Lipid Oxidation In Fish Feed

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Lipid oxidation in fish feed

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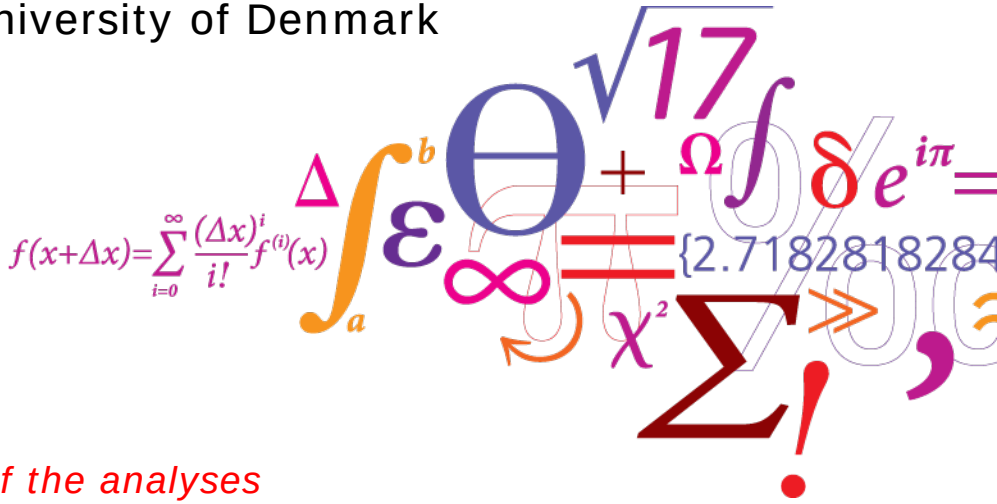

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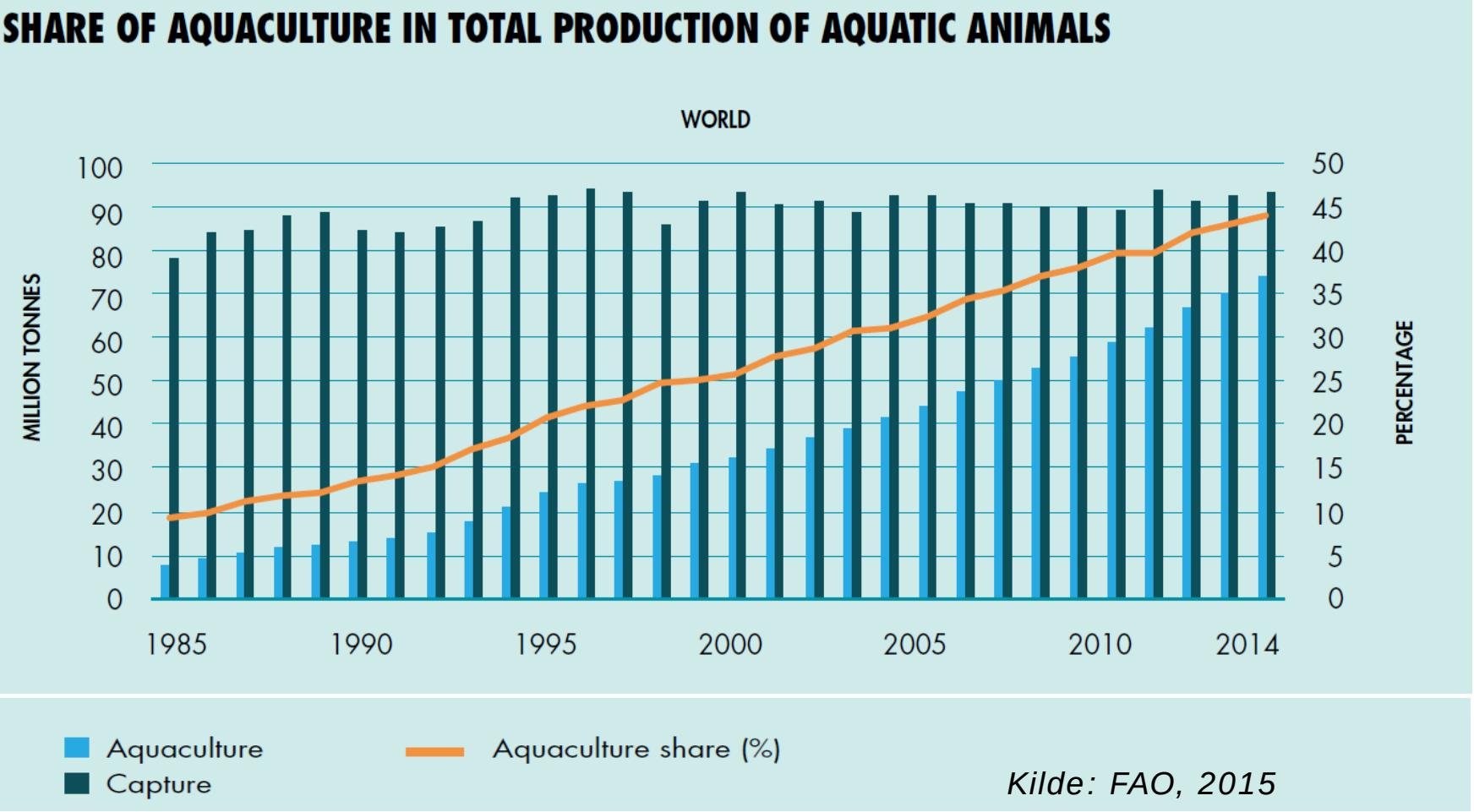
Outline

- Introduction to fish feed
- Lipid oxidation and antioxidants in fish feed
- Aims of our work
- The role of iron
- Oxipress and storage experiments
  - Effect of blood meal
  - Effect of antioxidants
- Conclusions and perspectives

The aquaculture production is increasing



Kilde: FAO, 2015

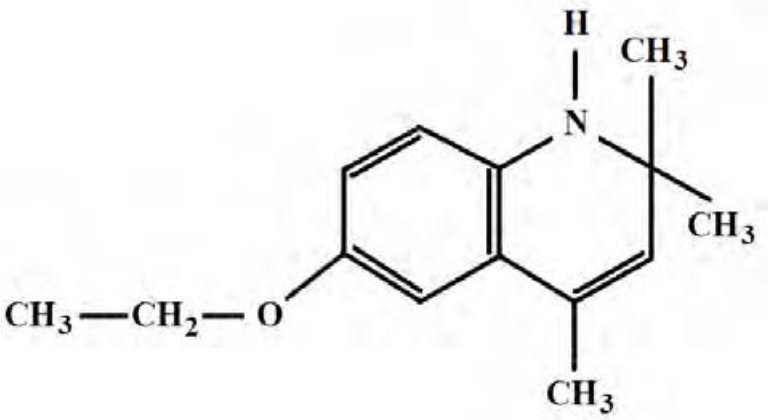
Rainbow trout fish feed – main components

Ingredient	Content
Fish meal	15-30%
Vegetable protein sources	15-50%
Bloodmeal (protein source)	0-10%
Fish oil	5-20%
Vegetable oil	0-25%
Binder (Starch – mainly wheat)	8-15%
Premix (vitamins, minerals, lecithin, amino acids)	<1%
Antioxidant	>150 ppm

Factors that can affect lipid oxidation in fish feed

- Fish oil/rapeseed oil content and quality
  - fish oil contains 20-25 % EPA and DHA
  - TOTOX (2 x PV + AV) usually <30, sometimes <40)
- Minerals – iron, cupper, magnesium
- Protein source
  - Fish meal contains heme iron in low levels
- Addition of blood meal
- Antioxidant addition – ethoxyquin and BHT are extensively used

Ethoxyquin (EQ)



- In 1950ies EQ developed by Monsanto as a rubber stabilizer
- In 1965 EQ registered as a pesticide to prevent browning of the scald of pears and apples
- Later applications expanded to antioxidant

Ethoxyquin continued

- EQ prevents heat and explosive danger during transport of fish meal due to lipid oxidation
- EQ added to fish feed alone or in combination with other antioxidants to prevent lipid oxidation
- The most efficient and widely used antioxidant in fish feed
- Maximum limit in fish feed 150 mg/kg
- A free radical scavenger
- EQ and decomposition products of EQ have been found in salmon muscle
- May be prohibited in the EU in the near future

Aims of the study

- To determine content of heme iron in feed and blood meal
- To investigate the role of heme iron in lipid oxidation of fish feed by producing feed with or without bloodmeal
- To evaluate the effect of addition of different antioxidants to the meal and/or oil fractions in fish feed
  - Compared with EQ
  - Compared with control without antioxidants

In this presentation only data from selected samples are shown

Analysis of iron in ingredients and fish feed

Non-heme iron determination

- Spectrophotometric method for non-heme iron: Reduction of iron in the ferric state (Fe3+) to iron in the ferrous state (Fe2+)

- Formation of a colored complex between the latter and bathophenanthroline

- Quantification was done by measuring absorbance at 540 nm

Analysis of iron in ingredients and fish feed

Heme iron determination

- Spectrophotometric method for heme iron: Measurements of hematin content

- Quantification was done by measuring absorbance at 640 nm where heme iron content was estimated using the calculation:

Heme iron (µg/g) = Hematin content (µg/g) x atomic weight of iron / molecular weight of hematin

Results of non-heme and heme iron analysis

	Heme iron (ug/g)		Non-heme iron (ug/g)		Total esstimated iron (ug/g)
Sample	avg	stdev	avg	stdev	
bloodmeal	805,75	1,97	483,30	45,22	1289,05
fish feed + bloodmeal	27,57	24,04	43,55	9,37	71,12
fish feed	6,85	11,96	35,82	7,57	42,68

Experimental details

Design

- MAOno: Antioxidant A in meal, no antioxidant in oil
- MnoOno: No antioxidant in meal and oil
- MBOno: EQ in meal, no antioxidant in oil
- MAOC: Antioxidant A in meal, antioxidant C in oil

With or without blood meal

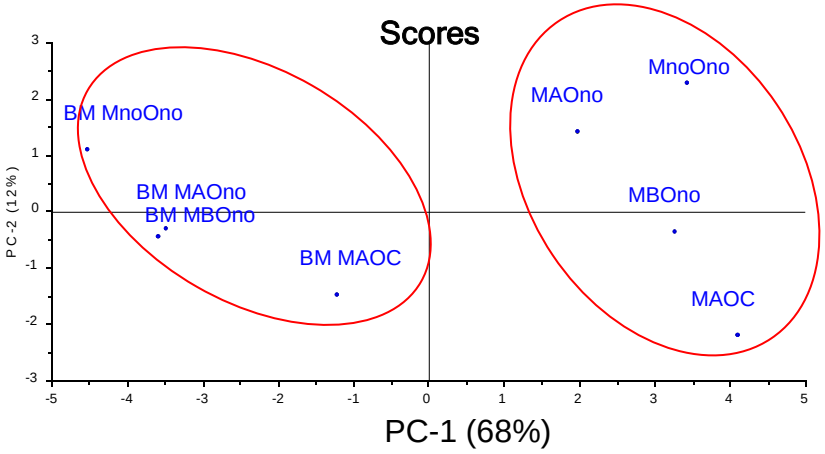
Storage experiment

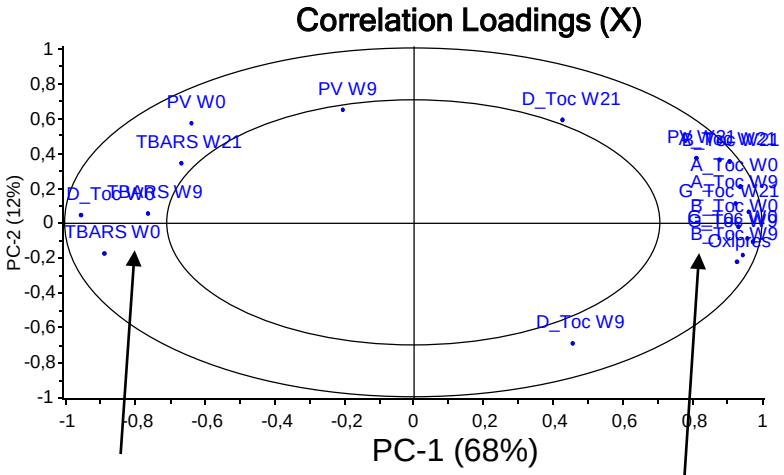
- Fish feed samples (≈500 g per sample) were stored at +35 ^{o}C in darkness
- Three sampling points: week 0, 9 and 21
- At each sampling point ≈30 g of each sample was taken out and stored at -40 ^{o}C prior to analysis

Oxipress: accelerated study at 90 ^{o}C

Principal components analysis

With bloodmeal **W.O bloodmeal**





BM = blood meal, M= antioxidant in meal

O= antioxidant in oil, B= EQ

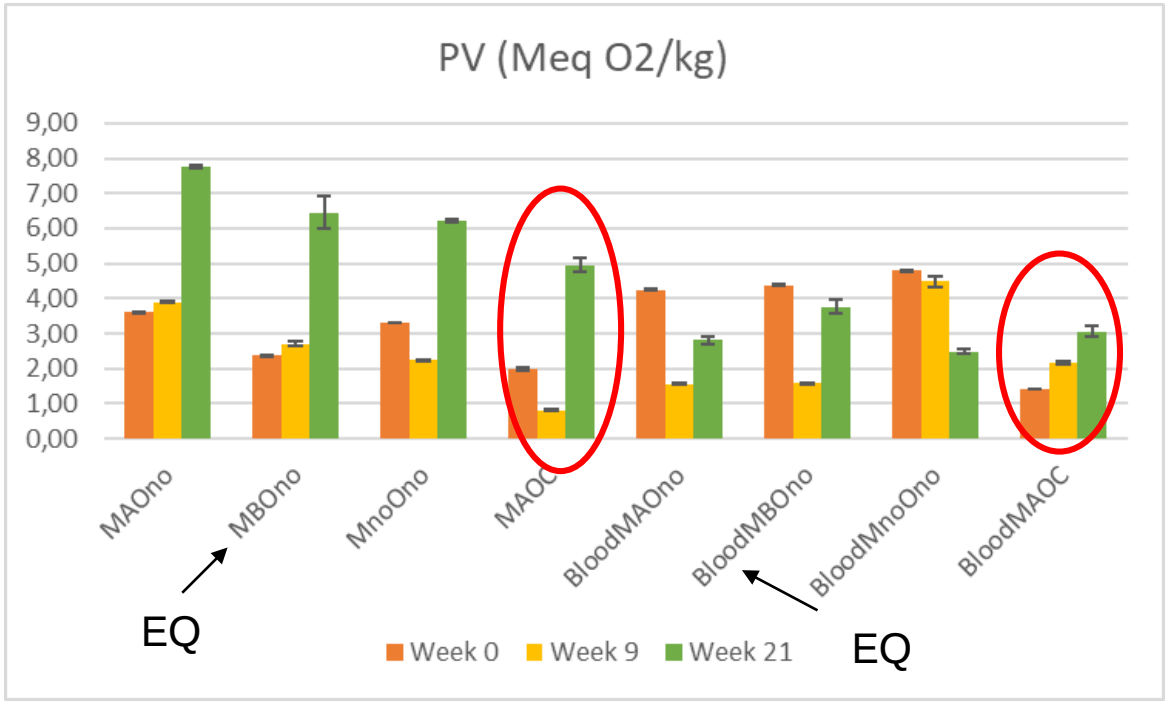
A, C = different Aos, No= no antioxidant

TBARS, PV (0 & 9)

Toco, Oxipress, PV21

- Samples with bloodmeal more oxidized
- Samples with MAOC seemed to oxidize less

Comparison of PV in samples with and without bloodmeal



M= antioxidant in meal

O= antioxidant in oil

B= EQ

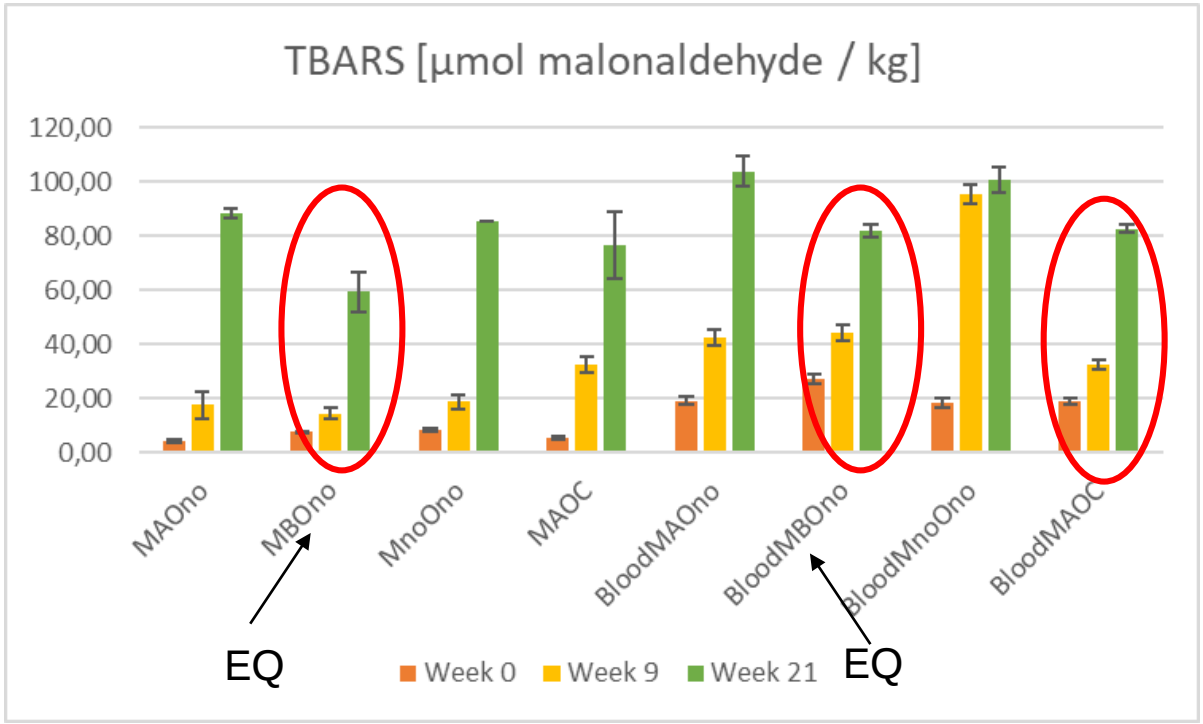
A, C = different AOs

No= no antioxidant

Blood = blood meal

- PV in samples without blood meal increased during storage
- PV in samples with blood meal in general decreased during storage
- Combination MAOC the most efficient (better than ethoxyquin)

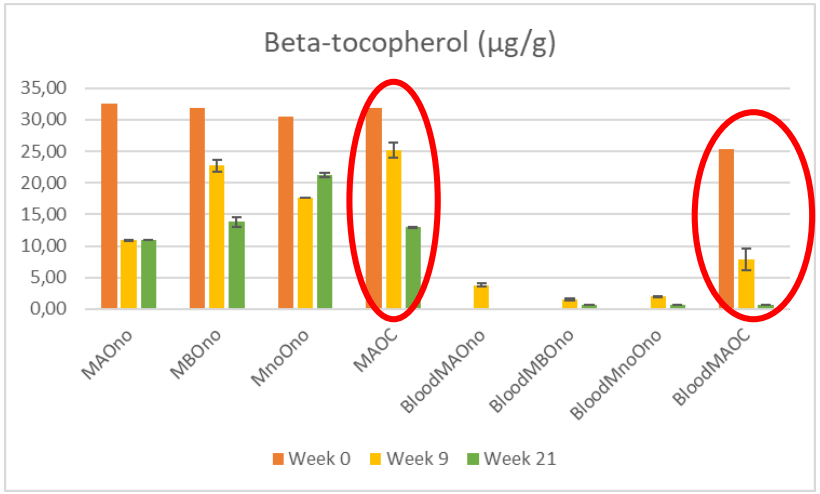
Comparison of TBARS in samples with and without bloodmeal

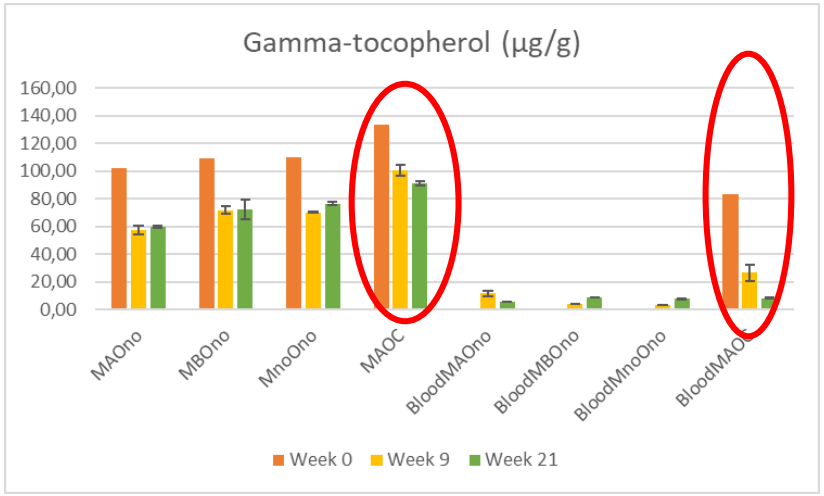


- M= antioxidant in meal
- O= antioxidant in oil
- B= EQ
- A, C = different AOs
- No= no antioxidant
- Blood = blood meal

- TBARS increased faster in samples with bloodmeal
- For samples w.o. bloodmeal EQ was the most efficient AO
- For samples w. bloodmeal MAOC was as good as EQ

Comparison of tocopherol in samples with and without bloodmeal





- Bloodmeal resulted in disappearance of toco
- MAOC resulted in slightly better protection of tocopherols in feeds w.o. bloodmeal and in markedly better production in feeds w. bloodmeal

M= antioxidant in meal

O= antioxidant in oil

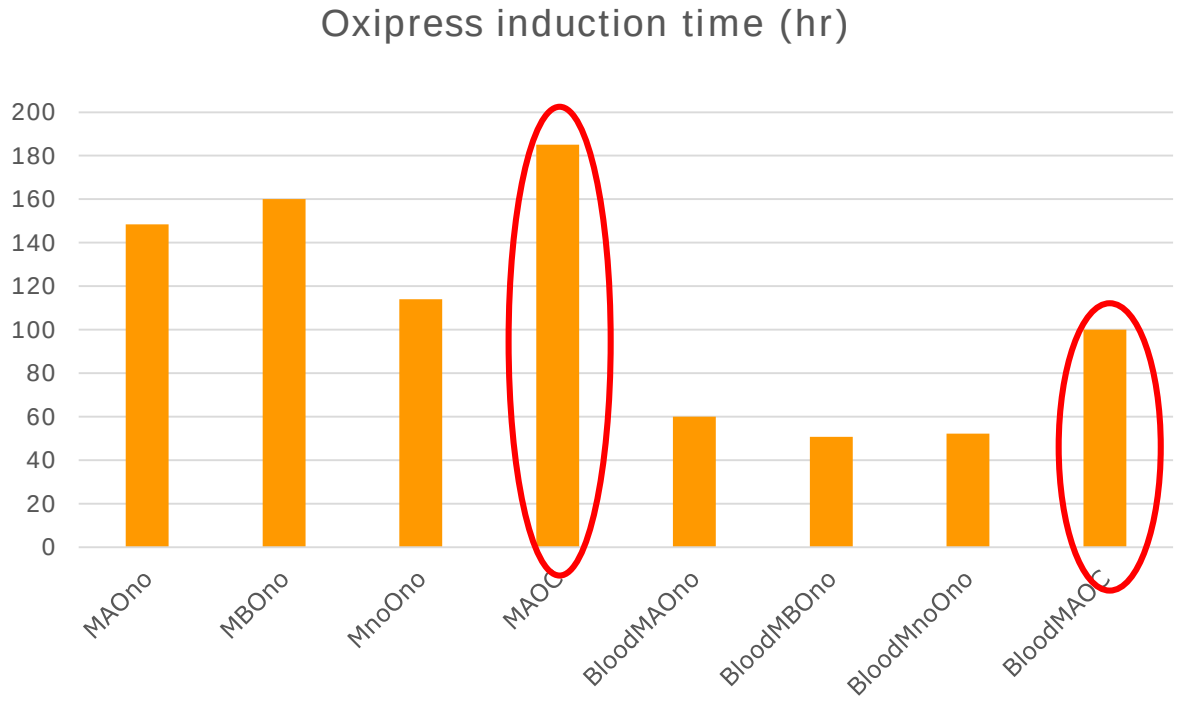
B= EQ

A, C = different AOs

No= no antioxidant

Blood = blood meal

Comparison of Oxypress data in samples with and without bloodmeal



M= antioxidant in meal

O= antioxidant in oil

B= EQ

A, C = different AOs

No= no antioxidant

Blood = blood meal

- Samples with bloodmeal oxidized faster
- MAOC was the most efficient antioxidant in both feed types (better than EQ (MBOno)

Conclusions and perspectives

- Oxipress data correlated well with TBARS and tocopherol, but not with PV
- Feeds with bloodmeal oxidized faster – due to high levels of iron
- Feed with antioxidants in both meal and oil (MAOC) oxidized less than when only EQ was added to the oil phase
- Feeds with bloodmeal may need both a metal chelator and radical scavenger to efficiently prevent lipid oxidation
- Further studies will involve a range of different antioxidants and a new set-up where effect of individual ingredients are studied