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Predicting fillet lipid composition of *Oncorhynchus mykiss* using different statistical tools

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HEALTH BENEFITS of OMEGA-3



Value has placed on **Long Chain Polyunsaturated Fatty Acids** (LC-PUFA) of the n-3 series due to their beneficial effects on **human health**

Fish is the main source of LC-PUFA and recommended intake doses are provided

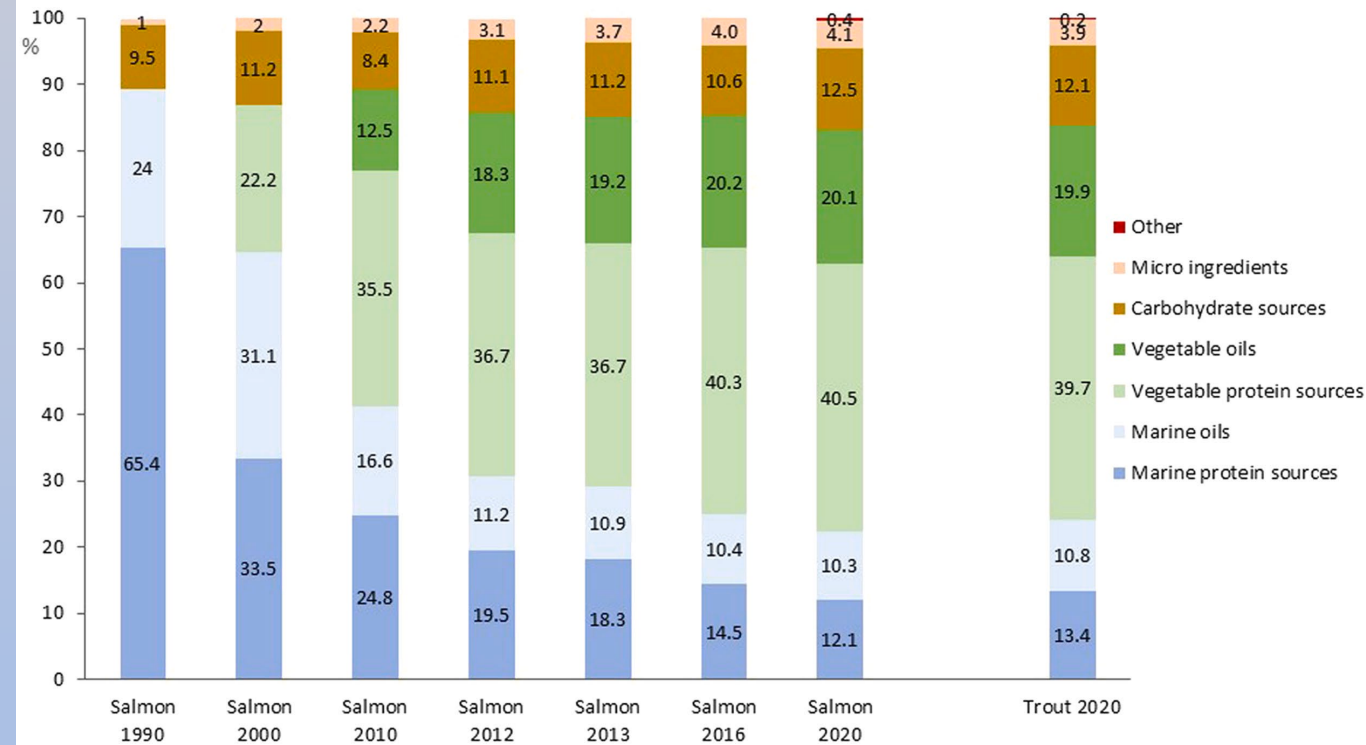
Food	Grams per serving		
	ALA	DHA	EPA
Flaxseed oil, 1 tbsp	7.26		
Chia seeds, 1 ounce	5.06		
English walnuts, 1 ounce	2.57		
Flaxseed, whole, 1 tbsp	2.35		
 Salmon, Atlantic, farmed, cooked, 3 ounces	1.24	0.59	
 Salmon, Atlantic, wild, cooked, 3 ounces	1.22	0.35	
 Herring, Atlantic, cooked, 3 ounces*	0.94	0.77	
Canola oil, 1 tbsp	1.28		
 Sardines, canned in tomato sauce, drained, 3 ounces*	0.74	0.45	
 Mackerel, Atlantic, cooked, 3 ounces*	0.59	0.43	
 Salmon, pink, canned, drained, 3 ounces*	0.04	0.63	0.28
Soybean oil, 1 tbsp	0.92		
 Trout, rainbow, wild, cooked, 3 ounces	0.44	0.40	
Black walnuts, 1 ounce	0.76		
Mayonnaise, 1 tbsp	0.74		
Oysters, eastern, wild, cooked, 3 ounces	0.14	0.23	0.30
 Sea bass, cooked, 3 ounces*	0.47	0.18	
Edamame, frozen, prepared, ½ cup	0.28		
Shrimp, cooked, 3 ounces*	0.12	0.12	
Refried beans, canned, vegetarian, ½ cup	0.21		
Lobster, cooked, 3 ounces*	0.04	0.07	0.10
 Tuna, light, canned in water, drained, 3 ounces*	0.17	0.02	
 Tilapia, cooked, 3 ounces*	0.04	0.11	
Scallops, cooked, 3 ounces*	0.09	0.06	
 Cod, Pacific, cooked, 3 ounces*	0.10	0.04	
 Tuna, yellowfin, cooked 3 ounces*	0.09	0.01	



Arcimboldi Giuseppe, Testa composta con pesci, crostacei, perle e coralli

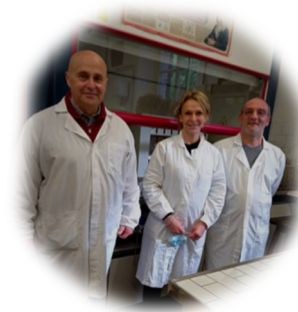
Clear role of the **diet** in
determining FA profile of
fish flesh

Marine ingredients shortage
leads the research for new
ingredients and new
formulation, thus **affected fish**
flesh quality

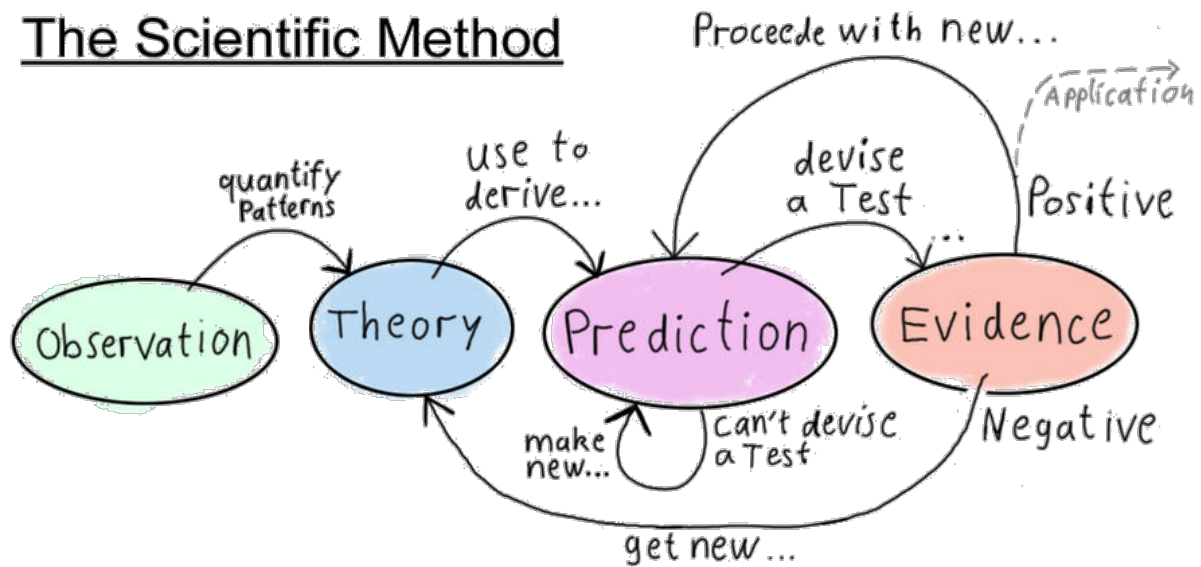


Aas et al., 2022 <https://doi.org/10.1016/j.aqrep.2022.101317>

We trust in the possible transition towards a sustainable aquaculture and fillet quality is our main expertise, thus we worked to develop new **sustainable diets** in which marine or vegetable ingredients are cut-off



The Scientific Method



Objective of this study was to benchmark different sources of information and statistical tools to **predict fillet lipid composition** in rainbow trout (*Oncorhynchus mykiss*).

173 observations (animals)

60 tanks

20 diets

5 diet groups as defined by protein source

5 random diet groups



Golden standard;

Vegetal proteins;

Alternative animal proteins;

Alternative microbial proteins;

Commercial diets.

Aquafeeds were characterized for their chemical composition, and ingredient abundance;

FA profile of both aquafeed and fillets was obtained by gas-chromatography



The **impact of different dietary factors** was tested; there were:

- “aa”: Ingredients
- “bb”: Crude Protein, Crude Lipids
- “cc”: Lipid composition, single FA
- “dd”: Lipid composition, groups of FA



These sets of variables were used as predictors

The model used was a linear regression of the fillet lipid component on the dietary factor:

$$\text{fillet} = \text{feed} + \text{block} + \text{error}$$

where ‘fillet’ is the fillet fatty acids (e.g. n-3 PUFA), ‘feed’ is the fixed effect of the chosen dietary component (e.g. Crude Protein), ‘block’ is the random effect of the tank (i.e. physical tank), ‘error’ is the random residual error (i.e. the fish).

One-way ANOVA was constructed based on the results from the mixed model.

All combinations of fillet lipid components and dietary components were tested, each combination represents a single ‘run of analysis’.

Prediction using three different models:

- Multiple Linear Regression (MLR)
- Partial Least Squares (PLS)
- Random Forest (RF)

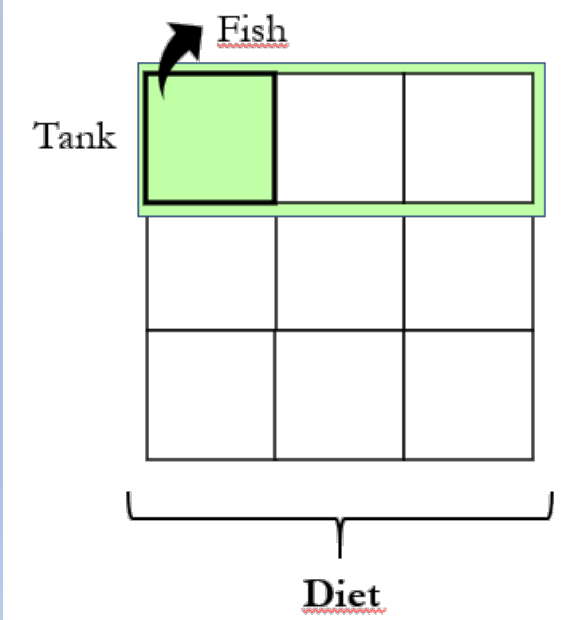
Prediction using three different models:

- Multiple Linear Regression (MLR)
- Partial Least Squares (PLS)
- Random Forest (RF)

Four cross-validation were utilized:

1. WITHIN-PROTEIN +
WITHIN-DIET + WITHIN-TANK

2. WITHIN-PROTEIN +
WITHIN-DIET + ACROSS-TANK



3. WITHIN-PROTEIN + ACROSS-
DIET + ACROSS-TANK

One diet per protein source are assigned to validation.

- o All protein sources appear in training.
- o Five replicates, one per random diet group.

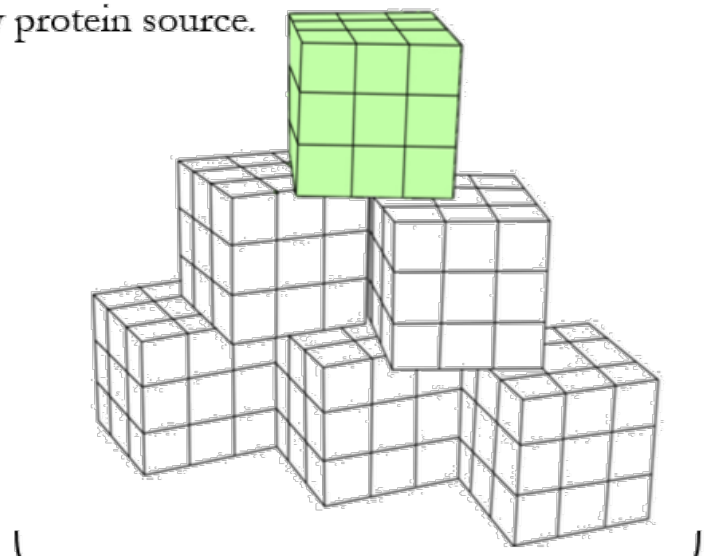


Protein «group»

4. ACROSS-PROTEIN + ACROSS-DIET +
ACROSS-TANK

One protein source is assigned to validation.

- o All tanks, all diets, all protein sources appear in training.
- o Five replicates, one per diet group ad defined by protein source.



All data

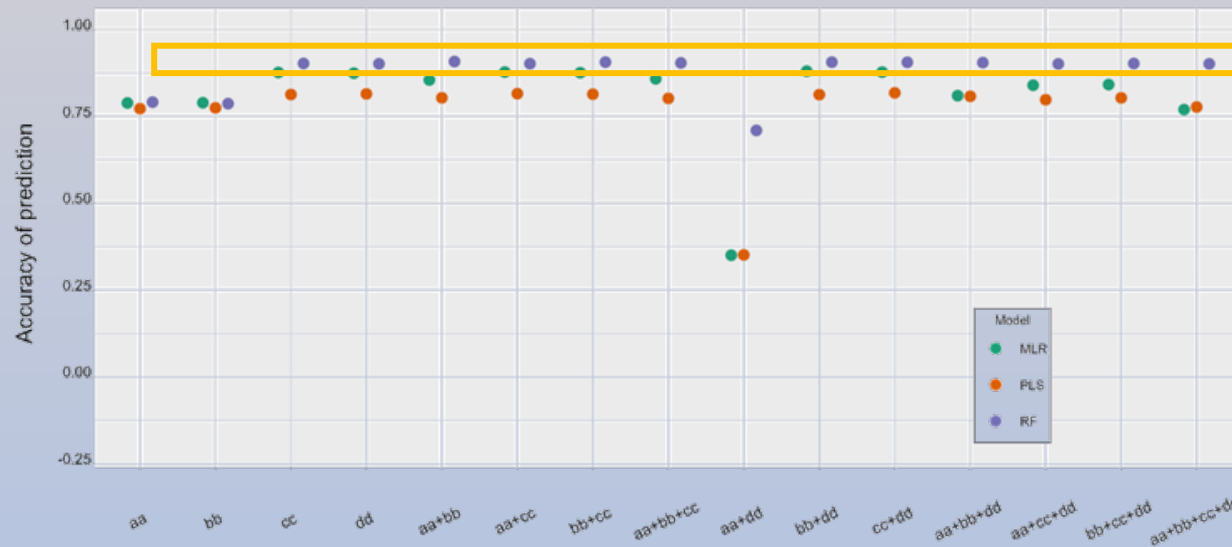
□ Training ■ Validation

Results

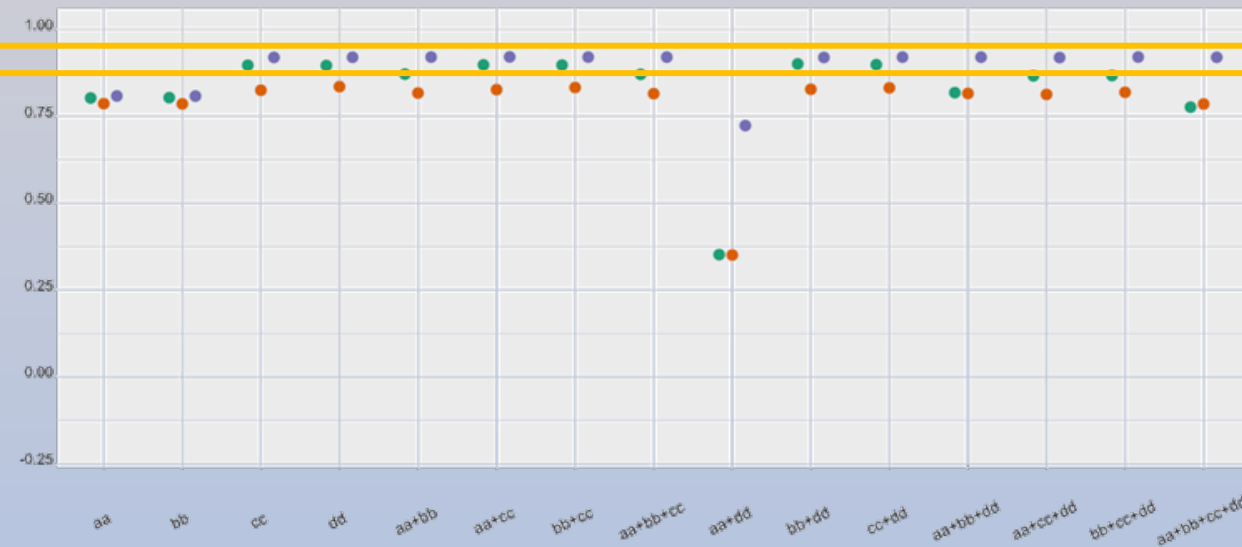


C20:5 n-3, eicosapentaenoic acid (EPA)

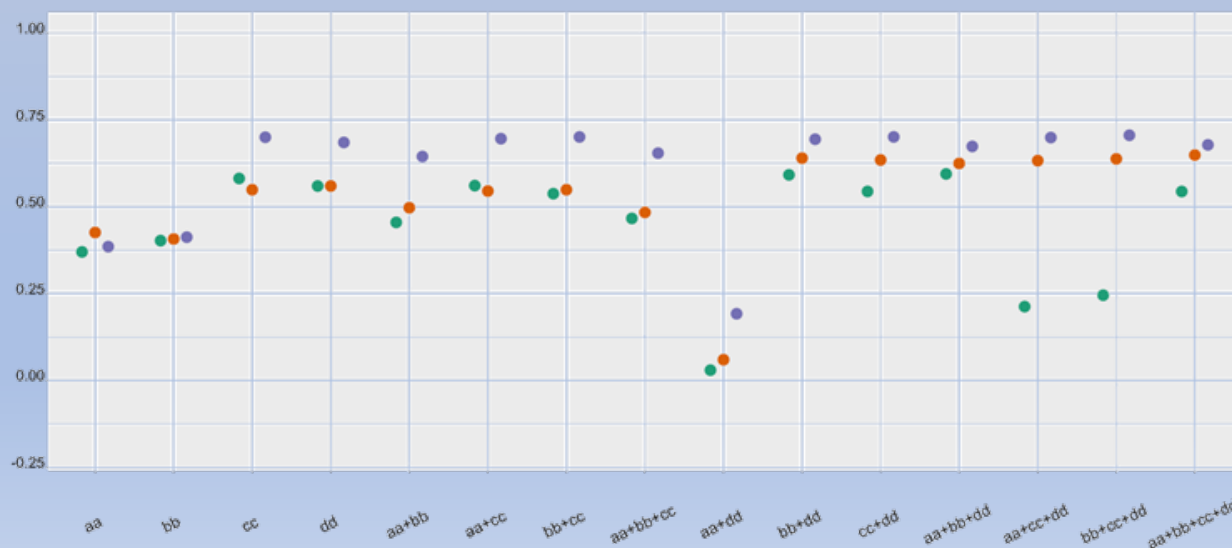
Within-Protein, Within-Diet, Within-Tank



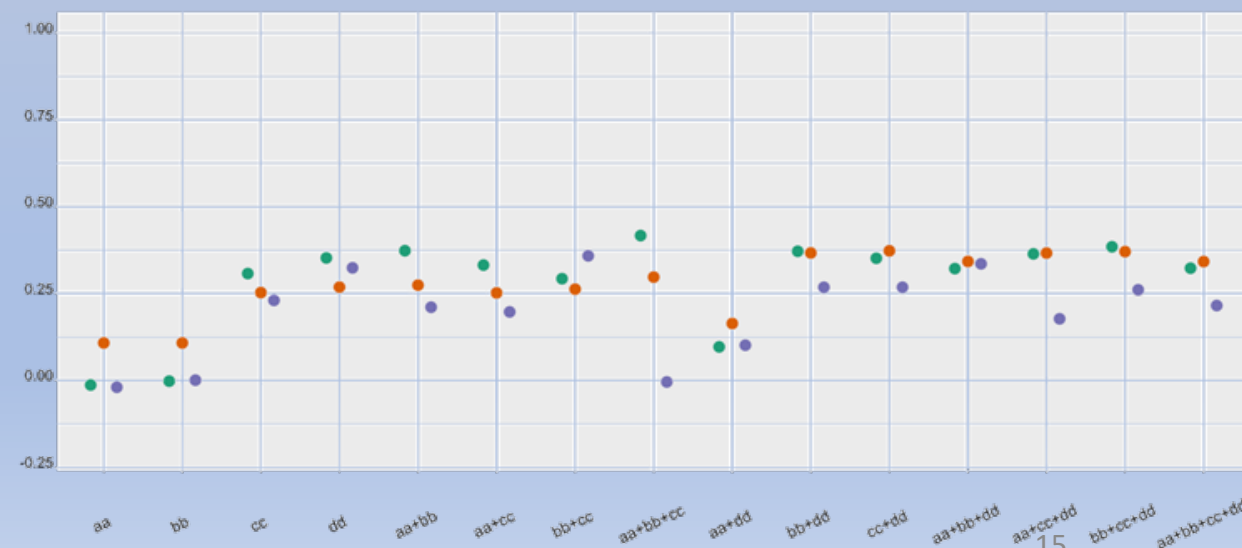
Within-Protein, Within-Diet, Across-Tank



Within-Protein, Across-Diet, Across-Tank



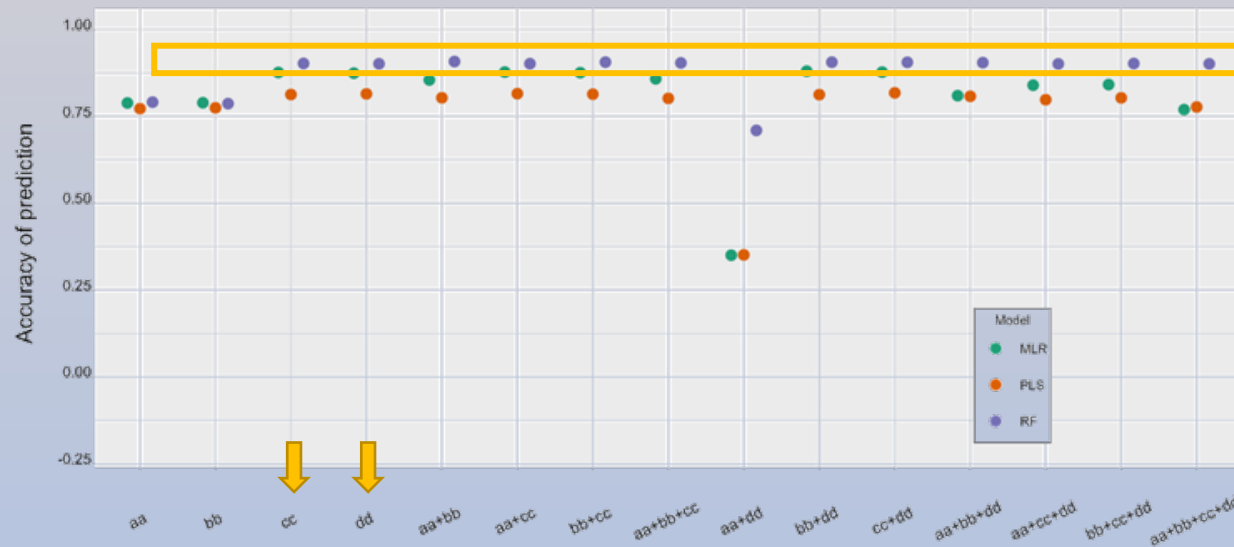
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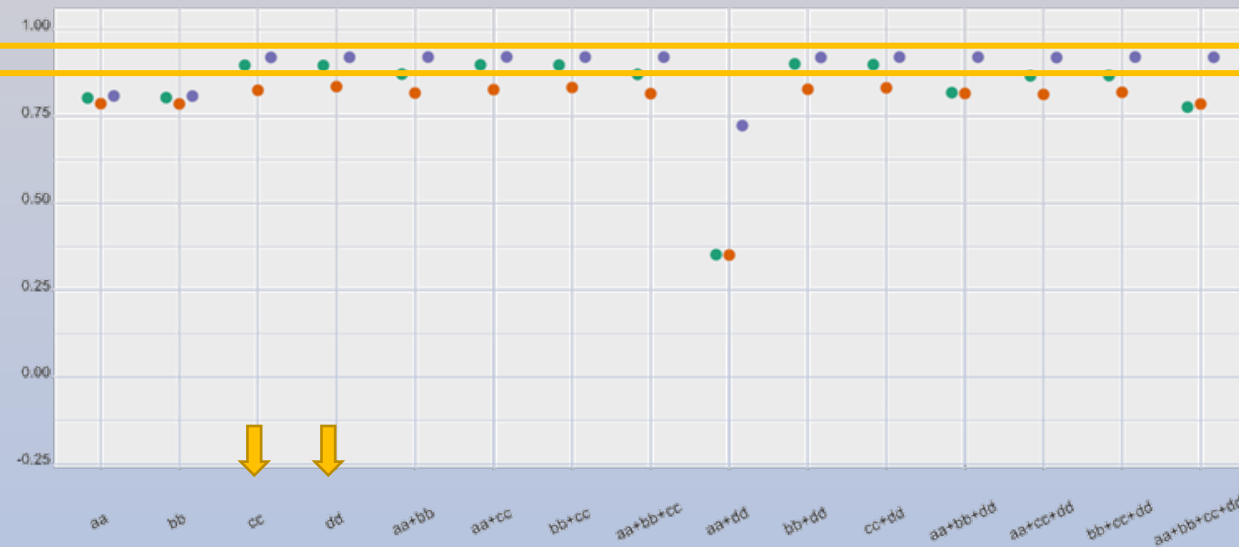
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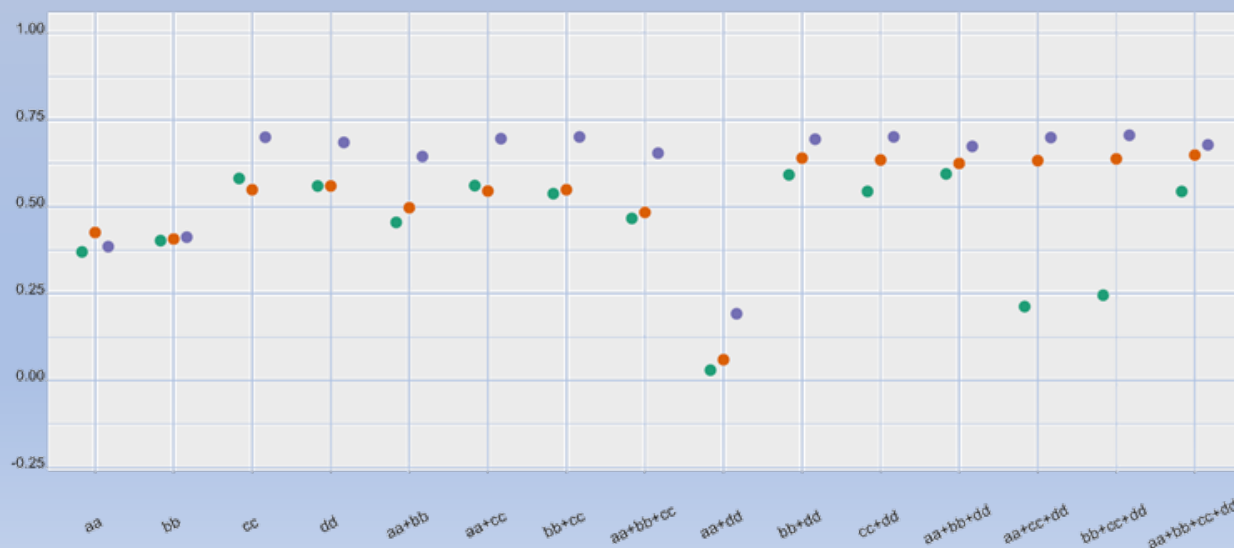
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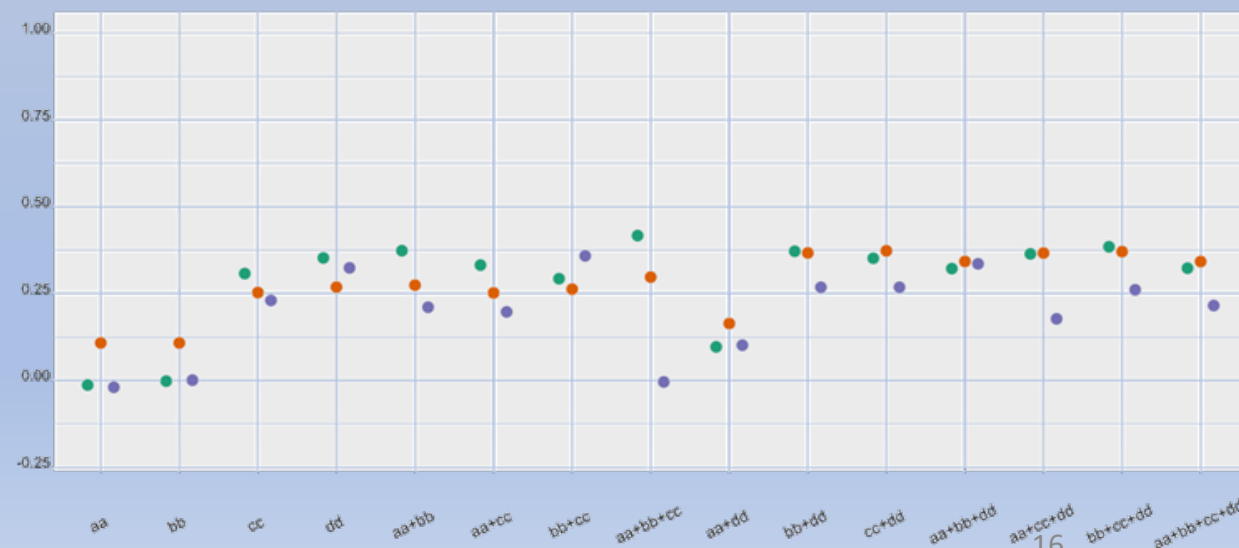
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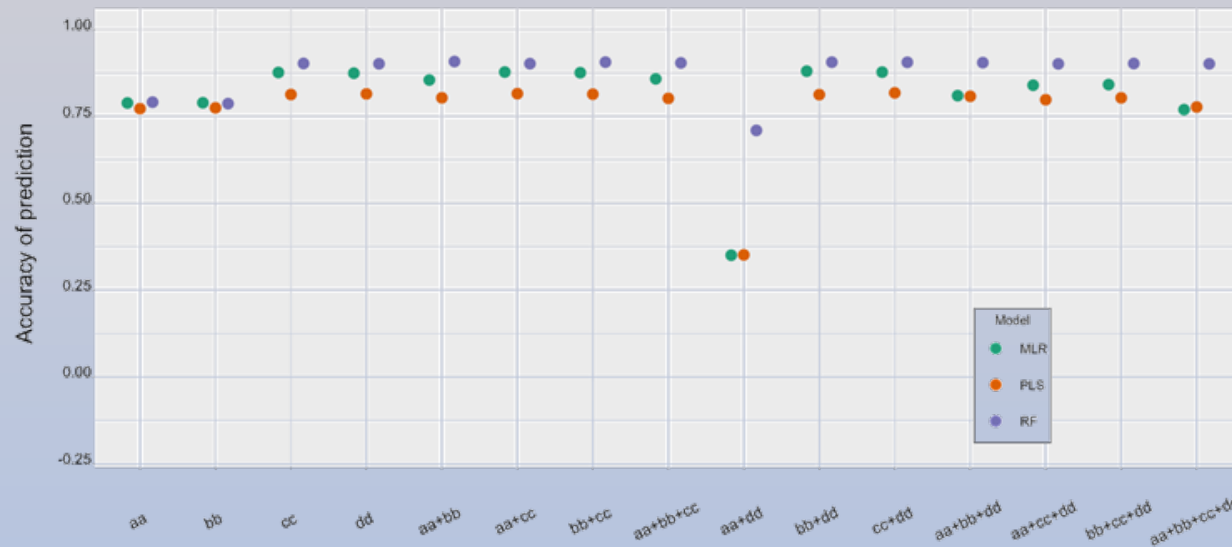
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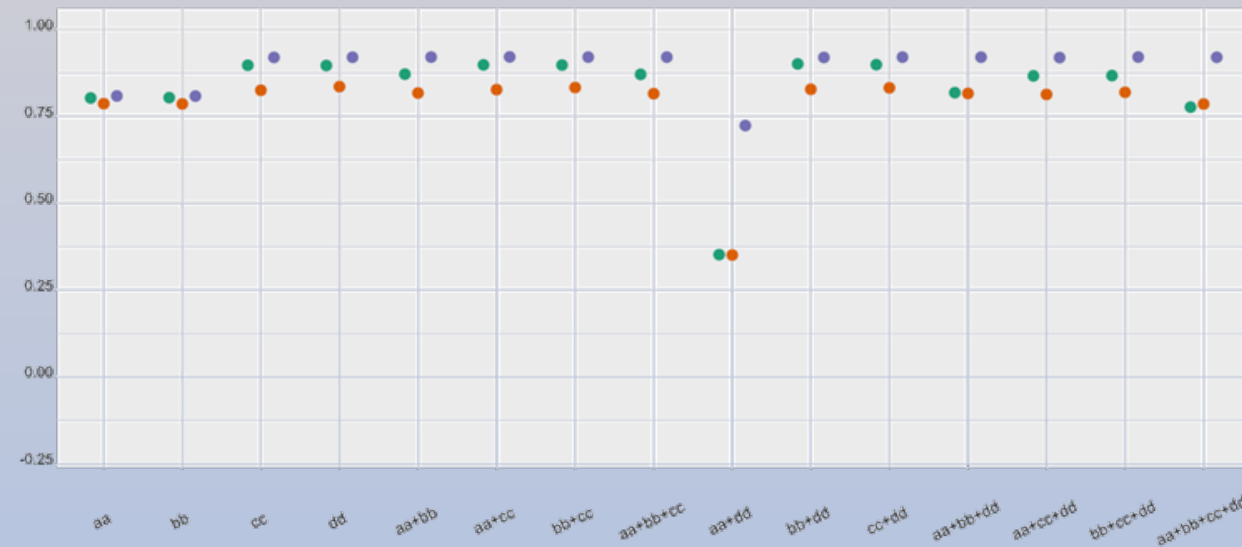
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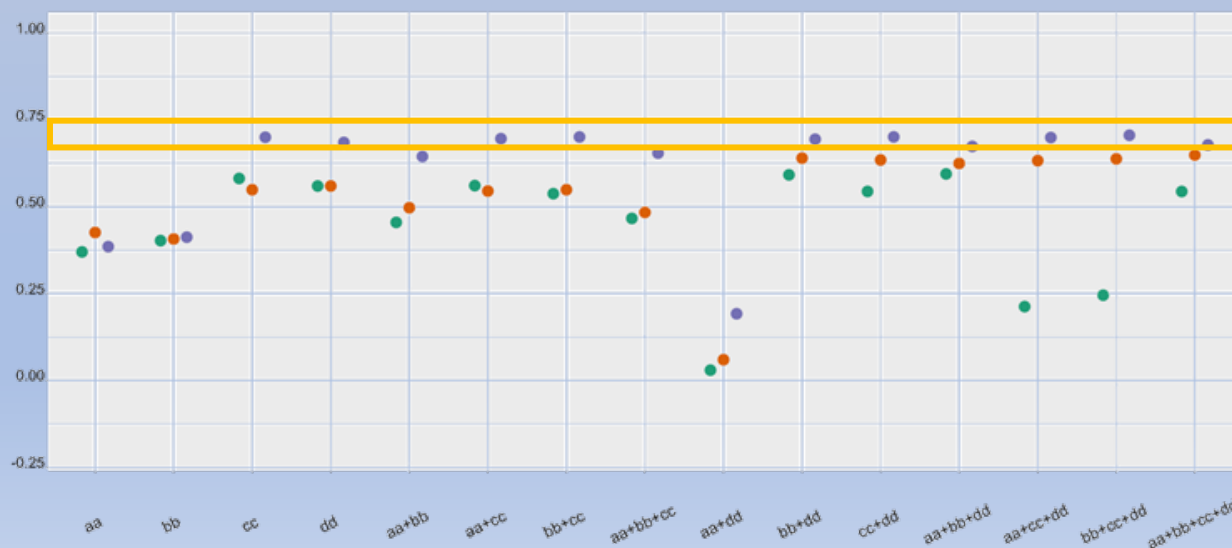
Within-Protein, Within-Diet, Within-Tank



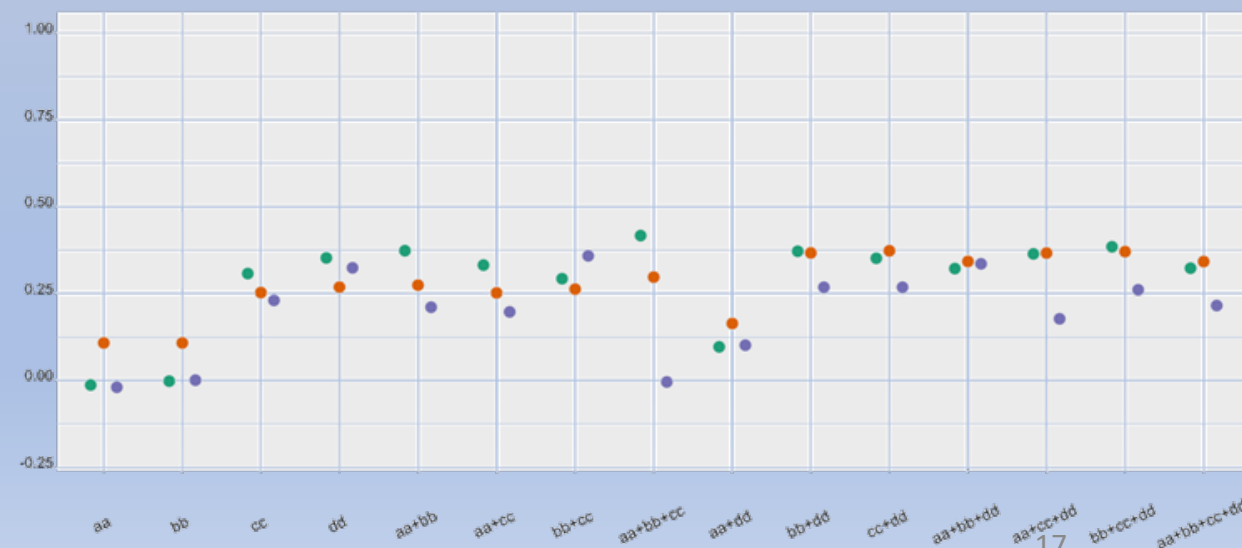
Within-Protein, Within-Diet, Across-Tank



Within-Protein, Across-Diet, Across-Tank



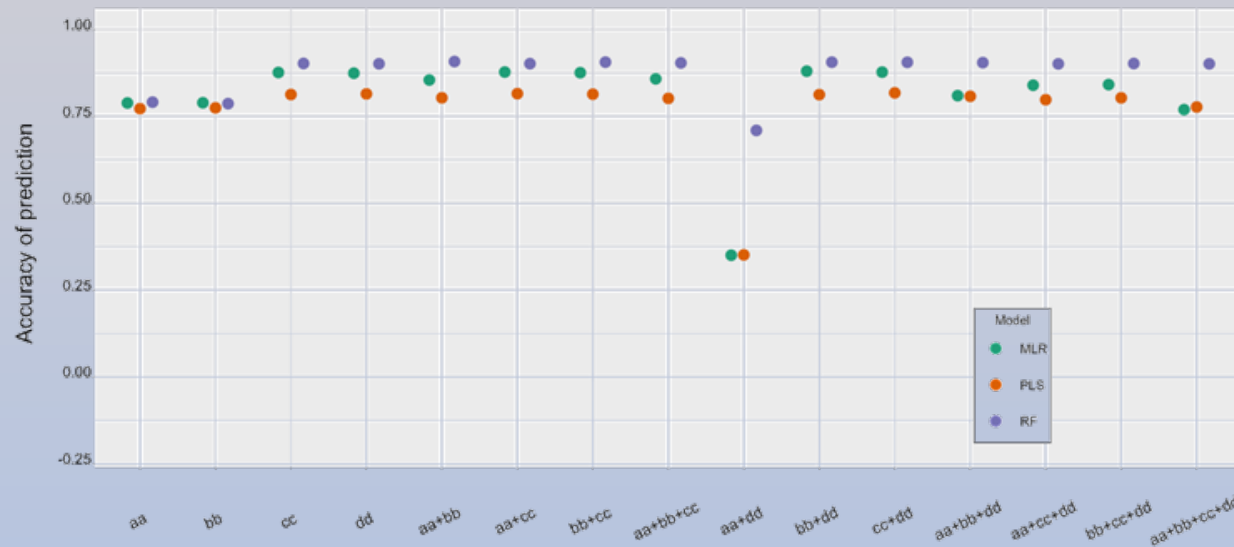
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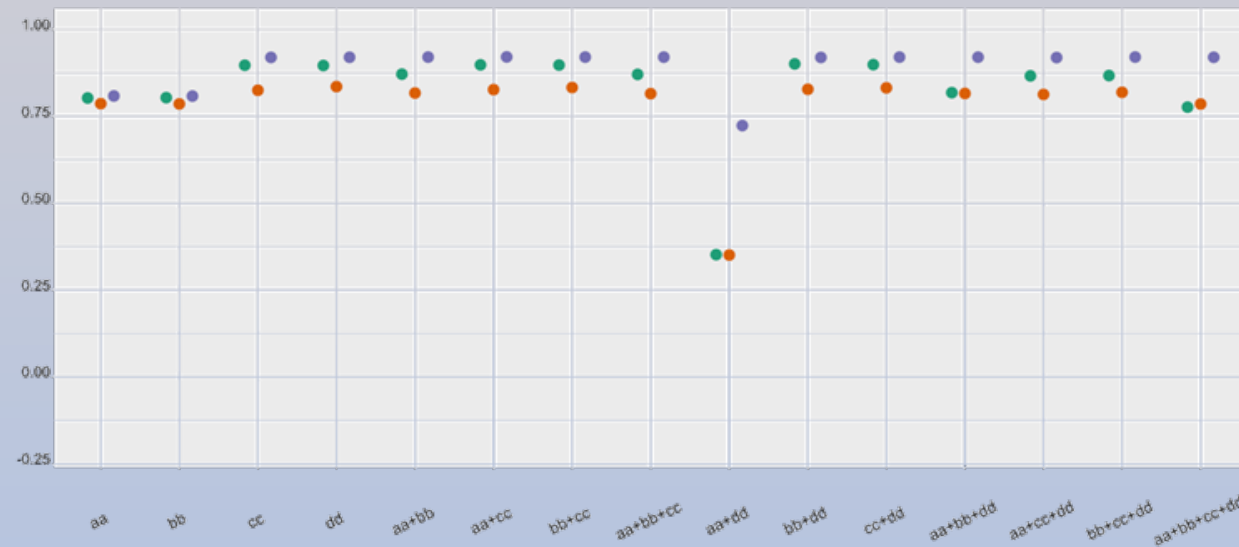
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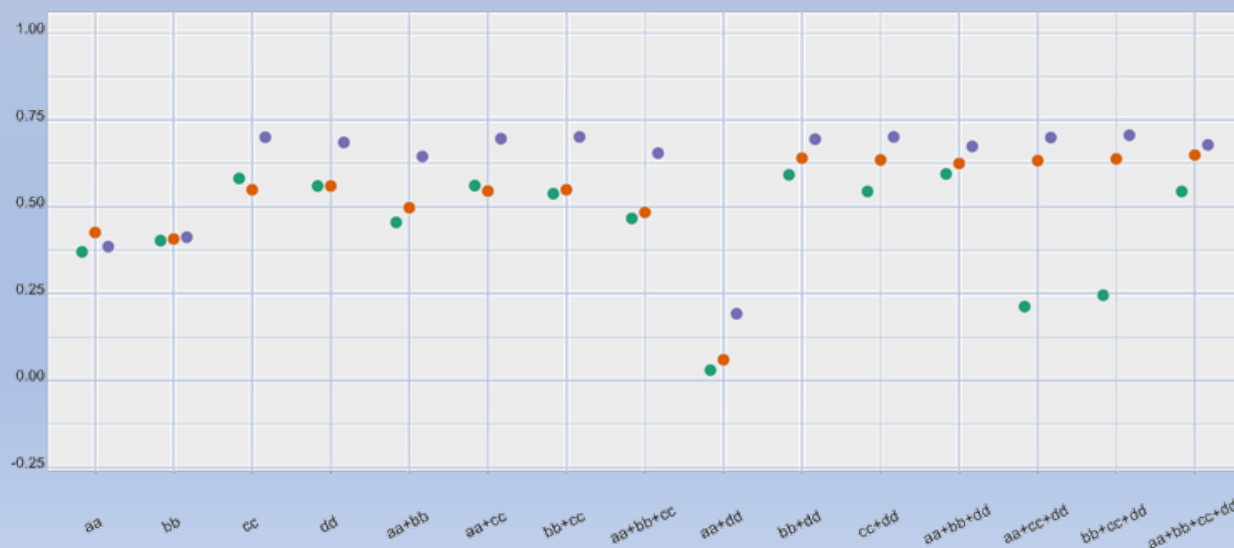
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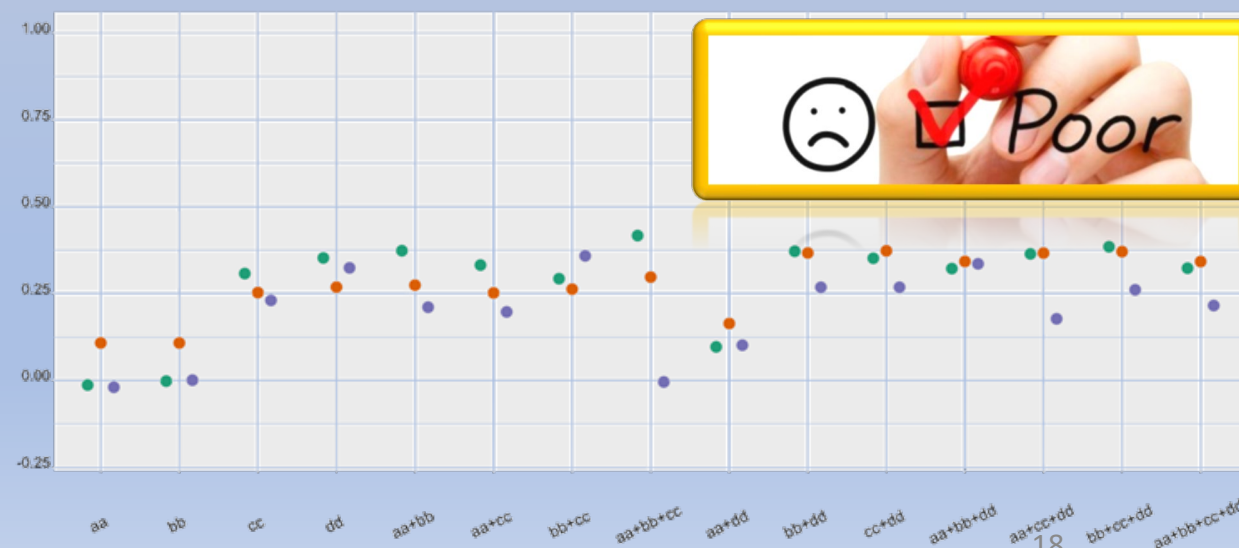
Within-Protein, Within-Diet, Across-Tank



Within-Protein, Across-Diet, Across-Tank



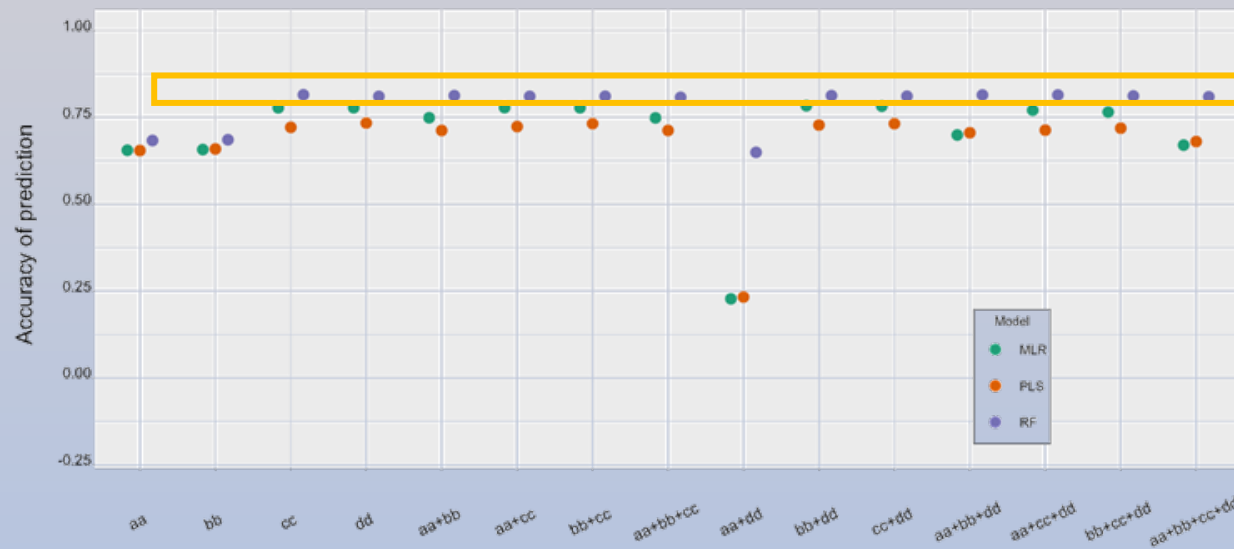
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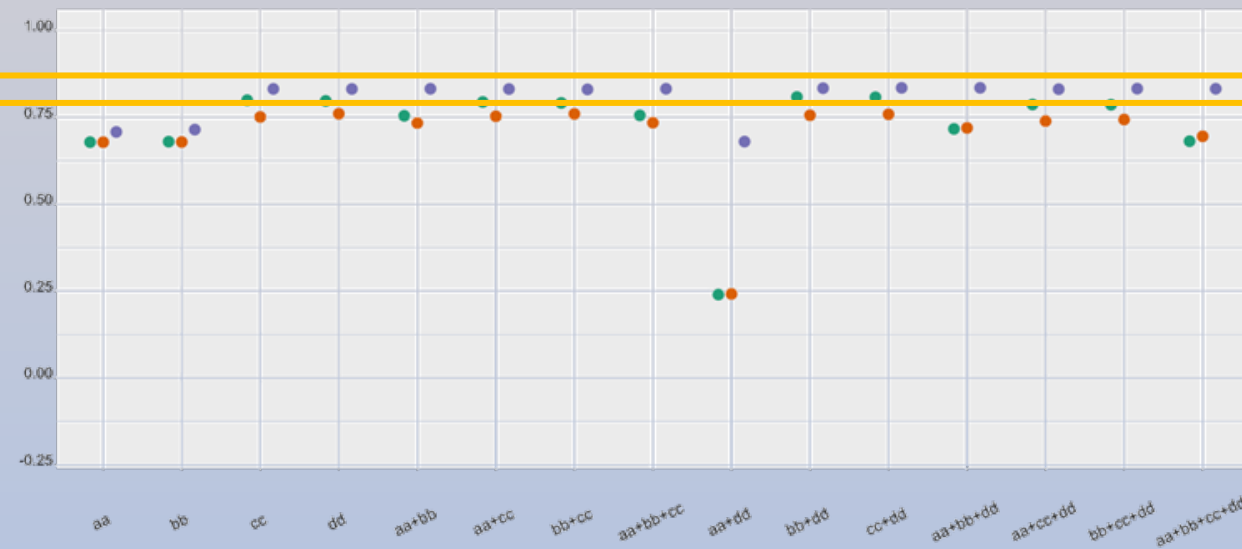
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C22:6 n-3, docosahexaenoic acid (DHA)

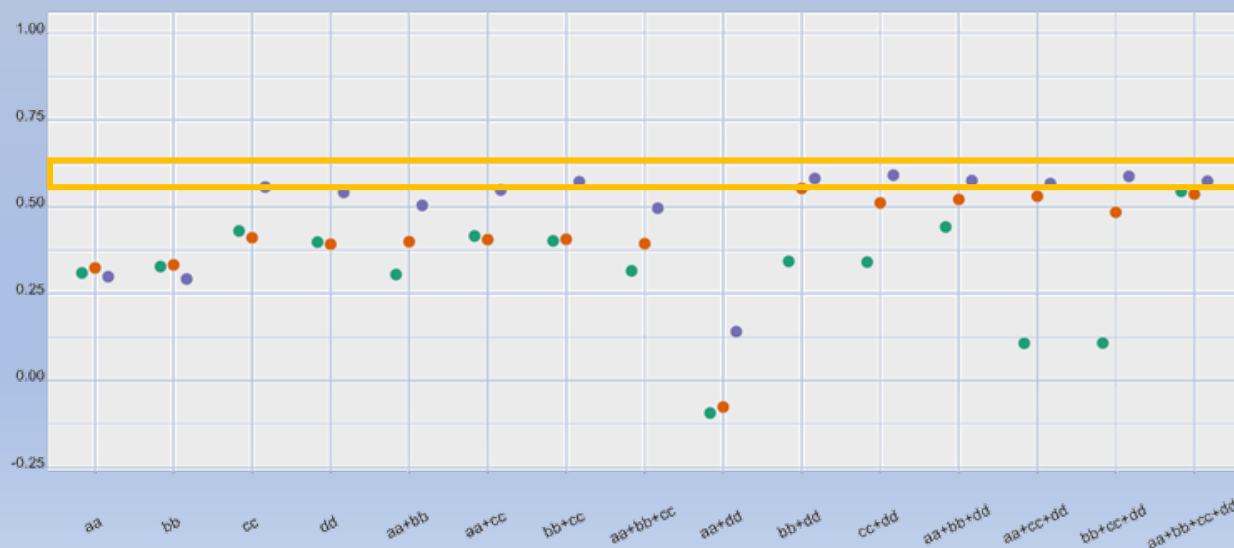
Within-Protein, Within-Diet, Within-Tank



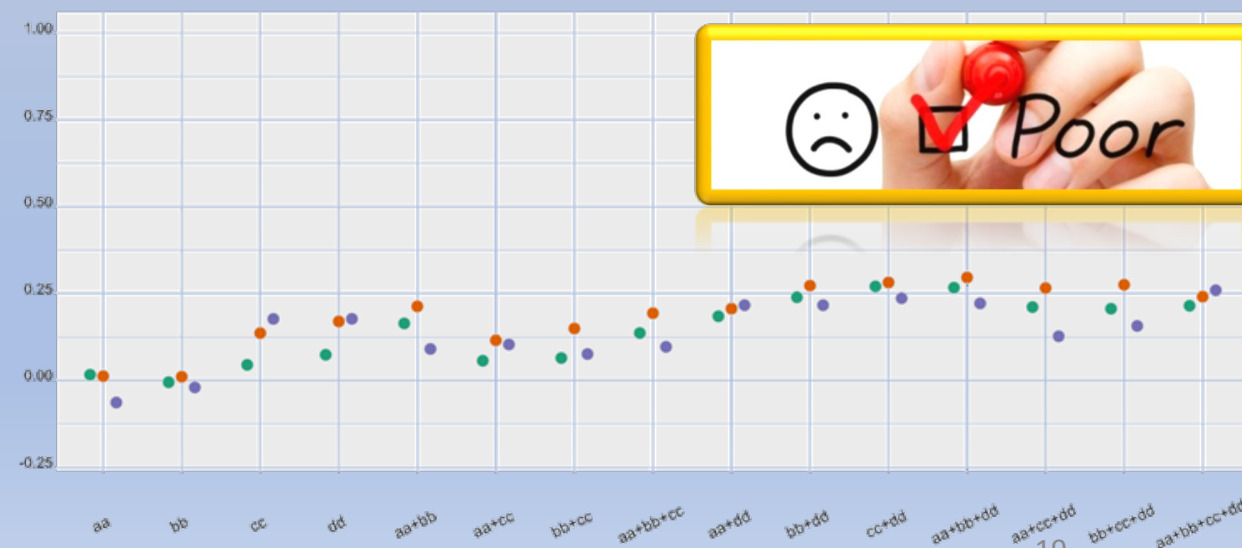
Within-Protein, Within-Diet, Across-Tank



Within-Protein, Across-Diet, Across-Tank



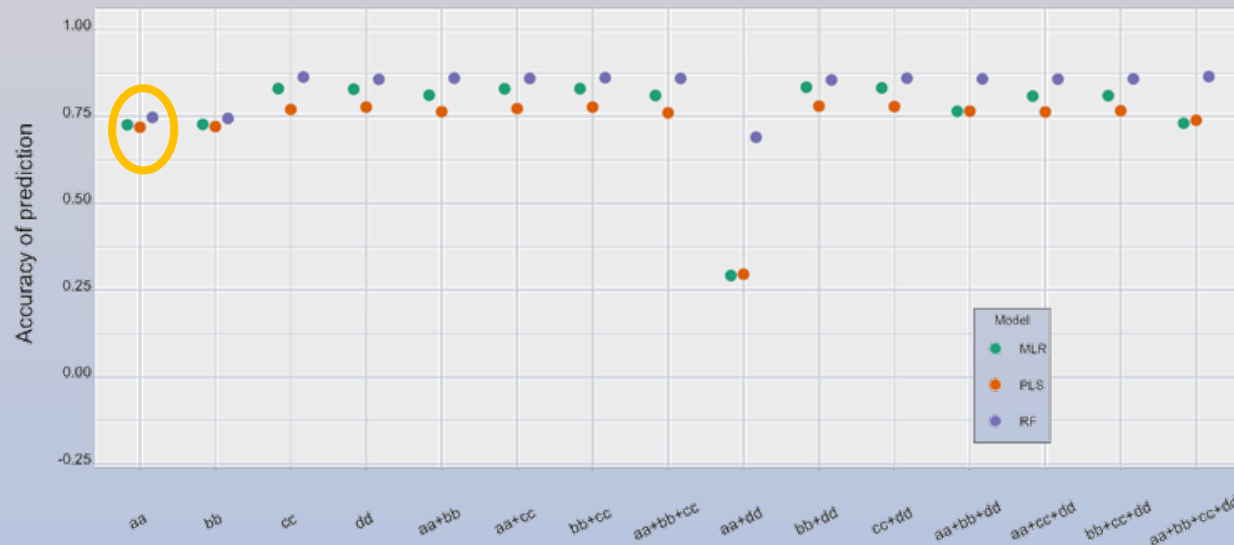
Across-Protein, Across-Diet, Across-Tank



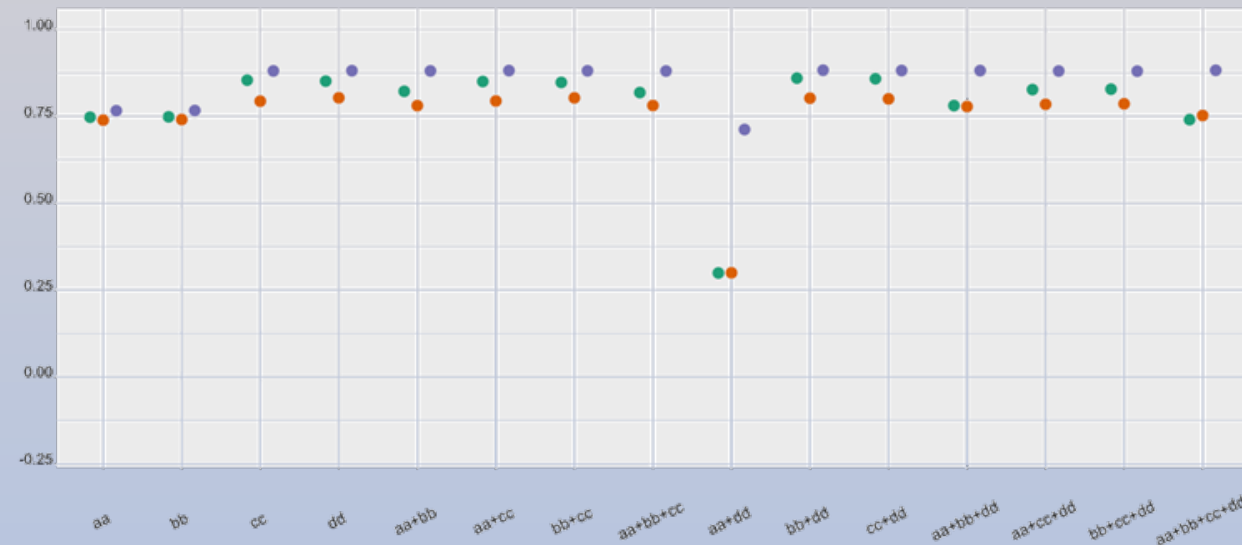
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Sum of EPA + DHA

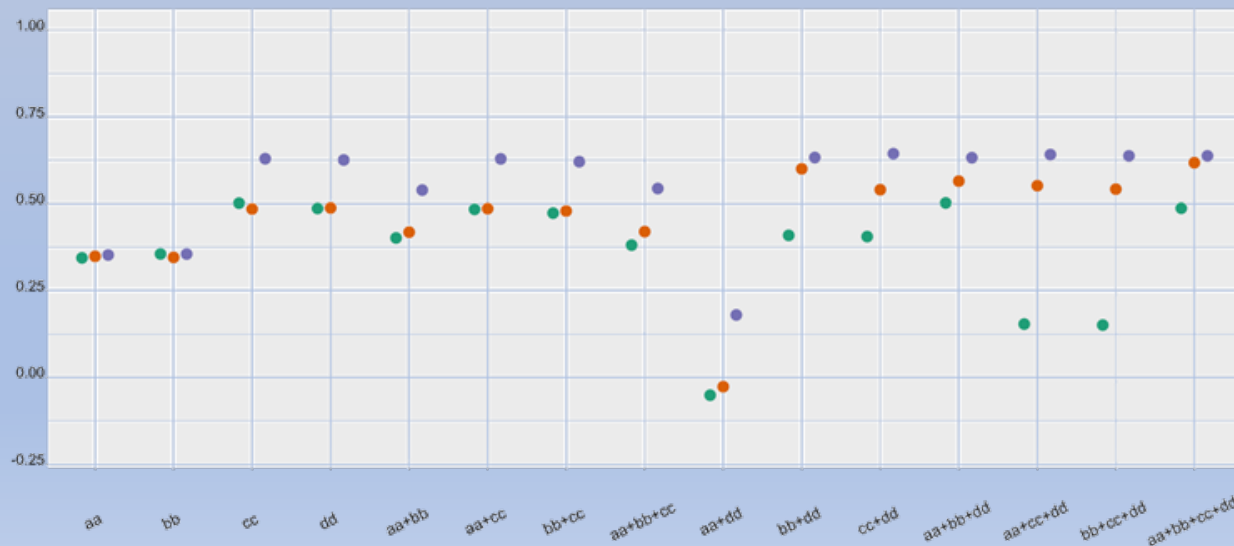
Within-Protein, Within-Diet, Within-Tank



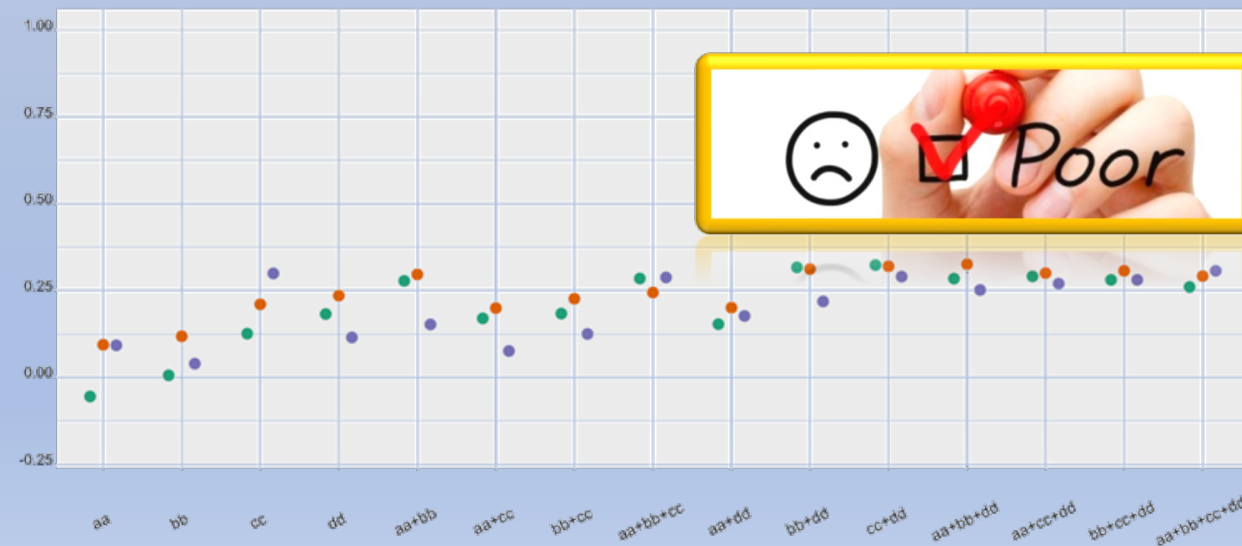
Within-Protein, Within-Diet, Across-Tank



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Across-Protein, Across-Diet, Across-Tank



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1. FA profile analysis of fish flesh cannot be avoided while studying a new feed formula



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2. The **EPA+DHA** of trout fillet could be predicted leveraging on diet description (**knowing ingredients' abundance!**)



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3. **Random Forest outperformed** the other statistical tools



1. FA profile analysis of fish flesh cannot be avoided while studying a new feed formula
2. The **EPA+DHA** of trout fillet could be predicted leveraging on diet description (**knowing ingredients' abundance!**)
3. **Random Forest outperformed** the other statistical tools
4. **FURTHER STUDIES** will focus on assessing the predictive ability of the same models when a specific diet composition is not being studied and yet included in the training set.





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