Technical Report: *DietaryBurdenCalculator 2.0.2*

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Introduction

Fish in aquaculture are fed based on a maximum reasonably balanced diet (MRBD) approach (Working Document on Residues on Fish). For this, components for fish feed are typically characterized by fixed percentages of carbohydrate concentrate, protein concentrate and fat of the feed components are regarded.

If several feed components are available many combinations of them would result in optimum protein and lipid concentrations for a given fish species. In order to calculate the maximum dietary burden calculation of residues in fish feed the simplex algorithm is considered. Georg Dantzig presented this solution method for linear programs in 1947 (Shenoy 2007, p.44). It is the most important method for linear programming (Zimmermann et al. 2001, p.48). Due to the development of computers, the simplex method offers the possibility to solve large-scale linear programming problems quickly (Shenoy 2007, p.44).

In this technical report, we present how to formulate the maximum dietary burden calculation as linear program such that the simplex method can be applied. As a result, we obtain the feed composition leading to the maximum dietary burden value.

Furthermore, we present the functionality of the software *DietaryBurdenCalculator*. For this, we calculate the dietary burden for an active substance with respect to two different important aquaculture species, rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*).

Mathematical background

In this section, we present the mathematical background of the maximum dietary burden calculation. For this, we explain the underlying assumptions and translate them into a linear equation system (modeling).

Assumptions

We calculate the maximum residue in fish feed. The following information shall be considered:

- 1. We consider two different fish species with specific requirements (different target compositions) that have to be satisfied.
- 2. The feed consists of several ingredients such as for example corn meal, peanut meal or olive cake.
- 3. These feed components have different characteristics according to their protein and lipid content (percentage) and their residue value (mg/kg).
- 4. The sum of the percentages of feed components is 100 %.
- 5. Each feed component has a percentage between 0 % and 100 %. The idea of a maximum reasonable balanced diet (MRBD) is considered, there exists a constant percentage smaller than 100 %. This value may differ according to the species.

We formulate above-mentioned conditions as a linear program that is solvable by the Simplex Method.

Modeling

The objective function is $\max S(x) = S_1 \cdot x_1 + \dots + S_n \cdot x_n$. The maximum residue of feed has to be calculated which contains of the sum of the product of the residue and the percentage of each single component. The constraints determining the set of all possible solutions (feasible set) are:

Protein content:	$P_1 \cdot x_1 + \dots + P_n \cdot x_n = P_{Fish}$
Lipid content:	$L_1 \cdot x_1 + \dots + L_n \cdot x_n = L_{Fish}$
Inclusion limit :	$x_i \leq (R_{Fish})_i, \ i = 1, \cdots, n$
Logical constraint:	$x_1 + \dots + x_n = 1$
Positive percentages:	$0 \le x_i, i = 1, \cdots, n$

The first couple of conditions guarantees that the protein and lipid content of feed correspond to the fish-specific requirements. The inclusion limit depends of the fish-specific maximum reasonable balanced diet (MRDB). The last couple of constraints depend of the properties of percentages. Negative percentages and percentages greater than 100 % are not allowed.

Parameter	Range	Description
L _{Fish}	[0,1]	Target content of lipid in feed with respect to the fish species (dry matter)
L_i	[0,1]	Lipid content of feed component $i = 1, \dots, n$ (dry matter)
P _{Fish}	[0,1]	Target content of protein in feed with respect to the fish species(dry matter)

Table 1: List and description of parameters of the dietary burden problem

P_i	[0,1]	Protein content with respect to dry matter of feed component $i = 1, \dots, n$ (dry matter)
$(R_{Fish})_i$	[0,1]	Maximum reasonable content of feed component $i = 1, \dots, n$ (dry matter)
S_i	\mathbb{R}_+	Residue value in mg/kg of feed component $i = 1, \dots, n$ (dry matter)
S	\mathbb{R}_+	Total residue value in mg/kg of feed (dry matter)
x _i	$[0, (R_{Fish})_i]$	content of feed component in diet $i=1,\cdots,n$ (dry matter)

The Simplex Algorithm either solves above discussed linear program in finite number of steps or proves the insolubility of the problem.

In more detail, we write the equations in a simplex tableau, a special matrix system. We put the objective function in the last row that shows the current objective function value. The objective function coefficient demonstrates if the current value is the optimal value or has to be adapted.

For a standardized simplex tableau, finding a start solution is straightforward. Way of proceeding:

- 1. Finding an initial solution or proving the insolubility of the problem.
- 2. Improving the solution as long as there is no possibly better solution.

In the first step, we design a synthetic objective function. Solving the problem as long as the synthetic objective function value is zero yields the start solution. Before starting the first phase the right site of the linear equation system has to be positive, otherwise the equation hast to be multiplied with -1. In our problem, it is always the case because we are dealing with percentages. In addition to that, slack variables arising from the restrictions have to be added: We replace the inequations $x_i \leq (R_{Fish})_i$, $i = 1, \dots, n$ by $x_i + x_{n+i} = (R_{Fish})_i$, $i = 1, \dots, n$. Considering above mentioned rules one obtain the following simplex tableau

Basis	x_1		x_n	x_{n+1}		x_{2n}	b
?	P_1		P_n	0		0	P_{Fish}
?	L_1		L_n	0		0	L_{Fish}
?	1		1	0		0	1
x_{n+1}	1	0	0	1	0	0	$(R_{Fish})_1$ ·
÷	0	·	0	0	·	0	÷
x_{2n}	0	0	1	0	0	1	$(R_{Fish})_n$
S	S_1		S_n	0	0	0	0

The simplex tableau has to be expanded by synthetic variables such that the start solution of the help problem can be found directly. This can be seen in the following tableau:

Basis	x_1		x_n	x_{n+1}		x_{2n}	x_{2n+1}	x_{2n+2}	x_{2n+3}	b
x_{2n+1}	P_1		P_n	0		0	1	0	0	P_{Fish}
x_{2n+2}	L_1		L_n	0		0	0	1	0	L_{Fish}
x_{2n+3}	1		1	0		0	0	0	1	1
x_{n+1}	1	0	0	1	0	0	0	0	0	$(R_{Fish})_1$
÷	0	·	0	0	·	0	0	0	0	÷
x_{2n}	0	0	1	0	0	1	0	0	0	$(R_{Fish})_n$
*	$P_1 + L_1 + 1$		$P_n + L_n + 1$	0	0	0	0	0	0	$P_{Fish} + L_{Fish} + 1$
S	S_1		S_n	0	0	0	0	0	0	0

The start basis is $(x_{2n+1}, x_{2n+2}, x_{2n+3}, x_{n+1}, \dots, x_{2n})^T$ and the simplex method may be applied to the problem. If the optimization value of the help problem is zero, then there exists a start solution to the original problem. Otherwise, the original problem is not solvable, because the restriction area is empty. In the second step, the original problem is solved. Therefore, the secondary objective function row and the column of the synthetic variables have to be deleted. The simplex method has to be applied to the resultant tableau.

Example

We consider a simple example to demonstrate the functionality of the simplex method.

$$\max 3x_{1} + 2x_{2}$$

$$2x_{1} + x_{2} = 8$$

$$x_{1} + x_{2} = 6$$

$$x_{1} \le 5, x_{2} \le 5$$

At first, we have to transform the inequations to equations by adding slack variables x_3 and x_4 . Doing so, we obtain the following constraints.

$$2x_1 + x_2 = 8x_1 + x_2 = 6x_1 + x_3 = 5x_2 + x_4 = 5$$

We insert these equations into the simplex tableau.

	Basis	x_1	x_2	x_3	x_4	b
	?	2	1	0	0	8
	- ?	1	1	0	0	6
	x_3	1	0	1	0	5
	x_4	0	1	0	1	5
_	OF	3	2	0	0	0

In a next step, we add two synthetic variables x_5 , x_6 and the synthetic objective function to get a start basis.

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b
x_5	2	1	0	0	1	0	8
x_6	1	1	0	0	0	1	6
x_3	1	0	1	0	0	0	5
x_4	0	1	0	1	0	0	5
OF	3	2	0	0	0	0	0
SF	3	2	0	0	0	0	14

Now, we have to choose the pivot column. The column is the defined by the smallest positive entry in the synthetic objective function row.

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b
x_5	2	1	0	0	1	0	8
x_6	1	1	0	0	0	1	6
x_3	1	0	1	0	0	0	5
x_4	0	1	0	1	0	0	5
OF	3	2	0	0	0	0	0
SF	3	2	0	0	0	0	14

Now we have to decide which row to choose to obtain the pivot element. For this, we focus on the quotients b_i/a_{ij^*} . The element j^* is the pivot column, i is a possible pivot row and b_i is the corresponding right side entry. In this case, the pivot element is a_{42} (interpreting the coefficients of the equations as matrix $A = a_{ij}$, $i = 2, \dots, 4$, $j = 1, \dots, 6$).

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b	Quotient
x_{5}	2	1	0	0	1	0	8	8/1 = 8
x_6	1	1	0	0	0	1	6	6/1 = 6
x_3	1	0	1	0	0	0	5	_
x_4	0	1	0	1	0	0	5	5/1 = 5
OF	3	2	0	0	0	0	0	
SF	3	2	0	0	0	0	14	
~ -	~	_	Ť	~	Ť	Ť		

After finding the pivot element, we have to standardize the pivot row by dividing the row by the pivot element. Then we generate zeros in the other entries in the pivot column. The original objective function row (OF) is not to be focused by finding the pivot element but has to be adapted as similar to the other rows.

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b
x_5	2	0	0	-1	1	0	3
x_6	1	0	0	-1	0	1	1
x_3	1	0	1	0	0	0	5
x_2	0	1	0	1	0	0	5
OF	3	0	0	-2	0	0	-10
SF	3	0	0	-2	0	0	4
	•						

We repeat this procedure. The first entry in the synthetic objective function row is greater than zero. Hence, the first column is the next pivot column. By regarding the quotients, we receive the new pivot element a_{21} .

Basis	x_1	x_2	x_3	x_4	x_{5}	x_6	b	Quotient
x_5	2	0	0	$^{-1}$	1	0	3	3/2 = 1.5
x_6	1	0	0	$^{-1}$	0	1	1	1/1 = 1
x_3	1	0	1	- 0	0	- 0	5	5/1 = 5
x_2	0	1	0	1	0	0	5	_
OF	3	0	0	-2	0	0	-10	
SF	3	0	0	$^{-2}$	0	0	4	

Again, we generate zeros in the pivot column except for the pivot element row. This entry has to be equal to one. By doing so, we obtain a new positive value in the synthetic objective function row, which identifies the new pivot column. The smallest quotient is in the first row, and thus a_{14} is the new pivot element.

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b	Quotient
x_5	0	0	0	1	1	-2	1	1/1 = 1
x_1	1	0	0	-1	0	1	1	_
x_3	0	0	1	1	0	-1	4	4/1 = 4
x_2	0	1	0	1	0	0	5	5/1 = 5
OF	0	0	0	1	0	-3	-13	
SF	0	0	0	1	0	-3	1	

Again, we generate zeros at the remaining pivot column entries.

Basis	x_1	x_2	x_3	x_4	x_5	x_6	b
x_4	0	0	- 0	1	1	-2	1
x_1	1	0	0	0	1	$^{-1}$	2
x_3	0	0	1	0	$^{-1}$	1	3
x_2	0	1	0	0	$^{-1}$	2	4
OF	0	0	0	0	$^{-1}$	$^{-1}$	-14
SF	0	0	0	0	-1	-1	0

We observe that there is no positive entry greater zero in the synthetic objective function row. Furthermore, the synthetic function value is equal to zero. That indicates that the original problem is solvable and the found basis is a permissible start solution. In addition to that, we can delete the synthetic variables and the synthetic objective function, such that we obtain a reduced simplex tableau.

Basis	x_1	x_2	x_3	x_4	b
x_4	0	0	0	1	1
x_1	1	0	0	0	2
x_3	0	0	1	0	3
x_2	0	1	0	0	4
OF	0	0	0	0	-14

There is no positive entry in the row of the original objective function such that the problem is already solved. The maximum value is 14 with $x_1 = 2$ and $x_2 = 4$. A problem like this with only two variables can also be solved graphically. As we consider in general more than two feed components, we cannot solve the dietary burden problem graphically.

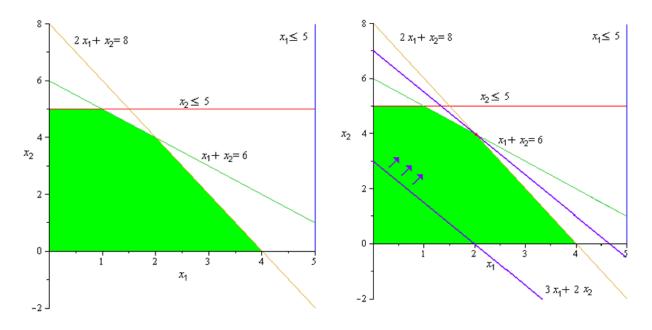


Figure 1: Graphical solution of the example

Working with the program *DietaryBurdencalculator*

If you run the *DietaryBurdenCalculator*, at first, a launch window appears (Figure 2). The user may continue or exit the program.

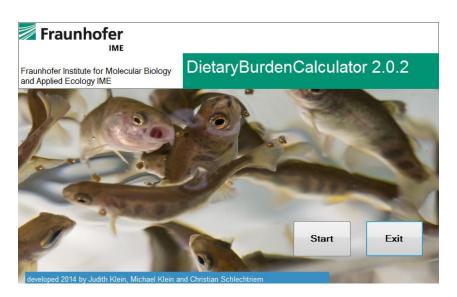


Figure 2: Start screen of the DietaryBurdenCalculator

The surface of the program is divided into three different steps.

- 1. Creating an active substance entry and assigning residues to feed components.
- 2. Selecting the relevant feed components for optimization.
- 3. Choosing the fish species.

Substance data

On the left, you may modify the substance database with a click on

- 1. Edit edit an existing substance,
- 2. Add add an additional substance,
- 3. Copy copy an existent substance or
- 4. Delete delete an existent substance.

You have here the possibility to assign the residue value and the residue input value of a substance to the components. Furthermore, you may edit your substance, add a new substance or copy an existing substance (Figure 3).

Figure 3: Substance database

The window (Figure 4) opens with a click on Edit. Here you can enter the corresponding residue values and the residue input values to the components. For this, the user shall insert the residue values with respect to fresh matter. The program calculates internally the residue value with respect to dry matter based on the dry matter content of the feed component. The user may decide on the residue input value (STMR or STMR-P).

Substance Subst	ance A					
Category	Сгор	Commodity	Residue input value	Residue value (mg/kg)		
By-Products	Brewer's grain	dried	STMR-P	0		
By-Products	Canola meal	Solvent extracted	STMR-P	0	=	
By-Products	Coconut/Copra	meal decorticated	STMR-P	0		
By-Products	Corn	feed meal	STMR-P	0.2634		
By-Products	Corn	bran	STMR-P	0		
By-Products	Corn	gluten feed	STMR-P	0		
By-Products	Corn	gluten meal	STMR-P	0		
By-Products	Corn	starch	STMR-P	0		
By-Products	Corn	Distiller's dried grains with solubles	STMR-P	0		
By-Products	Corn	Condensed distillers solubles	STMR-P	0		Cancel
By-Products	Cottonseed	meal decorticated	STMR-P	0		
By-Products	Linseed	meal, solv, extr.	STMR-P	0	-	

Figure 4: Adding residue values to the single feed components

For instance, we set the residue value of corn field (grain meal) to 0.263 mg/kg (fresh matter). According to the feedstuffs table in the Working Document of Pesticide Residues in Fish this feed component has a dry matter content of 87.8 %. The calculator calculates the residue by

$$S_{DW} = \frac{S_{FW}}{DM} \cdot 100.$$

In this particular case, we obtain $\left(\frac{0.263}{87.8}\right) \cdot 100 = 0.299544419 \approx 0.3$. In the report, the *DietaryBurdenCalculator* refers to both values: the by user inserted residue value (fresh weight) as well as the used residue value for calculation (dry weight).

Feed components

In the middle of the program's surface, you have a list box containing all components with a residue value greater than zero. This list box are the available components (Figure 5).

OietaryBurdenCalculator			
Program ?			
Substance database Substance A	Available Components Com field (Grain meal) Peanut (meal decorticated) Soybean (meal decorticated) Rice (Broken grains) Vegetable oil (oil)	->> Components in optimization>>>	Options © Common carp © Rainbow trout
Add substance Edit Copy	Show details	<	Optimize Exit

Figure 5: Available feed components

If you wish to get more information about the available components, you can click on "more details". This list contains the category of a feed component, the crop (commodity), the protein and lipid content based on dry matter, the dry matter content, the maximum reasonable content for a balanced died for both fish species, as well as the residue input value and the residue value.

With the arrow buttons between the middle and the right list box, the user may decide which components (all, or a smaller set) shall be considered in optimization (Figure 6).

OietaryBurdenCalculator				
Program ?				
Substance database Substance A Add substance Edit Copy Del	Available Components Com field (Grain meal) Peanut (meal decoficated) Soybean (meal decoficated) Rice (Broken grains) Vegetable oil (oil) Soybean Show details	->> < <	Components in optimization Com field (Grain meal) Peanut (meal decorticated)	Options Common carp Rainbow trout Optimize Exit

Figure 6: Feed components considered in calculation

In addition to the chosen feed components, fishmeal (75.00% protein, 5.00% lipid), starch (0.1 % protein, 0.1% lipid) and oil (100% lipid) are considered. These components have a residue value equal to zero and represent protein concentrate (PC, fishmeal), a carbohydrate concentrate (CC, starch) and fat (F, oil). The content of these components in diet is not restricted.

Options

In a third step, the user can change the fish species and choose between rainbow trout or common carp (Figure 7). They differ in their dietary needs.

M DietaryBurdenCalculator			
Program ?			
Substance database	Available Components Corn field (Grain meal) Peanut (meal decorticated) Soybean (meal decorticated) Rice (Broken grains) Vegetable oil (oil)	Components in optimization Com field (Grain meal) Peanut (meal decorticated) > <>	Options Options Common carp Rainbow trout
Add substance Edit Copy Del	Show details	<<	Optimize Exit

Figure 7: Options- choosing a fish species

Optimization

Clicking on the button "optimize" starts the calculation procedure (Figure 8). A short info result is given directly under the button. If the font is red the dietary burden is significant (>=0.1), else the font is black.

DietaryBurdenCalculator			
DietaryBurdenCalculator Program Substance database Substance A	Available Components Com (feed meal) Peanut meal (meal decorticated) Soybean (Meal decorticated) Rice (Broken grains) Vegetable oil (oil)	 Components in optimization Com (feed meal) Peanut meal (meal decorticated) Soybean (Meal decorticated) Rice (Broken grains) Vegetable oil (oil) 	Options Common carp Rainbow trout Maximum dietary burden based on Substance A is 0,134 mg/kg. Report
Add substance Edit Copy Del	Show details	<<	Optimize Exit



A more detailed calculation report containing all input and output information is created. It contains the fish species with corresponding target protein and lipid requirement, the MRDB values, substance residue of each component, the calculated dietary burden, and the respective composition of the feed and the dietary load of the substance caused by the individual components. The report opens if the user

clicks at "report". Furthermore, you can choose the data presentation and print the result or copy it into clipboard (Figure 9).

Report		
Substance Substance A	Species	Common carp
Report Chart Table		
		·····
Dietary Burden Calculation concerning	Substance A	L
DietaryBurdenCalculator 2.0.2		=
Fraunhofer Institute for Molecular Bi	ology and Ap	plied Ecology IME
INPUT		
Target content for Common carp:		
Crude fat Crude protein		10.00%
Clude protein		33.00%
Maximum principal content of componen	ts in the di	
Corn (feed meal)		35.00%
		Done
		Done

Figure 9: Calculation report

The result is presented as a pie chart, table or text report.

Parameter setting

We have the following parameters:

- 1. Substance specific parameters residue values
- 2. Species specific parameters target needs
- 3. Feed component data parameters lipid content, protein content, dry matter content, maximum reasonable content in diet

Substance specific values

The user has the possibility to enter the substance specific data:

- 1. A substance name,
- 2. residue values and residue input values of the feed components.

If the residue value of a feed component is equal to zero, the feed component is not available for the calculation.

Target needs of the fish species

Table 2 shows the needs of protein and lipid content of two different important aquaculture species reared for human consumption: Rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). Furthermore, these species represent inland aquaculture and different diets (omnivorous vs. carnivorous). The two species differ in their needs.

Table 2: Needs, lipid and protein content, of the chosen fish species common carp and rainbow trout

Fish species	CL in %	CP in %
Common carp	10	35
Rainbow trout	15	42

Feed component data

The feed component database of the dietary burden calculator rests upon the "Annex 2 Feedstuffs Table" of the Working Document of Pesticide Residues in Fish and the FAO paper 540. The database contains numerous feed components and their lipid content (CL crude lipid in percentage of dry matter (DM)), their protein content (CP crude protein in percentage of (DM)) and partially suggests of a Maximum Reasonable Balanced Diet (MRDB) for fish dependent on the species (Feedstuff table (Working Document on Pesticide Residues in Fish, Annex 2)

Table 7).

Special feed components

A classification of feed components is represented by CC (carbohydrate concentrate), PC (protein concentrate) and F (fat). In addition to the feed components in Feedstuff table (Working Document on Pesticide Residues in Fish, Annex 2)

Table 7, the program considers the unloaded feed components fishmeal (PC), starch (CC) and oil (F) in optimization (Table 3). These feed components are considered in diet between 0 % and 100 %.

Table 3: Special feed components

Feed Component	CL in %	CP in %
Fish meal (PC)	5	75
Starch (CC)	0.1	0.1
Oil (F)	100	0

Model functionality

With the *DietaryBurdenCalculator* a maximum burden value, the corresponding worst case feed composition and the dietary load of the substance caused by the individual components can be calculated. Furthermore, the calculator considers in the residue estimation a reasonable diet (MRDB). A toxic substance database can be created for different substances.

If the dietary burden calculation is not possible, the program informs the user and gives suggestions to solve the problem.

As result a report is created that contains the input data and results as text report, as table and as pie chart.



Figure 10: Overview of program functionality

Examples

We repeat the example given in "Annex 3 Dietary Burden Calculation" in the Working Document of Pesticide Residues in Fish for both fish species, carp and trout.

A feed shall be arranged using five feed components (Table 4): peanut (meal decorticated), soybean (meal decorticated), corn field (grain meal), rice (broken grains) and vegetable oil (oil). At first, we assign the residue values (Table 4).

Table 4: Chosen feed components with residue values

Feed component	Residue value (STMR)
Corn field (Grain meal)	0.2634
Peanut (meal decorticated)	0.08118
Soybean (meal decorticated)	0.04475
Rice (Broken grains)	0.00176
Vegetable oil (oil)	0.01

In addition to these feed components, we add the unloaded feed components fishmeal (PC), starch (CC) and oil (F).

We calculate the maximum dietary burden for both species, carp and trout. Due to the different needs of the species, we obtain different results. We need more lipid and protein content in diet of trout.

For carp, we get a feed composition leading to 0.141 mg/kg burden (Table 5). The feed composition for rainbow trout leads to a much lower and not significant burden namely 0.087 mg/kg (Table 5).

Feed component	Feed composition in % (carp)	Feed composition in % (trout)
Corn field (Grain meal)	35.00	20.00
Peanut (meal decorticated)	15.00	10.00
Soybean (meal decorticated)	30.00	15.00
Rice (Broken grains)	0.02	6.64
Vegetable oil (oil)	7.30	11.96
Fishmeal (PC)	12.68	36.40
Starch (CC)	0.00	0.00
Oil (F)	0.00	0.00

The diet for carp is mainly based on corn field and peanut, which is included as much as reasonable (MRBD). These are the components with the highest residue value. Corn field is responsible for three quarter of the burden (Table 6). This feed component is also responsible for the main dietary load for the trout (68.78 %). Again, corn field is maximal in feed composition. However, the content is lower for trout, as the maximum reasonable dietary limit is lower for trout (20 %). Additionally, peanut and soybean are added maximally. Fishmeal is added in both diets, for carp and trout, but do not lead to any burden, as its residue is equal to zero. Starch and oil are not considered for both diets.

Feed component	Dietary load in % (carp)	Dietary load in % (trout)
Corn field (Grain meal)	78.22	77.09
Peanut (meal decorticated)	10.06	11.56
Soybean (meal decorticated)	11.17	9.64
Rice (Broken grains)	0.00	0.17
Vegetable oil (oil)	0.54	1.54
Fishmeal (PC)	0.00	0.00
Starch (CC)	0.00	0.00
Oil (F)	0.00	0.00

The reports generated by the *DietaryBurdenCalculator* can be seen in Figure 11 and Figure 12.

Discussion of results

Above-mentioned examples give an overview about the benefit and limits of the program *DietaryBurdenCalulator*. The program describes the maximum dietary burden but cannot interpret the result if it is a reasonable or realistic feed.

Caused of the differences in protein and lipid content the different fish types lead to different dietary burden and hence different feed compositions are constructed.

Although the aim is the calculation of *maximum burden*, a large proportion of unpolluted fishmeal (12.68 % for carp, 36.40 % for trout) is added into feed (Table 5: Worst case feed composition for carp and trout). The essential role of fishmeal in case of this problem can be justified by the maximum restrictions. Fishmeal is for trout the main protein supplier in corresponding feed composition and thus necessary for this particular optimization problem to fulfill the needs of the species.

The development of a dietary burden calculator for fish metabolism studies based on simplex method offers substantial advantages in interpreting and predicting dietary burden in fish feed. However, the simplex method is not common in this moment for this particular problem. As fish in aquaculture is fed on the maximum reasonable balanced diet approach, it is necessary for interpreting residues in feed to consider the different nutrition preferences of fish species.

However, the program has no possibility to evaluate if the input data are rational. Although the program recognizes obvious mistakes like negative percentages or percentages greater than 100 %.

The program is based on the importance of protein and lipid content in diet, but in general, it is no problem to expand the dietary burden calculator by adding additional conditions for the diet, for example, if the vitamin content shall also be considered.

More conditions may lead to a more realistic feed but also to a more complex model and hence maybe to a more confusing program. That leads to higher data requirements and thus to probably incomplete data sets.

The program calculates a worst feed composition concerning the residue without considering economic conditions. Therefore, the result may be different to actual feeding diets used for aquaculture species. However, the use of feed components is restricted by their maximum limit intake and thus their contents in feed are realistic.

Anyway, the calculator offers a possibility to calculate the maximum burden of feed of a substance and thus may help of the assessment of the necessity of fish metabolism studies to validate the residue value.

Appendix Reports generated by the software *DietaryBurdenCalculator*

Dietary Burden Calculation concerning Substance A	
DietaryBurdenCalculator 2.0.2	
Fraunhofer Institute for Molecular Biology and Applied Ecology IME	
INPUT	
Target content for Common carp:	10,000
Crude fat Crude protein	10.00% 35.00%
Maximum principal content of components in the diet:	
Corn (feed meal)	35.00%
Peanut meal (meal decorticated) Soybean (Meal decorticated)	15.00% 30.00%
Rice (Broken grains)	50.00%
Vegetable oil (oil)	10.00%
Fish meal(PC)	100.00%
Starch(CC) Oil(F)	100.00% 100.00%
	100.000
Percent dry matter of components:	
Corn (feed meal)	87.8%
Peanut meal (meal decorticated) Soybean (Meal decorticated)	90.2% 89.5%
Rice (Broken grains)	88.0%
Vegetable oil (oil)	-
Substance A residues in the components:	
Corn (feed meal)	0.263 mg/kg (STMR-P)
Peanut meal (meal decorticated) Soybean (Meal decorticated)	0.081 mg/kg (STMR-P)
Rice (Broken grains)	0.045 mg/kg (STMR-P) 0.002 mg/kg (STMR-P)
Vegetable oil (oil)	0.010 mg/kg (STMR-P)
Substance A residues in the components (dry matter): Corn (feed meal)	0,200 mg/lcg (CEMID D)
Peanut meal (meal decorticated)	0.300 mg/kg (STMR-P) 0.090 mg/kg (STMR-P)
Soybean (Meal decorticated)	0.050 mg/kg (STMR-P)
Rice (Broken grains)	0.002 mg/kg (STMR-P)
Vegetable oil (oil)	0.010 mg/kg (STMR-P)
RESULTS	
Maximum dietary burden based on Substance A is 0.134 mg/kg (dry matter).	
The respective composition of the feed is:	25,000
Corn (feed meal) Peanut meal (meal decorticated)	35.00% 15.00%
Soybean (Meal decorticated)	30.00%
Rice (Broken grains)	0.02%
Vegetable oil (oil)	7.30%
Fish meal(PC) Starch(CC)	12.68% 0.00%
Oil(F)	0.00%
The dietary load of Substance A caused by the individual components is: Corn (feed meal)	78.22%
Peanut meal (meal decorticated)	10.06%
Soybean (Meal decorticated)	11.17%
Rice (Broken grains)	0.00% 0.54%
Vegetable oil (oil) Fish meal(PC)	0.54%
Starch (CC)	0.00%
Oil(F)	0.00%

Figure 11: Report for Common Carp

Dietary Burden Calculation concerning Substance A	
DietaryBurdenCalculator 2.0.2	
Fraunhofer Institute for Molecular Biology and Applied Ecology IME	
:NPUT	
Target content for Rainbow trout: Crude fat	15,000
Crude fat Crude protein	15.00% 42.00%
Maximum principal content of components in the diet:	
Corn (feed meal) Peanut meal (meal decorticated)	20.00%
Soybean (Meal decorticated)	10.00% 15.00%
Rice (Broken grains)	10.00%
Vegetable oil (oil) Fish meal(PC)	15.00% 100.00%
Starch(CC)	100.00%
Oil(F)	100.00%
Percent dry matter of components:	
Corn (feed meal) Peanut meal (meal decorticated)	87.8% 90.2%
Soybean (Meal decorticated)	90.2% 89.5%
Rice (Broken grains) Vegetable oil (oil)	88.0%
Substance A residues in the components:	
Corn (feed meal)	0.263 mg/kg (STMR-P)
Peanut meal (meal decorticated) Soybean (Meal decorticated)	0.081 mg/kg (STMR-P) 0.045 mg/kg (STMR-P)
Rice (Broken grains)	0.002 mg/kg (STMR-P)
Vegetable oil (oil)	0.010 mg/kg (STMR-P)
Substance A residues in the components (dry matter):	
Corn (feed meal)	0.300 mg/kg (STMR-P)
Peanut meal (meal decorticated) Soybean (Meal decorticated)	0.090 mg/kg (STMR-P) 0.050 mg/kg (STMR-P)
Rice (Broken grains)	0.002 mg/kg (STMR-P)
Vegetable oil (oil)	0.010 mg/kg (STMR-P)
RESULTS	
Maximum dietary burden based on Substance A is 0.078 mg/kg (dry matter)).
The respective composition of the feed is:	20.000
Corn (feed meal) Peanut meal (meal decorticated)	20.00% 10.00%
Soybean (Meal decorticated)	15.00%
Rice (Broken grains) Vegetable oil (oil)	6.64% 11.96%
Fish meal(PC)	36.40%
Starch(CC) Oil(F)	0.00% 0.00%
	0.000
The dietary load of Substance A caused by the individual components is: Corn (feed meal)	77.09%
Corn (Ieed meal) Peanut meal (meal decorticated)	11.56%
Soybean (Meal decorticated)	9.64%
Rice (Broken grains) Vegetable oil (oil)	0.17% 1.54%
Fish meal(PC)	0.00%
Starch(CC) Oil(F)	0.00% 0.00%
	0.00%

Figure 12: Report for rainbow trout

Feedstuff table (Working Document on Pesticide Residues in Fish, Annex 2) Table 7: Feed components

				CL	СР	DM	max carp	max trout
Category	Crop	Commodity	IFN Code	(%)	(%)	(%)	(%)	(%)
By-Products	Brewer's grain	dried	5-00-516	7	25.9	92.3	15	10
By-Products	Canola meal	Solvent extracted	5-05-146	1.9	37.3	91	10	8
By-Products	Coconut/Copra	meal decorticated	5-01-572	2.2	21.9	90.2	15	10
By-Products	Corn	feed meal	4-02-880	4.8	10.2	87.8	35	20
By-Products	Corn	bran	4-02-841	5.7	15	87.5	20	5
By-Products	Corn	gluten feed	5-02-903	3.5	24.7	90.1	20	10
By-Products	Corn	gluten meal	5-02-900	3.6	59.9	91.3	20	15
By-Products	Corn	starch	4-02-889	0.4	0.4	90.2	35	15
By-Products	Corn	Distiller's dried grains with solubles	5-02-843	10	27.8	90.8	35	10
By-Products	Corn	Condensed distillers solubles	5-12-211	11.3	29.5	90.6	10	3
By-Products	Cottonseed	meal decorticated	5-01-627	1.2	44.2	90.8	15	5
By-Products	Linseed Lupin seed	meal, solv. extr.	5-02-048	2	35	90	7	5
By-Products	white	meal (treated)	5-27-717	6.1	34.5	89.5	15	9
By-Products	Mustard	Meal, solv. extr.	5-12-149	1.8	42.4	89.9	10	5
By-Products	Palm	kernel meal	5-03-486	1.4	16.3	90	10	8
By-Products	Peanut meal	meal decorticated	5-03-649	1	46.5	90.2	15	10
By-Products	Potato	protein	-	2.8	81.8	89.4	3	2
By-Products	Rice	polishing	4-03-943	14.5	13.6	100	50	10
By-Products	Rice	hulls	1-08-075	1	3.1	100	5	0
By-Products	Sesame seed	meal	5-04-220	4.8	45	92.4	20	10
By-Products	Safflower	Meal decorticated	5-26-095	6.9	45.2	91	7	5
By-Products	Soybean	Meal decorticated	5-20-638	0.8	49.8	89.5	30	15
By-Products	Sunflower	meal decorticated, solv. extr.	5-30-024	3.2	43.5	92.6	20	10
By-Products	Wheat	bran	4-05-190	4.7	15.6	88.7	5	2
By-Products	Wheat	flour	4-05-199	1.7	14.3	88	15	10
By-Products	Wheat	germ	5-05.218	8.8	28.5	88.7	5	2
By-Products	Wheat	middlings	4-05-205	4.4	16.9	89.4	20	10
By-Products	Wheat	gluten meal	-	1.5	80.1	91.4	5	2

Cereal Grains/ Crop Seeds Cereal Grains/	Cow pea	seed	5-01-661	4.9	25.1	88	15	15
Crop Seeds Cereal Grains/	Faba bean	seed	5-09-262	8.4	28.3	88	15	15
Crop Seeds Cereal Grains/	Lupin (white)	seed	5-02-707	6.1	34.5	88	15	15
Crop Seeds Cereal Grains/	Pea	seed	5-03-600	1.7	23.7	90	15	15
Crop Seeds Cereal Grains/	Lupin	seed, head processed	5-64-610	38.9	4.6	88	9	3
Crop Seeds Cereal Grains/	Rice	Broken grains	4-03-938	0.6	8.1	88	50	10
Crop Seeds Fat	Wheat Vegetable oil	Grain (extruded) oil	4-12-208	1.9 100	13.5 0	87.7 0	15 10	10 15

List of Abbreviations

CC	Carbohydrate concentrate
СР	Crude lipids in percentage of dry matter
CL	Crude protein in percentage of dry matter
DM	Dry matter
F	Fat
FAO	Food and Agriculture Organization of the United Nations
MRBD	Maximum reasonable balanced diet
РС	Protein concentrate
STMR	Supervised trials median residue
STMR-P	Supervised trials median residue in processed commodity
IFN	Code International Feed Nomenclature code

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