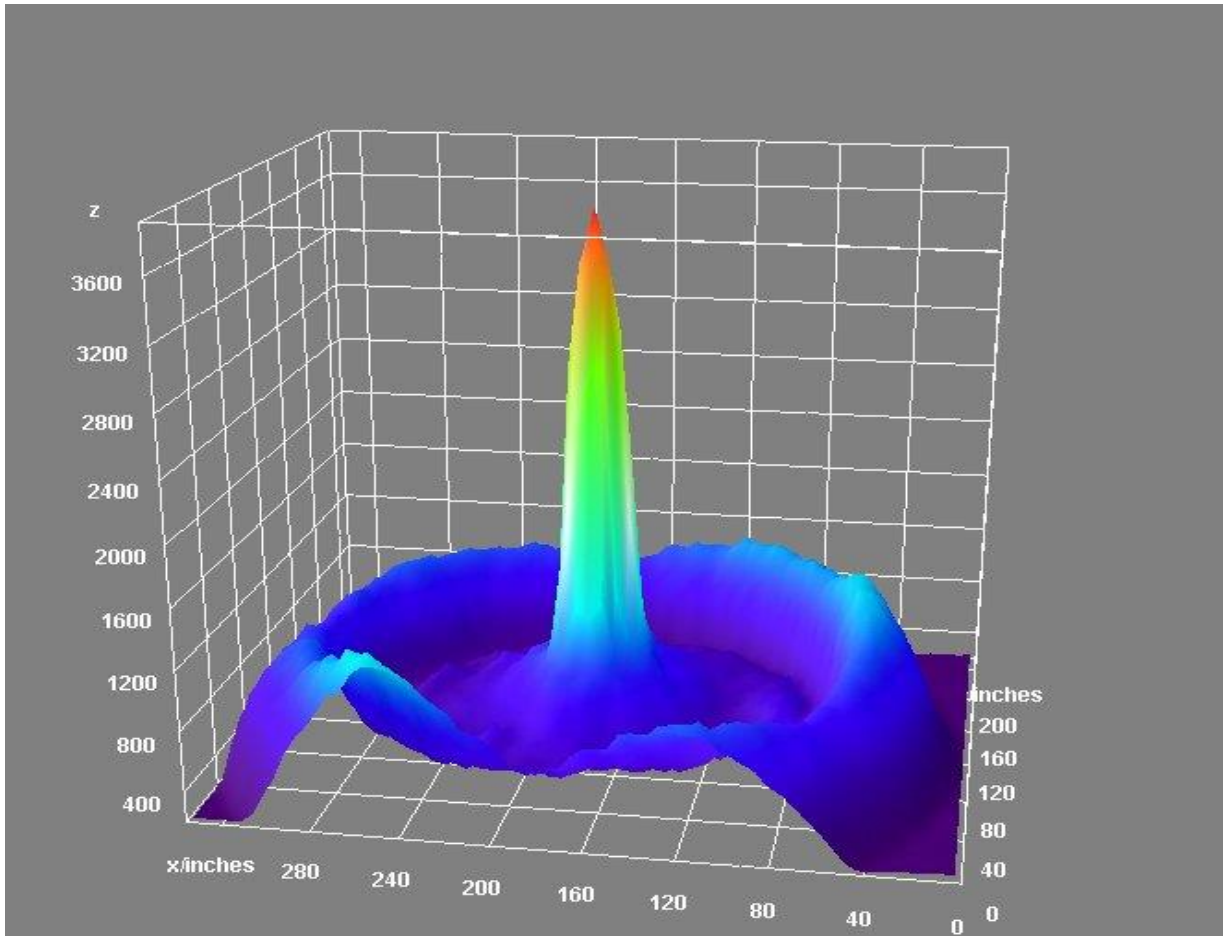
Six different diets including graded levels of *H. illucens* meal (0,5 – 5,0 ng/ul) and a negative control (M3) were provided from CycleFarms and tested with the Bio-Sensor system. The signal was considered as negative if below 700 counts.



Conclusions

- ✓ Results of the analysis confirm the accuracy of the qPCR method for insect detection in complex matrix like practical feeds.
- ✓ DNA primers result in **high** target **specificity**.
- ✓ Both primers allowed the use of the assay also in feeds subjected to heat-temperature processing.
- ✓ Bio-Sensor based results are **coherent** with the ones obtained using qPCR.

Thanks for your attention ...



In collaboration with

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