Investigating Nutrient Requirements of Grouper Fish for Feed Formulation

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Abstract—**High nutrients and desirable taste of grouper fish affect the high demand in grouper fish in the current prevailing market. Nevertheless, wild catch grouper fish in current prevailing market supply is limited and insufficient to meet the cyclical nature of high demand in grouper fish consumption leading to the high price. For that reason, grouper fish is worth farming. Hence, there is a need to formulate the fish feed since it is important to have correct nutrients requirement for feed formulation to ensure the adequacy of the diet for the grouper fish. Therefore, the aim of this study is to investigate the proper range of nutrients need in the fish feed. Data was collected from 30 manufacturers of grouper fish feed meal and analysis were done by SPSS. Quantitative analysis includes descriptive statistics, coefficient of variance, one sample test, pearson analysis and correlation were used for analysing nutrients data. Results show that grouper fish require seven approximate nutrients that are crude protein 40-45%, crude fat 1-10%, crude fibre 0.5-8%, moisture 0.8-95.5%, ash 0.4-18%, phosphorus 0.1-1.8%, calcium 2-4%, and nine essential amino acids (EAA) which are Arginine 2.06-4.21%, Histidine 0.66-1.26%, Isoleucine 1.2-2.57%, leucine 2.23-4.23%, lysine 1.96-4.04%, methionine 0.89-1.81%, Phenylalanine 0.89-2.46%, Threonine 1.29-2.59% and Valine1.46-2.86%. This vital information can be counted in such a further study in fish feed formulation to reduce cost and maximize profitability in grouper fish farming.**

Index Terms—**Grouper Fish; Fish Feed Formulation; Nutrients Requirements; Essential Amino Acid; Fish Feed Meal.**

I. INTRODUCTION

Nutrients are essential to all life in terms of health and to keep certain basic functions such as metabolic, mental, physical or chemical well [1]. All living beings need food to survive and reproduce, and they must balance their consumption, storage and use of food. Evidence of this can be seen as the bear's life in preparation for hibernation, so they will store energy primarily in the form of body fat. Therefore, adequate feed is very important for all living beings to survive and meet the standard of nutritional needs and daily functions.

To farm grouper fish, it is necessary to understand the nutritional needs and nutritional balance estimated for the healthy growth of grouper fish. Balance of nutrients means that the nutrient is provided with the necessary nutrients that living being can have the same basic nutritional needs as described by Campbell *et al*. [2] that are (1) fuels to supply all physical activities; (2) the establishment of the animal's own molecules of organic molecules; and (3) essential nutrients.

These objectives can be achieved via understanding the nutrients composition analyses, and ultimately led to our interest to undertake the fish feed problems for grouper fish as our research focus to fill in the gap. Grouper fish is selected as demand in the current trend of restaurants and price are high compared to other species of fish [3]. Nevertheless, it is difficult to find the relevant data because there is no exact data available [4]. Therefore, by doing these analyses, it enables us to develop grouper fish feed formula feeding nutrients priorities outlined. The formulation of grouper feed can be done using various mathematical method such as linear programming, chance constrained programming and heuristics as reviewed in [5].

II. METHODOLOGY

In this present study, data come from two sources: Data source (1) are from 30 manufacturers' grouper fish feed meal as illustrated in Table 2. These results are with the experts' input summarized in Table 1. Data sources (2) are from literature reviews such as journal papers and experts' opinions which are summarized in Table 6. SPSS was used to perform the data analysis in this study.

The main nutrients needed for grouper fish are crude protein, crude fat, crude fibre, moisture, crude ash, phosphorus and calcium. Fish feed meal with nutrients component (percentage) are demonstrated in Table 1 (experts' input) and Table 2 (from manufacturers) below. For example, the maximum nutrient requirement of crude protein is 45% for grouper fish while the minimum nutrients requirements of crude protein is 40 %.

Table 1
Fish feed meal with its range components (percentage) from experts' input

EAA	Minimum (%)	Maximum (%)
Crude protein	40	45
Crude fat	1	10
Crude fiber	0.5	8
Moisture	0.8	95.5
Ash	0.4	18
Phosphorus	0.1	1.8
Calcium	0.4	18

Table 2
Fish feed meal with its range components (percentage) from manufactures

	Crude_protein	Crude_fat	Crude_fibre	Moisture	Crude_ash	Phosphorus	Calcium
1	44.00	7.00	3.00	11.00	16.00		
2	52.00	8.00	6.00	12.00	16.00	1.30	4.00
3	38.00	5.00	3.00	11.00	16.00	1.50	2.50
4	48.00	10.00	3.00	10.00	17.00	1.80	2.00
5	52.00	2.00	6.00	10.00	10.00		
6	46.00	13.00	2.00	10.00	13.00		
7	40.00	5.00	5.00				
8	38.00	2.00	8.00	10.00		0.60	
9	35.00	7.00	4.00	8.00		1.20	
10	5.00	1.00	0.50	0.80			
11	11.00	1.20	5.00	79.00			
12	46.00	14.00	3.00	11.00	13.00		
13	12.00	1.50	3.50	79.00			
14	52.00	9.40	2.00	19.00	18.00		
15	52.00	20.00	1.00	8.50	12.00		
16	43.00	7.00	4.00	9.00	16.00	1.20	
17	60.00	8.00	3.00	5.00			
18	47.00	24.00	1.80	8.50	12.00		
19	7.80	1.60	0.50	87.00			
20	4.00	0.50	1.00	90.00			
21	44.00	10.00	3.00	5.00		1.00	
22	47.00	5.50	0.50	6.00		0.10	
23	14.80	1.01	0.50	1.00			
24	65.00	5.00	3.50	6.50		0.10	
25	6.00	0.50	1.00	90.00			
26	47.00	5.00	3.00	10.00	17.00	1.00	
27	4.70	0.80	0.50	92.00			
28	9.00	8.00	3.00	45.00			
29	37.00	2.00	8.00	12.00			
30	7.00	0.70	0.70	95.50	0.40	0.10	

The main nutrients needed for grouper fish are crude protein, crude fat, crude fibre, moisture, crude ash, phosphorus and calcium. Fish feed meal with nutrients components (percentage) are illustrated in Table 1 (experts' input) and Table 2 (from manufacturers). For example, the maximum nutrient requirement of crude protein is 45% for grouper fish while the minimum nutrients requirements of crude protein is 40 %.

In this present study, quantitative descriptive statistics include the coefficient of data comes from variance (CV), mean, standard deviation, range of sample, maximum and minimum sample. These are shown as in Table 3. All the CV of nutrients except moisture are less than 1.0. It shows that crude protein, crude fat, crude fibre, ash, phosphorus and calcium are less dispersed than moisture. In other words, the smaller CV of crude protein, crude fat, crude fibre, ash, phosphorus and calcium, the smaller residuals to the predicted value are. This is good indication of the variable. Moisture with a CV of 1.189 can still be considered a lower CV by itself. Therefore, it can be concluded that this is suggestive of good nutrients.

Table 3
Coefficient of variance of nutrients for grouper fish

	N	Range	Min	Max	Mean	Std. Deviation	CV
Calcium	3	2.00	2.00	4.00	2.8333	1.04083	0.367
Crude_ash	13	17.60	.40	18.00	13.5692	4.64173	0.342
Crude_fat	30	23.50	.50	24.00	6.1903	5.78614	0.935
Crude_fiber	30	7.50	.50	8.00	2.9667	2.12089	0.715
Crude_protein	30	61.00	4.00	65.00	33.8100	19.55319	0.578
Moisture	29	94.70	.80	95.50	29.0276	34.51512	1.189
Phosphorus	11	1.70	.10	1.80	.9000	.59498	0.661
Valid N (listwise)	3						

Using SPSS to perform the one sample test to check its nutrients are significant to the grouper fish feed as in Table 4. The seven types of nutrients are significant ($p < 0.05$), so it can be concluded that crude protein, crude fat, crude fibre, moisture, ash, phosphorus and calcium have a significant to the fish feed for grouper. These nutrients have a positive relation in order to increase fish growth in weight (kilogram) or length (centimetre).

Table 4
One sample test in analyzing nutrients in grouper feed formulation

	One-Sample Test					
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Crude_protein	9.471	29	.000	33.81000	26.5087	41.1113
Crude_ash	10.540	12	.000	13.56923	10.7643	16.3742
Crude_fat	5.860	29	.000	6.19033	4.0298	8.3509
Calcium	4.715	2	.042	2.83333	.2478	5.4189
Crude_fiber	7.661	29	.000	2.96667	2.1747	3.7586
Moisture	4.529	28	.000	29.02759	15.8987	42.1564
Phosphorus	5.017	10	.001	.90000	.5003	1.2997

SPSS was used to perform the correlation nutrients to analysis which nutrients are positive correction with other nutrients as explicit in Table 5. A positive value indicates that there is a positive correlation between each other. For example, if crude protein has a positive value on increase in crude protein will also increase crude ash, crude fat and crude fibre indirectly. If crude protein has a negative value, which means an increase in crude protein will reduce the phosphorus content.

Table 5
Correlation nutrients in grouper feed formulation

| | Correlation Matrix[a,b] | | | | | | |
	C	CA	CF	CFi	CP	M	P
C	1.000	-.693	-.127	.971	.533	.961	-.923
CA	-.693	1.000	.803	-.500	.240	-.866	.918
CF	-.127	.803	1.000	.115	.771	-.397	.500
CFi	.971	-.500	.115	1.000	.721	.866	-.803
CP	.533	.240	.771	.721	1.000	.277	-.165
M	.961	-.866	-.397	.866	.277	1.000	-.993
P	-.923	.918	.500	-.803	-.165	-.993	1.000

Table 6
Pearson analysis for nutrients in grouper feed formulation

| | | Correlations | | | | | | |
		C	CA	CF	CFi	CP	M	P
C	Pearson	1	-.693	-.127	.971	.533	.961	-.923
	Sig. (2-tailed)		.512	.919	.154	.642	.179	.252
	N	3	3	3	3	3	3	3
CA	Pearson	-.693	1	.155	.336	.755[**]	-.816[**]	.885[*]
	Sig. (2-tailed)	.512		.613	.261	.003	.001	.019
	N	3	13	13	13	13	13	6
CF	Pearson	-.127	.155	1	-.095	.580[**]	-.483[**]	.653[*]
	Sig. (2-tailed)	.919	.613		.618	.001	.008	.029
	N	3	13	30	30	30	29	11
CFi	Pearson	.971	.336	-.095	1	.371[*]	-.303	.297
	Sig. (2-tailed)	.154	.261	.618		.043	.110	.375
	N	3	13	30	30	30	29	11
CP	Pearson	.533	.755[**]	.580[**]	.371[*]	1	-.774[**]	.150
	Sig. (2-tailed)	.642	.003	.001	.043		.000	.660
	N	3	13	30	30	30	29	11
M	Pearson	.961	-.816[**]	-.483[**]	-.303	-.774[**]	1	-.400
	Sig. (2-tailed)	.179	.001	.008	.110	.000		.223
	N	3	13	29	29	29	29	11
P	Pearson	-.923	.885[*]	.653[*]	.297	.150	-.400	1
	Sig. (2-tailed)	.252	.019	.029	.375	.660	.223	
	N	3	6	11	11	11	11	11

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

A Pearson correlation coefficient was computed to assess the relationships between crude protein with crude ash, crude fat and crude fibre as shown in Table 6. There is a positive correlation between crude protein with crude ash, r =0.755, n=13, p =0.03, crude fat r =0.580, n =30, p =0.01 and crude fibre r =0.371, n =30, p =0.043. Overall, there is a strong and positive correlation between crude protein with crude ash, crude fat and crude fibre.

The increase in crude protein was associated with an increase in crude ash, crude fat and crude fibre. It applies also to phosphorus with crude ash and crude fat.

Essential amino acid (EAAs) required for grouper fish are Arginine, Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine. These nine essential amino acids (EAAs) are adopted and adapted from [6]. The minimum and maximum values for EAAs components as recommended by experts are summarized in Table 7.

All the CV of Essential amino acid (EAAs) are at most 0.3 as shown in Table 8. This shows that Arginine, Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine are less dispersed. In other words, the

smaller CV of Arginine, Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine and Phenylalanine, the smaller residuals for the predicted value are. This is a good indication of the variable. Thus, it can be concluded that these are good suggestive Essential amino acid (EAAs).

Table 7
Components of essential amino acids (EAAs) (percentage)

EAA	Minimum (%)	Maximum (%)
Arginine	2.06	4.21
Histidine	0.66	1.26
Isoleucine	1.37	2.57
Leucine	2.23	4.23
Lysine	1.96	4.04
Methionine	0.89	1.81
Phenylalanine	1.2	2.46
Threonine	1.29	2.59
Valine	1.46	2.86

Table 8
Coefficient of essential amino acids (EAAs) for grouper fish

Descriptive Statistics	N	Range	Min	Max	Mean	Std. D	C V
Arginine	10	2.15	2.06	4.21	2.8900	.64934	.225
Histidine	10	.60	.66	1.26	.9500	.21489	.226
Isoleucine	10	1.20	1.37	2.57	2.0340	.38260	.188
Leucine	10	2.00	2.23	4.23	3.0780	.59745	.194
Lysine	10	2.08	1.96	4.04	2.7540	.58839	.214
Methionine	10	.92	.89	1.81	1.2660	.26005	.205
Phenylalanine	10	1.26	1.20	2.46	1.5480	.46406	.300
Threonine	10	1.30	1.29	2.59	1.7680	.37809	.214
Valine	10	1.40	1.46	2.86	2.1420	.38236	.179
Valid N (listwise)	10						

SPSS was used to perform the one sample test to analysis which essential amino acids are positive correction with other nutrients as shown in Table 9. The nine types of essential amino acids (EAAs) are significant (p< 0.05). Thus, it can be concluded that Arginine, Histidine, Threonine, Valine,

Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine have a significant to the fish feed for grouper. These essential amino acids (EAAs) have a positive relation to increase fish growth in weight (kilogram) or length (centimetre).

Table 9
One sample test in analyzing essential amino acids (EAAs) in grouper feed formulation

	One-Sample Test					
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Histidine	13.980	9	.000	.95000	.7963	1.1037
Isoleucine	16.812	9	.000	2.03400	1.7603	2.3077
Leucine	16.292	9	.000	3.07800	2.6506	3.5054
Lysine	14.801	9	.000	2.75400	2.3331	3.1749
Methionine	15.395	9	.000	1.26600	1.0800	1.4520
Phenylalanine	10.549	9	.000	1.54800	1.2160	1.8800
Threonine	14.787	9	.000	1.76800	1.4975	2.0385
Valine	17.715	9	.000	2.14200	1.8685	2.4155
Arginine	14.074	9	.000	2.89000	2.4255	3.3545

SPSS was used to perform the correlation essential amino acids (EAAs) as depicted in Table 10. A positive value indicates that there is a positive correlation between each other. For instance, if Arginine has a positive value on increase in Arginine will also increase Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine indirectly.

Table 10
Correlation essential amino acids (EAAs) in grouper feed formulation

Correlation Matrix[a,b]									
	A	H	I	Le	Ly	M	P	T	V
A	1.000	.810	.833	.932	.964	.988	.331	.978	.846
H	.810	1.000	.671	.778	.759	.748	.263	.831	.794
I	.833	.671	1.000	.959	.869	.819	.481	.841	.931
Le	.932	.778	.959	1.000	.967	.910	.525	.950	.958
Ly	.964	.759	.869	.967	1.000	.941	.467	.969	.875
M	.988	.748	.819	.910	.941	1.000	.377	.968	.833
P	.331	.263	.481	.525	.467	.377	1.000	.485	.654
T	.978	.831	.841	.950	.969	.968	.485	1.000	.903
V	.846	.794	.931	.958	.875	.833	.654	.903	1.000

a. Determinant = .000

b. This matrix is not positive definite.

A Pearson correlation coefficient was computed to assess the relationships between Arginine with Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine as demonstrated in Table 11. There is a positive correlation between Arginine with Histidine, $r =0.810$, $n =10$, $p =0.05$, Isoleucine, $r =0.833$, $n =10$, $p =0.03$, Leucine, $r =0.932$, $n =10$, $p =0$, Lysine, $r =0.964$, $n =10$, $p =0$, Methionine, $r =0.988$, $n =10$, $p =0$, Threonine, $r =0.978$, $n =10$, $p =0$ and Valine $r =0.846$, $n =10$, $p =0.02$. Overall, there is a strong and positive correlation between Arginine with Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine and Methionine. Increases in Arginine are correlated with increases in Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, and Methionine. However, Phenylalanine merely has a positive correlation with Valine $r =0.654$, $n =10$, $p =0.04$.

Table 11
Pearson analysis for essential amino acids (EAAs) in grouper feed formulation

		Correlations								
		H	I	Le	Ly	M	P	T	V	A
H	PC	1	.671[*]	.778[**]	.759[*]	.748[*]	.263	.831[**]	.794[**]	.810[**]
	Sig.		.034	.008	.011	.013	.463	.003	.006	.005
	N	10	10	10	10	10	10	10	10	10
I	PC	.671[*]	1	.959[**]	.869[**]	.819[**]	.481	.841[**]	.931[**]	.833[**]
	Sig.	.034		.000	.001	.004	.159	.002	.000	.003
	N	10	10	10	10	10	10	10	10	10
Le	PC	.778[**]	.959[**]	1	.967[**]	.910[**]	.525	.950[**]	.958[**]	.932[**]
	Sig.	.008	.000		.000	.000	.119	.000	.000	.000
	N	10	10	10	10	10	10	10	10	10
Ly	PC	.759[*]	.869[**]	.967[**]	1	.941[**]	.467	.969[**]	.875[**]	.964[**]
	Sig.	.011	.001	.000		.000	.173	.000	.001	.000
	N	10	10	10	10	10	10	10	10	10
M	PC	.748[*]	.819[**]	.910[**]	.941[**]	1	.377	.968[**]	.833[**]	.988[**]
	Sig.	.013	.004	.000	.000		.282	.000	.003	.000
	N	10	10	10	10	10	10	10	10	10
P	PC	.263	.481	.525	.467	.377	1	.485	.654[*]	.331
	Sig.	.463	.159	.119	.173	.282		.156	.040	.351
	N	10	10	10	10	10	10	10	10	10
T	PC	.831[**]	.841[**]	.950[**]	.969[**]	.968[**]	.485	1	.903[**]	.978[**]
	Sig.	.003	.002	.000	.000	.000	.156		.000	.000
	N	10	10	10	10	10	10	10	10	10
V	PC	.794[**]	.931[**]	.958[**]	.875[**]	.833[**]	.654[*]	.903[**]	1	.846[**]
	Sig.	.006	.000	.000	.001	.003	.040	.000		.002
	N	10	10	10	10	10	10	10	10	10
A	PC	.810[**]	.833[**]	.932[**]	.964[**]	.988[**]	.331	.978[**]	.846[**]	1
	Sig.	.005	.003	.000	.000	.000	.351	.000	.002	
	N	10	10	10	10	10	10	10	10	10

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

III. RESULTS AND DISCUSSION

Quantitative analysis includes CV, mean and correlation on nutrients for grouper fish have been done. From the statistical analysis, the main findings are as follows: (1) There are five main nutrients and nine main essential amino acids needed for grouper fish: (i) crude protein, (ii) crude fat, (iii) crude fibre, (iv) ash and (v) moisture (vi) Arginine, (vii) Histidine, (viii) Threonine, (viiii) Valine, (x) Isoleucine, (xi) Leucine, (xii) Lysine, (xiii) Methionine, and (xiiii) Phenylalanine. (2) All other fish feed meals have a range of between 30-59% of protein content, and the average of crude protein for all fish feed meals is 33.81%. But, sample 17 and sample 24 from Table 1 are outliners from others which are 60% and 65% of crude protein, respectively. There are 7 samples of less than 10%. After consulting with experts, crude protein less than 10% is not recommended. It should be in the range of 40-45%. Although more percentage of crude protein is encouraged, it is not recommended because of its high cost. (3) For crude fat, a range of 1-10% is advisable. Fish feed meal with less crude fat would avoid excessive growth of lipids in the fish's body. Sample 8 and 29 both have a crude fibre content of 8%, which is the highest content compared to the rest of the fish feed meals and the mean (2.9667%). The rest of the fish feed meals have a content of crude fibre ranging between 0.5-8%. Crude fibre plays a role in the fish's body by eliminating toxins and waste products with greater efficiency. It is a known fact that fibre helps to eliminate waste from its gastrointestinal tract because of its ability to bind water and thus produce firmer stools. It is important to include crude fibre in a fish's daily feed for the healthy growth of the maximum length and size. (5) The moisture of the fish feed ranges from 0-95.5% and an average of 29.03%. The food with higher moisture would be suitable for groupers that live in the oceans and salty water habitat. This is because when the food is left on salty water, it does not become wet and dissolve away quickly due to its moisture whereas it is different for the fish feed with a lower moisture. The fish feed with a low moisture are such as sample 24 and 21 with merely 6.5% and 5% moisture. It is most suitable for a grouper feed in the clear water and non-sea water. This is due to the neutral pH of the non-sea water that does not change the texture of the feed even though the feed has low moisture. (6) Lastly, some fish feed meals also contain other nutrients such as ash, phosphorus and calcium. 13 types of fish feed meals have ash which ranges from 0.4-18%, and a mean of 13.57%, others 0%. (7) 11 types of fish feed meals have phosphorus which ranges from 0.1-1.8% and an average of 0.9%, others 0%. (8) Only 3 types of fish feed meals have calcium which ranges from 2-4%, and an average of 2.83%, others 0%. (9) There is a strong and positive correlation between crude protein with crude ash, crude fat and crude fibre. It is also a strong and positive correlation between phosphorus with crude ash and crude fat. (10) There is a strong and positive correlation between Arginine with Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, and Methionine. (11) There is a strong and positive correlation between Phenylalanine with Valine alone. Therefore, we would like to suggest that the ideal nutrient for grouper fish is a high crude protein content in order to promote healthy growth in length and size. The experts' opinion about the crude protein is falls within the range of nutrients (40%-45%) from statistical analyses (4%-65%). It is same with crude fat falls within the range of nutrients (1%-10%) from statistical analyses (0.5%-24%). The range of experts' opinion about calcium falls overlap (0.4%-18%) with statistical analyses (2%-4%). Overall, the results from analysis and experts opinion are conclusive and in the similar viewpoints. However, there is a slightly different result from analysis and experts' opinion especially for crude protein and crude fat, but yet is still falls within the range from statistical analyses. For the nutrients requirements, it can't take into account for crude protein even though as it is 4% from analysis is applicable. In fact, 4% is too low and would affect the actual grow of grouper fish according to experts. Maximum of 65% of crude protein from analysis is perfect and idealistic, however, in reality, the maximum of crude protein as it is 65% is too high and cannot absorb totally 65% of crude protein and wasted. Additionally, in reality 65% of crude protein is too costly.

IV. CONCLUSION

Nutrient requirements of grouper fish has been investigated because of the high catch demand in the current prevailing market. This study shows that the main nutrients needed for grouper fish are crude protein, crude fat, crude fibre, moisture, ash, phosphorus and calcium. Crude protein is the most important nutrients, followed by crude fat and crude fibre. It also can be concluded that the main essential amino acids required for grouper fish are Arginine, Histidine, Threonine, Valine, Isoleucine, Leucine, Lysine, Methionine and Phenylalanine. This information can be further studied in the fish feed formulation using Evolutionary Algorithm in order to minimise cost and maximize profit in farming grouper fish. Most importantly, this study can be a reference to other research related to fish diet.

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