### Enzyme Analysis Matrix Development

BroilerOpt<sup>™</sup> Feed Program and Matrix Values from the Enzyme Study-

Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets

Bernardes et al, R. Bras. Zootec., 51:e202120211, 2022

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#### After reviewing the Quality of this Study, we felt it was ideal for showing the Enzyme Values that resulted.

- We appreciate and thank the authors of "Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets".
  - These Scientists are Romário Duarte Bernardes1\*,
  - Carlos Henrique de Oliveira, Arele Arlindo Calderano,
  - Rafael de Sousa Ferreira, Kelly Morais Maia Dias,
  - Bruno Figueiredo de Almeida, Pedro Eleutério Aleixo,
- Luiz Fernando Teixeira Albino from Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil
- This following work is by Feed2Gain, LLC only.

#### Feeding Trial with Seven Treatments:

- Treatment 1 is a Positive Control (PC). Energy is the same in all starter diets and grower diets but protein and amino acids are highest in this dietset.
- Treatment 2 is NC1; PC minus 0.16% calcium, 0.15% aP, and 0.5% crude protein [CP] in each diet.
- Treatment 3 is NC1 plus an enzyme blend CBE at 250 g per tonne.
- Treatment 4 is NC1 plus an enzyme blend SE at 250 g per tonne.
- Treatment 5 is NC2; PC minus 0.16% calcium, 0.15% aP, and 1% CP.
- Treatment 6 is NC2 plus an CBE at 250 g per tonne
- Treatment 7 is NC2 plus an enzyme blend SE at 250 g per tonne.

### Our Analysis works with the NC1 and NC2 treatments.

- First, we calibrate BroilerOpt.exe to each of the feeding programs of NC1 and NC2. The chicks are all Cobb males.
- Then, we use the calibrated treatment to analyze the Contribution of the Enzyme treatments with the feeding results reported.
- We are using a matrix set of corn and soybean meal that are not the values that were used in the study as we do not have those.
- Knowing the basic diet allows us to determine what must have changed to get the enhanced growth with the enzyme mixture.
- With the changed diet, we can estimate the benefit of the enzyme treatment.

The Enzymes increased the growth and feed conversion of the test animals.

	Published Resu	ults at 42 days of age	
Diets	Enzyme	Flock Weight Gain, kg	Feed/Gain
PC	2 none	3.245	1.516
NC1	none	3.146	1.571
	CBE	3.167	1.547
	SE	3.267	1.513
NC2	2 none	3.044	1.567
	CBE	3.206	1.54
	SE	3.241	1.528

### NC1 was Calibrated and then Used in Current Flock

- The diets and intakes are as given in the paper.
- Growth is as given in the paper and feed conversion close (1.571 given). Note the age is too high, suggesting a difficult calibration.
- Now, we can use our matrix to select ingredients to evaluate.



### Setting Up NC1 for Ingredient Testing

- Now we have our matrix ingredients closely matching the Published Nutrients in the Diets.
- We can go to the Ingredient Testing Tab

AI	mino Acids							
Diet Type			^		Formulas			
Diet Change				2	Corn, Grain 7.86% CP	54.41	56.36	
Feed Cost	824.19	809.31		20	Soybean Meal 46% CP	39.19	37.51	
Feed Weight	1.2430	3.6980		32	Oil, Soybean	2.960	2.594	
Energy	3000.0	3150.0		40	Disodium Phosph.	0.986	0.998	
Protein	22.520	19.500		37	Calcium Carbonate	1.038	1.046	
Arginine	1.4040	1.1840		41	Salt	0.515	0.516	
Histidine	0	0		45	DL Methionine	0.296	0.310	
Lysine	1.2380	1.0520		44	L-Lysine HCl	0.176	0.226	
Tryptophan	0.2570	0.2160		47	L-Threonine	0.012	0.022	
Phe + Tyrosine	0	0		51	Vitamins Premix	0.130	0.130	
Phenylalanine	0	0		54	Mineral premix Starter	0.130	0.130	
Met_Cys	0.8930	0.7560		43	Cl. Choline - 70%	0.100	0.100	
Methionine	0	0		58	Antibiotic	0.055	0.055	
Threonine	0.7610	0.6620		57	Anticoccid agent.	0.001	0.001	
Leucine	0	0						
Isoleucine	0	0						
Valine	0.9360	0.8050						

Ether Extract (EE) %

With Ingredient Testing, We choose the Ingredients that are most affected and Enter the Performance Values for the Treatment.

- There are only two bulk ingredients, corn and soybean meal.
- We enter the growth data.
- We will let the computer find what nutrients in corn and soy can be increased in sync to get the observed Performance.



#### Clicking Run Analysis Shows the Changes Needed in Corn and Soy to get the performance

To get the Increase in live weight and improvement in Feed Conversion, Corn was increased 1.8 % in energy and 4.89 % in protein. Soybean Meal contributed no additional energy and 2 % more Protein.



## We can Use "Diet Display" Window to get the Feed Ingredients.

- We enter the ingredient number from our matrix to select an ingredient.
- Then we put in the weight o the ingredient in each diet.
- There are two diets in the publication.
- We Export to the Current Calc. Tab.

ig. No Ing									
	g. Name	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	^	Calculate Nutrients	Hide
Co	ost	796.72	756.39					- On and the set	
En	nergy	3050.7	3190.3					Upen Ing. File	
Pro	otein	22.851	19.794						Load All Diets in Or
Arg	ginine	1.4313	1.2048					Transfer	
His	istidine	0.5469	0.4772					FRUM	Import from clipboa
Lys	vsine	1.2533	1.1209						and Add to Existin
Try	yptophan	0.2639	0.2216					Exportr TO	0 04 5
Ph	ne + Tyrosine	1.7843	1.5350					Current	Upen Uther Feed
Ph	henylalanine	1.0261	0.8799						1 1105
Me	et_Cys	0.9060	0.7646					Export to	Import Specs from
Me	ethionine	0.5986	0.4933					Calibration	Clipboard
Th	nreonine	0 7849	0.6824				~		
							>	Show The	Save As

## Save Outcome Gives Matrix Values for NC1 + CBE and Can Save them to your Matrix.

- The Program finds the apparent matrix of the enzyme for all given amino acids, protein and energy.
- The program can create a new ingredient for Corn and Soybean plus enzyme.
- One can choose which to use when testing value of the enzyme impact.



You can save this analysis TWO ways. Create a Matrix Value for your Test Ingredient by setting an input weight and cost. OR You can Add New Ingredients to the Matrix for the Ingredients that were analyzed with an addition to the name and new cost. You will see the values before saving. You can update Names in the Grid

	NC1 + CBE		New Corn, Grain 7.86% CP	New Soybean Meal 46% CP		^
Price		Dose 0.025 %				
Energy	145671		3425	2396		
Protein	2169		8.244	47.44		
Arginine	132.6		0.357	3.244		
Histidine	0		0.231	1.112		
Lysine	117.6		0.199	2.652		
Tryptophan	24.22		0.063	0.602		
Phe + Tyrosine	0		0.629	3.795		
Phenylalanine	0		0.346	2.203		
Met_Cys	84.60		0.304	1.183		
Methionine	0		0.157	0.581		
Threonine	73.55		0.304	1.602		
Leucine	0		0.954	3.264		
Isoleucine	0		0.252	1.969		
Valine	89.71		0.325	2.030		~
<						>
		0.025			Enter the Dose then Click Here	

#### We repeat for NC1 with SE enzyme product.

- We use the published weight gain and feed conversion for the fourth treatment, NC1 plus SE.
- We Solve for the Impact of the enzyme on Corn and Soybean Meal.
- The impact on energy is the same as NC1 + CBE but more protein is released to achieve the observed growth and feed conversion.

		Formulas		Diet No. 1	Diet No. 2	
	2	Corn, Grain 7.86% CP	✓	54.41	56.36	
	20	Soybean Meal 46% C	◄	39.19	37.51	
	32	Oil, Soybean		2.960	2.594	
	40	Disodium Phosph.		0.986	0.998	
	37	Calcium Carbonate		1.038	1.046	
	41	Salt		0.515	0.516	
	<					
Put Your Res	ults fron	n Test Ingredient Additi	on Below			
k Final	Flo	ck Feed				
ght	Co	nversion F	inal Age			
7	1.51	3 43			Results	
	,	,				
	Ing. Na	me	Energ	gy Increase	Protein/Lysine Increa	u.
	20 Corr	, Grain 7.86% CP		2.000	15.64	4
	Soybe	an Meal 46% CP		0	8.355	5
Have th						
Run Ar						
	Best Fit	t		3.267 kg	1.510 f2c	43.19 days
	Adjuste	d Diets				
	Energy	1		3037	3188	3
	Protein			24.71	21.65	5
	Arginin	Ð		1.541	1.315	5
	Histidin	e		0	(	)
	lvsine			1 358	1 168	3
	<					

3.2

### We can then Generate Matrix Values from this outcome with N1 + SEC .

- We can see the matrix values that could be used for the Enzyme Product and for Corn and Soy plus the enzyme.
- The program would save these values with just a click.
- We will look at the average results later.



You can save this analysis TWO ways. Create a Matrix Value for your Test Ingredient by setting an input weight and cost. OR You can Add New Ingredients to the Matrix for the Ingredients that were analyzed with an addition to the name and new cost. You will see the values before saving. You can update Names in the Grid

	NC1 + SE		New Corn, Grain 7.86% CP	New Soybean Meal 46% CP		^
Price		Dose 0.025 %				
Energy	150356		3431	2396		
Protein	8643		9.089	50.39		
Arginine	528.4		0.393	3.446		
Histidine	0		0.254	1.181		
Lysine	468.5		0.220	2.817		
Tryptophan	96.47		0.069	0.639		
Phe + Tyrosine	0		0.694	4.031		
Phenylalanine	0		0.382	2.340		
Met_Cys	337.0		0.335	1.257		
Methionine	0		0.173	0.618		
Threonine	293.1		0.335	1.701		
Leucine	0		1.052	3.467		
Isoleucine	0		0.278	2.091		
Valine	357.4		0.358	2.156		~
<						>
NC1 + SE		0.025			Enter the Dose then Click Here	

#### We Calibrate and Process NC2 the same way

- We Calibrate and then run on Current Calc.
- We add the ingredients via the Open Diets window.
- Here we are set to run the Evalution of an ingredient.

Calibration	of Location	l l	Current	Flock		ſ	C	)ptir	nization	Ĩ	
Click on Desired Location TestNC2	Current Calculation	Open Diets	When Protein Cha Amino Acids Dr When Lysine C Amino Acids	anges, Ratio Hanges, ratio	🔲 Show Pink 0	Changes	□ si	how 1	emperatures Lean Bre	ed	
		Diet Type					^		Formulas		
Flock Sex Males	Save Diets	Diet Change						2	Corn. Grain 7.86% CP	56.36	63.70
		Feed Cost	0	0				20	Sovbean Meal 46% CP	37.51	29.83
Flock Size 1000		Feed Weight	1.1970	3.5730				32	Oil. Sovbean	2.594	3,489
	Save All Current	Energy	3000.0	3150.0				40	Disodium Phosph.	0.998	0.693
No of Diets 2		Protein	22.060	19.000				37	Calcium Carbonate	1.046	0.831
		Arginine	1.4040	1.1450				41	Salt	0.516	0.472
Flock Final 3.044	ka	Histidine	0	0				45	DL Methionine	0.310	0.236
Weight	Ny Use Calibration	Lysine	1.2380	1.0520				44	L-Lysine HCl	0.226	0.317
	Diets	Tryptophan	0.2480	0.2090				47	L-Threonine	0.022	0.022
Flock Feed 1.550		Phe + Tyrosine	0	0				51	Vitamins Premix	0.130	0.130
Conversion ,	GIVEN COST -	Phenylalanine	0	0				54	Mineral premix Starter	0.130	0.130
Final Age 42.2 0	ays Rup IP	Met_Cys	0.8930	0.7560				43	CL Choline - 70%	0.100	0.100
	- Itan Er	Methionine	0	0				58	Antibiotic	0.055	0.055
Cost / Bird	View	Threonine	0.7610	0.6450				57	Anticoccid agent	0.000	0.000
	Report	Leucine	0	0					r intooodid agona	0.001	0.001
Cost/Tonne	- 1	Isoleucine	0	0				_			
Costronne	Enzyme	Valine	0.9260	0.7830				_			
	1 op Off	Ether Extract (EE) %						_		-	
Profit/Bird		Dry matter %						_		-	

### We have set up the Ingredient Testing for NC2 with CBE added.

- This treatment shows 2 % energy from Corn and none from Soybean Meal. Then an increase in Corn Protein by 14.5% and Soybean Meal by 2.1 %.
- We can average the two outcomes for CBE (in NC1 and NC2) later.

This Section finds the change in energy and protein contribution of ingredients affected by an Enzyme. It searches for the changes that best explain the Growth Differences observed between the Control and Test Outcomes.

#### Evaluate the impact of an Enzyme / Process on up to 4 Ingredients



# We next generate the Matrix values for the Enzyme and for Corn and Soy Plus CBE

• The values look similar to the NC1 plus CBE.

🖏 Ingredient Analysis - Save Results	- 🗆 ×
Add a New Test Add Existing but Ingredient Upgraded Ingredients	Cancel
You can save this analysis TWO ways	Create a Matrix Value for your Test Ingredient by

You can save this analysis TWO ways. Create a Matrix Value for your Test Ingredient by setting an input weight and cost. OR You can Add New Ingredients to the Matrix for the Ingredients that were analyzed with an addition to the name and new cost. You will see the values before saving. You can update Names in the Grid

	NC2 + CBE		New Corn, Grain 7.86% CP	New Soybean Meal 46% CP	·
Price		Dose 0.025 %			
Energy	166472		3431	2396	
Protein	4052		9.000	47.47	
Arginine	247.6		0.389	3.246	
Histidine	0		0.252	1.113	
Lysine	225.1		0.218	2.654	
Tryptophan	44.81		0.069	0.602	
Phe + Tyrosine	0		0.687	3.798	
Phenylalanine	0		0.378	2.205	
Met_Cys	161.9		0.332	1.184	
Methionine	0		0.172	0.582	
Threonine	138.1		0.332	1.603	
Leucine	0		1.042	3.267	
Isoleucine	0		0.275	1.970	
Valine	167.7		0.355	2.031	
Ether Extract (EE) %			3.810	2.850	
/					>

### We have set up the Ingredient Testing for NC2 with SE added.

lock Veig

 Energy seems to come from Corn and not Soy while this enzyme mix shows a response to soy protein and a large response to Corn protein.

				Click Here to <sup>-</sup>	ransier From	Control D	iet	
		Formulas		Diet No. 1	Diet No. 2			^
	2	Corn, Grain 7.86% CP	✓	56.36	63.70			
	20	Soybean Meal 46% C	✓	37.51	29.83			
	32	Oil, Soybean		2.594	3.489			
	40	Disodium Phosph.		0.998	0.693			
	37	Calcium Carbonate		1.046	0.831			
	41	Salt		0.516	0.472			~
	<							>
Your Res	sults fron	n Test Ingredient Additio	n Below					
_	Co 1.52	nversion Fin 8 42	ial Age		P	esults		
	Col 1.52 Ing. Nat	nversion Fin 8 42 me	Energy	Increase	Protein/Lysir	esults		
	Col 1.52 Ing. Nat 20 Corr	nversion Fin 8 42 me	Energy	Increase	Protein/Lysir	esults e Increa:		
	Col 1.52 Ing. Nat 20 Com Soybe	nversion Fin 8 42 me 1, Grain 7.86% CP an Meal 46% CP	Energy	Increase 1.800 0	<b>P</b> rotein/Lysir	esults e Increa: 21.38 2.570		
Have th	Col 1.52 Ing. Nai 20 Com Soybe	nversion Fin 8 42 me 1, Grain 7.86% CP an Meal 46% CP	Energy	Increase 1.800 0	Protein/Lysir	e Increa: 21.38 2.570		For Help
Have th Run Ar	Col 1.52 Ing. Na 20 Com Soybe	nversion Fin 8 42 me 1, Grain 7.86% CP an Meal 46% CP	Energy	Increase 1.800 0	<b>P</b> rotein/Lysir	e Increa: 21.38 2.570		For Help Dbl Click
Have th Run Ar	Col 1.52 Ing. Na 20 Com Soybe Best Fit	nversion Fin 8 42 me 1, Grain 7.86% CP an Meal 46% CP	Energy	Increase 1.800 0 3.241 kg	<b>P</b> rotein/Lysir	e Increa: 21.38 2.570	43.07 days	For Help Dbl Click
Have th Run Ar	Co 1.52 Ing. Na 20 Corr Soybe Best Fit Adjuste	nversion Fin 8 42 me 1, Grain 7.86% CP an Meal 46% CP d Diets	Energy	Increase 1.800 0 3.241 kg	<b>P</b> rotein/Lysir	e Increa: 21.38 2.570 1.508 f2g	43.07 days	For Help Dbl Click
Have th Run Ar	Co 1.52 Ing. Na 20 Corr Soybe Best Fit Adjuste Energy	nversion Fin 8 42 me , Grain 7.86% CP an Meal 46% CP d Diets	Energy	Increase 1.800 0 3.241 kg 3034	<b>P</b> rotein/Lysir	e Increa: 21.38 2.570 1.508 f2g 3189	43.07 days	For Help Dbl Click
Have th Run Ar	Col 1.52 Ing. Nat 20 Corre Soybe Best Fit Adjuste Energy Protein	nversion Fin 8 42 me , Grain 7.86% CP an Meal 46% CP	Energy	Increase 1.800 0 3.241 kg 3034 23.46	Protein/Lysir	e Increa: 21.38 2.570 1.508 f2g 3189 20.43	43.07 days	For Help Dbl Click
Have th Run Ar	Col 1.52 Ing. Na 20 Corr Soybe Best Fit Adjuste Energy Protein Arginin	nversion Fin 8 42 me , Grain 7.86% CP an Meal 46% CP d Diets	Energy	Increase 1.800 0 3.241 kg 3034 23.46 1.493	Protein/Lysir	e Increa: 21.38 2.570 1.508 f2g 3189 20.43 1.231	43.07 days	For Help Dbl Click
Have th Run Ar	Col 1.52 Ing. Nat 20 Corre Soybe Best Fit Adjuste Energy Protein Arginine Histidin	nversion Fin 8 42 me , Grain 7.86% CP an Meal 46% CP d Diets e e	Energy	Increase 1.800 0 3.241 kg 3034 23.46 1.493 0	Protein/Lysir	esults 21.38 2.570 1.508 f2g 3189 20.43 1.231 0	43.07 days	For Help Dbl Click

Evaluate the impact of an Enzyme / Process on up to 4 Ingredients

### Matrix Values for NC2 plus SE

- We now have two estimates for the matrix that one could use for CBE and SE enzyme addition to very similar diets.
- This would be an excellent way to judge the enzyme values in your own matrix.
- Run a trial or use the enzyme for a period and then compare results.

🖏 Ingredient Analysis - Save Results

NC2 + SE



Cancel

You can save this analysis TWO ways. Create a Matrix Value for your Test Ingredient by setting an input weight and cost. OR You can Add New Ingredients to the Matrix for the Ingredients that were analyzed with an addition to the name and new cost. You will see the values before saving. You can update Names in the Grid

	NC2 + SE		New Corn, Grain 7.86% CP	New Soybean Meal 46% CP	^
Price		Dose 0.025 %			
Energy	149825		3425	2396	
Protein	5675		9.540	47.70	
Arginine	346.8		0.413	3.262	
Histidine	0		0.267	1.118	
Lysine	315.3		0.231	2.667	
Tryptophan	62.77		0.073	0.605	
Phe + Tyrosine	0		0.728	3.816	
Phenylalanine	0		0.401	2.216	
Met_Cys	226.8		0.352	1.190	
Methionine	0		0.182	0.585	
Threonine	193.4		0.352	1.610	
Leucine	0		1.105	3.282	
Isoleucine	0		0.291	1.980	
Valine	235.0		0.376	2.041	
Ether Extract (EE) %			3.810	2.850	
Crumpttor %			00 an	01 10	>

0.025

## Combined NC1 and NC2 Results for Matrix Values

- The differences between the two matrix sets is obvious. More Energy and Protein is released by SE. Of course, that was obvious from the better growth performance seen with SE.
- BroilerOpt <sup>™</sup> Feed Program can put numerical values on a Test Ingredient and show which ingredients appear to be most affected.
- Formulation with the Corn Plus Enzyme may work better as there are diets that will have more corn and less soybean meal as we go higher in age fed.

	CBE	SE
Energy	145671	166472
Protein	2169	4042
Arginine	132.6	274/6
Histidine	0	0
Lysine	117.6	225.1
Tryptophan	24.22	44.81
Phe + Tyrosine	0	0
Phenylalanine	0	0
Met_Cys	84.6	161.9
Methionine	0	
Threonine	73.55	138.1
Leucine	0	0
Isoleucine	0	0
Valine	89.71	167.7

#### BroilerOpt<sup>™</sup> Feed Program is A Great Resource

- It can formulate feed and accurately predict Flock Growth, carcass content and Feed Efficiency for Males and Females.
- It does feed formulation and can show graphs of cost, feed conversion, and age with Protein or Lysine and Energy content of each feed.
- It can Find the Optimum Feeding Program for your Feeding Locations based on Ingredient Cost as well as final age, live weight and carcass composition.
- Now, It can help with identifying Matrix Values for low addition weight Feed Ingredients that have Significant Effects on Growth and Feed Efficiency.

### Using the Created Matrix Values to Measure Value

- The program can save the Bulk Ingredients that were created to match the growth observed and/or the matrix for the Test Ingredient.
- We chose the N1 diets and the addition of SE enzyme mix from slide 13.
- We priced the ingredients so they would not be used and looked at the Shadow Prices found by the Least Cost Calculations.
- These are the highest prices that would be needed to just get the ingredients into the diets and are not evaluated if the ingredient is blocked from use in the Diets (restricted to no addition).

### Shadow Prices tell the value Ingredients when Used in the Diets

- The Prices of Corn and soybean meal are important. The Corn used by the program was priced at 454 per tonne (units were not important). The Soybean meal was 1150 per tonne.
- The program valued the New Corn at about 496.1 or 9.2% more.
- Soy was 1195 or 3.4% more value.
- The Enzyme Product was valued at 187274 per tonne.

Ingred.Name	Current Price	Diet 1	Diet 2
8 Limestone	175.0	175.0	175.0
9 Salt	125.0	125.0	125.0
10 DL Methionine	6500	6500	6500
11 BioLis, 54.5%	6500	6500	6500
12 L-Threonine	4450	4450	4450
13 Vitamins Premix	400.0	400.0	400.0
14 Mineral premix Starter	400.0	400.0	400.0
15 Cl. Choline - 60%	249.0	249.0	249.0
16 Salinomycin3 (12%)	6000	6000	6000
17 Antioxidant (BHT)	6000	6000	6000
18 New Corn, Grain 7.86%	500.0	496.1	500.0
19 New Soybean Meal 46	1300	1195	1197
20 NC1 SE	200000	187274	200000

#### Just for Interest

- We know that every study has variation. So, we looked at NC1 and NC2 with the Calibration for PC, the positive Control, using the given diets for each. Then with NC1 Calibration and then NC2 Calibration.
- The differences between Observed and Calibrated is the 42 day restriction on the program.
- NC1 seemed to perform worse than expected on Feed Conversion (f2g) expected 1.524 – 1.571 observed.
- NC2 was poorer on growth expected 3.127, observed 3.044.

		Expected Performance <u>at 42</u> days			
Observed	Calibration	РС	NC1	NC2	
3.245	PC	3.245	3.174	3.127	
1.516		1.516	1.524	1.539	
3.146	NC1	3.1	3.059	3.027	
1.571		1.535	1.55	1.562	
3.044	NC2	3.158	3.112	3.035	
1.528		1.496	1.512	1.542	

### Conclusion: A Matrix Set of Values was Possible with BroilerOpt ™.exe

- In this Experiment, Both Enzyme Products provided value when added to the lower protein diets.
- The Product coded SE provided more value than the CBE product.
- Because, in this case, the Matrix Values were not present, not all amino acids could be evaluated.
- A Research Trial would be expected to have more variation in its results because there are fewer animals involved, a Commercial Trial will have only two outcomes, with and without the enzyme.
- Either way, the comparison of nutrients required to generate the growth response will provide the needed information for BroilerOpt.exe to evaluate the Added Ingredient.