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(54) Title: A FISH FODDER FOR FRESHWATER FISH AND USE OF SUCH FODDER

(57) Abrégé/Abstract:
A rearing fodder of extruded fodder type, comprising proteins, fats and carbohydrates, for freshwater fish, wherein the fish is larger than 2 grams, and wherein the freshwater has a content of CO2 being above 5 milligrams/litre, wherein the fodder has an increased content of at least one of the minerals: phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) beyond what exists naturally in the used protein raw materials, fat raw materials and carbohydrate raw materials, wherein the collective amount of minerals in the extruded fodder is at least 10 %, on a dry substance basis, of the total weight of the fodder, and wherein one or more of the minerals phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) is/are added beyond what exist in the used protein raw materials, fat raw materials and carbohydrate raw materials.

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(54) **Title:** A FISH FODDER FOR FRESHWATER FISH AND USE OF SUCH FODDER

(57) **Abstract:** A rearing fodder of extruded fodder type, comprising proteins, fats and carbohydrates, for freshwater fish, wherein the fish is larger than 2 grams, and wherein the freshwater has a content of CO2 being above 5 milligrams/litre, wherein the fodder has an increased content of at least one of the minerals: phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) beyond what exists naturally in the used protein raw materials, fat raw materials and carbohydrate raw materials, wherein the collective amount of minerals in the extruded fodder is at least 10 %, on a dry substance basis, of the total weight of the fodder, and wherein one or more of the minerals phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) is/are added beyond what exist in the used protein raw materials, fat raw materials and carbohydrate raw materials.

A FISH FODDER FOR FRESHWATER FISH AND USE OF SUCH FODDER

The invention concerns a fodder for freshwater fish, more particularly a fodder having an increased content of minerals relative to that previously described as necessary for providing fish with a good growth.

Some salmon fish are anadromous fish. Sexually mature fish migrate from seawater to freshwater to spawn, while the offspring migrate back to the sea to grow. The eggs are hatched at the river bottom, and the juvenile salmon (fry) spend their initial lifetime in the river water. In the spring, great physiological changes occur in the fry as it prepares for the transition from living in freshwater to living in seawater. The preceding autumn, when subjected to natural conditions, the fry determines whether it is large enough to migrate as smolt the next spring. In nature, the time between hatching and migrating may vary from more than one year to 5 years, depending on water temperature and food availability.

Rearing of salmon and sea trout must follow the natural mode of living for the fish. Having fertilized the eggs, the eggs are hatched in freshwater. The fish go through the yolk sack stage and the fry stage in tanks in a hatchery. In a hatchery, it is possible to manipulate both water temperature and lighting conditions, so as to control the timing of smoltification in a different manner than in nature. As such, smolt is referred to as 0-yearlings, 1-yearlings and 2-yearlings. 0-yearlings are planted as smolt the first autumn after hatching in the winter, while the 1-yearlings spend more than one year in the hatchery.

After smoltification, reared fish are put into fish cages in seawater to grow until slaughtering. A daily weight increase depends on body weight and may be compared to the effect of capital size in an interest calculation. Two smolt of equal quality concerning health and physiological adaptation and planted in the sea simultaneously, may grow equally fast in terms of percentages (specific growth rate), but the larger one will reach a slaughtering size prior to the other one.

In intensive rearing of fish, the duration from hatching to smoltification, and the size of the smolt at smoltification, is of great economic importance. The size of tied-up capital, in the form of fish, is of great importance to the profitability of both the fingerling producer and the producer of consumable fish.

Rearing of salmon has become more intense. The industry has grown fast, and the need for smolt has been on the increase. Many fingerling producers are restricted in terms of withdrawing more freshwater from their water sources. This has caused the fish density in the rearing tanks of the hatcheries to increase. Among other things, this has been made possible by adding oxygen (O_2) to the water. Consequently, the amount of carbon dioxide (CO_2) in the water also has increased dramatically, up to as much as 40 milligrams CO_2/litre (mg CO_2/l) and above. The concentration of CO_2 in the water depends on the water quality. Water having a low conductivity, typical of for example the coastal region of Norway, has a low buffer quality, and therefore the content of CO_2 in this water becomes larger than that of more ion-rich water. Thus, watercourses in Southern and Western Norway receiving acid rain possess a water quality having conductivities down towards 10 mikroSiemens/centimetre (μS/cm). Most common in Norwegian watercourses is a water quality between 25-75 μS/cm. In comparison, regions of Eastern Norway having calcareous bedrock possess a water quality having conductivities of ca. 300 μS/cm. In order for the water to possess a certain buffer capacity, the conductivity should be above 30 μS/cm.

Fish being exposed to a high CO_2-level (5 mg/l and above) over an extended time, will attain an increased level of CO_2 in their blood (*hypercapnia*), and the amount increases with the amount in the water. This results in an increased content of bicarbonate in order to compensate for the CO_2-increase, the result being that the pH-value of the blood decreases (*respiratory acidosis*). Fish having acidosis will seek to counteract this condition by mobilising ions from the bone structure and secrete phosphate via the kidney.

A high content of CO_2 in the water provide a negative influence on growth and health of the fish. Fish exposed to high CO_2-levels may develop *nefrocalcinosis*, which is distinguished by calcium precipitating and depositing in the kidney. This is observed already at 5-10 mg/l and has been described from 15 mg/l and up (Fivelstad, S. et al.; "The effects of carbon dioxide on salmon smolt"; in "Norsk Fiskeoppdrett", pages 40-41, no. 16, 1998).

In intensive rearing of fish, extruded fish fodder is used most commonly. This is composed of proteins, carbohydrates and fats. The protein raw materials may consist of animal protein sources, such as fish meal, bone meal, blood meal and feather meal, and of vegetable protein sources, such as soy, corn gluten, wheat gluten and lupines. Carbohydrates are primarily added as a binding agent to provide the fodder pellet with a sustainable shape and mechanical strength. The carbohydrate source may be whole or ground up wheat, potato starch or other starch sources. In order to increase the energy content of the fish fodder, animal oil, such as fish oil or vegetable oil, including rapeseed oil or soybean oil, is generally added after forming in the extruder step and the subsequent drying step. These raw materials also contain minerals. Thus, phosphate and other minerals, for example, are included in fishbone remnants of fish meal. Phosphate also occurs as phosphate lipids in the protein sources and in the oil sources.

The objective of the invention is to improve the growth of reared fish in freshwater. In connection with intensive rearing of fish, the objective is particularly to improve the growth of fingerlings of salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), and of other anadromous fish species of salmon.

The objective is achieved by means of features disclosed in the following description and in subsequent claims.

Surprisingly, the objective is achieved by increasing the mineral content of formulated fish fodder beyond what the literature describes as necessary for ensuring a good growth.

In the following, non-limiting examples of preferred embodiments are described, in which the effect is shown through test results referred to.

In the following examples, the use of a standard fodder and customised fodder mixtures are described. Common to these fodder types is that they initially have added thereto what is considered to be necessary amounts of minerals for maintaining a good growth. For the specific minerals, the following target numbers for the fodder recipes are to be used for freshwater fish:

phosphate (P) - 1.09 %, potassium (K) - 0.72 %, calcium (Ca) - 1.34 %, magnesium (Mg) - 0.17 %, sodium (Na) - 0.64 %.

Deviations from this may occur, both in terms of dosing inaccuracy during production, and also in terms of analysis inaccuracy.

The invention concerns addition of minerals beyond these levels generally considered to be adequate. Addition of extra mineral amounts is termed as addition of a premix. The person skilled in the art will know that the term premix also is used for addition of trace minerals considered necessary for growth. Such mineral mixes will contain for example the trace elements copper (Cu), zinc (Zn), manganese (Mn), iodine (I), and also relatively small amounts of calcium (Ca), magnesium (Mg) and potassium (K). This description is based on the fact that premix refers to the extra addition, and that minerals already have been added in a common manner without making reference thereto.

In the examples, an inert filling material denoted "Diamol" is used. This has been done for the recipes to be equivalent concerning protein level and fat level. Among other things, Diamol contains Ca (1.2 %), P (0.7 %), Mg (0.5 %), K (0.72 %), Na (0.47 %) and Fe (2.4 %). Contrary to mineral premixes containing digestible mineral compounds, Diamol contains indigestible mineral compounds. Up to 4 % of Diamol is added. Thus, the specified minerals will contribute little to the total amount thereof. For total ash content, however, Diamol will contribute considerably, inasmuch as almost the entire addition will remain in the ash analysis.

Example 1

The study was carried out on salmon (*Salmo salar*). The average start weight was 0.15 grams (g), and the fish was observed for 231 days until smoltification. The water temperature was constant at 13.1 ± 1.7 degrees Centigrade (°C). The fish was stocked at ca. 300 fish per tank in a total of 12 tanks. Each tank contained 60 l of water.

For the test, two diets were made. One fodder was a standard fodder containing 9.3 % of minerals (ash; normal mineral group = NM-group). Initially, the other fodder was of the same composition, but an additional 4 % of pre-ashed fish meal was added to constitute a total mineral content of 12.3 % (high mineral group = HM-group).

When the fish was from 0.15 g to ca. 2.5 g, an agglomerated fodder was used. This was subjected to sieving, forming nutritionally equivalent compositions of the used fractions: 0.3-0.5 millimetres (mm); 0.5-0.8 mm; and 0.8-1.2 mm.

Table 1.1. Analysis of fodder composition. The values are provided on a dry substance basis.

Analysis (%)	**Standard fodder**			**Standard fodder + 4 % of pre-ashed fish meal**		
		Extruded (mm)			**Extruded (mm)**	
	Agglomerated	**2**	**3**	**Agglomerated**	**2**	**3**
Protein	56.5	48.5	48.8	54.6	49.2	49.0
Fat	21.5	21.8	23.2	22.1	22.8	22.7
Water	6.9	7.0	6.3	6.2	5.7	7.2
Total minerals (ash)	8.1	9.2	8.9	9.7	12.7	12.0
P	1.24	1.44	1.48	1.08	2.08	1.87
K	0.75	0.79	0.80	0.71	1.05	0.95
Ca	1.57	2.06	2.08	1.32	3.02	2.67
Mg	0.18	0.13	0.13	0.16	0.19	0.17
Na	0.65	0.58	0.60	0.99	0.90	0.80

Moreover, the fish groups were divided in two with respect to CO_2-amount in the water. In 6 tanks, no extra CO_2 was added, the CO_2-level therefore corresponding to the CO_2-secretion from the fish. In the other 6 tanks, an increasing CO_2-amount was added as the fish were growing. Until the fish reached 10 g, no extra CO_2 was added. For the sizes 10-15 g; 15-20 g; and from 20 g to smolt; CO_2 was added to reach a concentration in the water of 10; 20; and 35 mg CO_2/l, respectively. Thus, the study comprised 4 groups, each group randomly distributed between 3 tanks.

River water along the coast of Norway is acidic and possesses a low buffer capacity. In Norwegian rearing of fingerling, it is therefore common practice to add small amounts of UV-radiated seawater to buffer the ion-deficient freshwater. In this study, 0.5 ‰ seawater (5 l seawater per m^3) therefore was added to the freshwater as long as the desired CO_2-amount was up to 10 mg/l in some of the tanks. The admixing of seawater was increased to 1.5 ‰ when the CO_2-amount increased to 20 mg/l, and to 2.5 ‰ when the CO_2-amount was increased to 35 mg/l. Inasmuch as the pipeline network for supply of water was the same for all tanks, the admixing of seawater was identical for all tanks, irrespective of the amount of supplied CO_2.

Table 1.2. Measured CO_2-level (lowest and highest; mg/l) and pH-value in the water.

Desired amount of CO_2 (mg/l) in the water	Fish weight (g)							
	<10		10-15		15-20		>20	
	CO_2 (mg/l)	pH	CO_2 (mg/l)	pH	CO_2 (mg/l)	pH	CO_2 (mg/l)	pH
No addition	-	-	2.7-5.1	6.50-6.82	4.7-6.0	6.63-6.77	6.6-7.8	6.58-6.72
10			8.7-11.3	6.17-6.27				
20					17.1-20.0	6.11-6.17		
35							33.5-37.3	5.72-5.88

-: not measured

Table 1.3. Weighing results in grams (g).

	Time of testing (days after start)					
	97		190		231	
Group	Average	Sd.	Average	Sd.	Average	Sd.
NM, no CO_2	8.1	1.4	52.4	3.6	80.2	6.3
HM, no CO_2	9.2	0.8	55.9	2.3	82.3	7.3
NM, increased CO_2	8.1 [(a)]	0.6	42.8	1.9	54.8	2.4
HM, increased CO_2	10.5 [(a)]	0.3	51.8	0.7	65.1	2.2

NM: normal level of minerals in the fodder

HM: high level of minerals in the fodder

Sd: standard deviation
[a] no addition of CO_2

The study shows that an increased content of minerals beyond what is recommended provides a surprising, positive effect on the growth. For fish growing from 0.15 g to 10 g, the fish receiving extra minerals have a better growth. In this phase, no extra CO_2 was added to the water. When no extra CO_2 was added to the water, the fish pervasively exhibited a better growth during the entire study, but this difference is not statistically significant. Also, the study clearly showed that an increased amount of CO_2 in the water restrained the growth. An increased amount of minerals could not completely compensate for this, but the group receiving an increased amount of minerals had an average weight being 20 % better than that of the group receiving a standard fodder of good nutritional quality. An increased content of CO_2 fall within ordinary production requirements. The NM-group without and with addition of CO_2 had a specific growth of 2.72 and 2.56 %/day, respectively, while the HM-group without and with addition of CO_2 had a specific growth of 2.74 and 2.64 %/day, respectively. This growth is estimated from 6 g to smoltification. The growth was good for all groups.

Example 2

The study was carried out on salmon (*Salmo salar*). The average start weight was 49.1 g, and the fish was observed for 41 days until smoltification. The water temperature was constant at 14.0 ± 1.4 °C. The fish was stocked at 90 fish per tank in a total of 20 tanks. The tanks were circular tanks with a diameter of 1.0 meter.

The diameter of the fodder particles was 3 mm. For the test, 10 diets were made. One fodder was a control fodder containing 10.9 % of minerals. 4 % of Diamol was added as filler in this fodder. Initially, the other fodder had the same composition, but an additional 4 % of pre-ashed fish meal was added and a further 0.5 % of Diamol. This contained 12.2 % of minerals. Initially, the remaining fodders were also the same as the control fodder, but 4 % of mineral premixes customised for this study were added. These fodders contained 11.6 % of minerals, and no Diamol was added thereto.

A series of mineral premixes were produced in order to study whether the collective amount of minerals is of significance, or whether single components provide the advantageous effect observed in Example 1. A complete premix was tested together with premixes in which one of the elements: P, Ca, K and Mg was removed. Additionally, one premix was tested in which both P and Ca were removed; one premix in which P, Ca and Mg were removed; and one premix in which P, Ca, Na and Cl were removed.

Table 2.1. Composition of the test fodders. The values are provided on a dry substance basis.

Analysis	**Standard fodder**	**Fodder + 4 % ash**	**Complete premix**	**Premix - P**
Protein (%)	51.6	49.8	48.8	50.6
Fat (%)	22.5	23.7	24.5	23.9
Water (%)	4.5	5.0	5.5	4.5
Total minerals (ash) (%)	10.9 [a]	12.2 [b]	11.6	11.6
P (%)	1.4	1.9	1.8	1.4
K (%)	0.95	1.1	1.1	1.1
Ca (%)	1.7	2.5	2.4	2.3
Mg (%)	0.17	0.21	0.19	0.20

[a] 4 % of Diamol added

[b] 0.5 % of Diamol added

Table 2.2. Weighing results in grams (g) at start and after 41 days.

Fodder type	**Start weight (g)**	**Final weight (g)**	**Growth in %**	**Relative growth in relation to control**
Control	48.3	83.7	73.3	100
Control + pre-ashed fish meal	50.6	89.7	77.2	105.3
Control + complete premix	48.6	88.1	81.4	111.0
Control + premix - P	47.9	85.5	78.3	106.7
Control + premix - Ca	47.7	85.9	80.2	109.4
Control + premix - K	49.3	87.9	78.4	106.9
Control + premix - Mg	49.6	91.4	84.5	115.3
Control + premix - (P + Ca)	51.0	91.4	79.0	107.8
Control + premix - (P + Ca + Mg)	49.3	89.0	80.6	109.9
Control + premix - (P + Ca + Na + Cl)	48.6	87.8	80.9	110.3

In relation to the fish receiving control fodder, the fish receiving pre-ashed fish meal had a 5.3 % higher body weight, and the groups receiving different variants of premix all had a better growth than that of the groups receiving control fodder or fodder with pre-ashed fish meal. The growth was from 6.7 % to as much as 15.3 % better, which is very surprising during a time as short as 41 days. Accordingly, the study showed that the customised premixes replaced the pre-ashed fish meal, and that the growth became even better. Surprisingly, the study also showed that it is the collective amount of extra minerals that is of significance. Individual minerals may be taken out of the premix without the growth becoming worse than that of the control fodder.

Example 3

The study was carried out on salmon (*Salmo salar*). The average start weight was 2.5 g, and the fish was observed for 182 days until smoltification. The water temperature was constant at 14.0 ± 0.9 °C. The fish was stocked at ca. 300 fish per tank in a total of 24 tanks. Each tank contained 60 litres of water. For the test, 2 diets were made. One fodder was a control fodder containing 9.0 % of ordinary minerals (NM-group), but wherein an additional 4 % of Diamol was added. Initially, the other fodder had the same composition, but an additional 4 % of the same mineral premix as that described in Example 2 (HM-group) was added. The mineral content was 12.0 %.

At start-up of the study, two tanks, in which the fish received a control fodder having an addition of 4 % of Diamol, and two tanks, in which the fish received fodder of increased mineral content, were restrained with respect to addition of extra CO_2 to the water. In these tanks, no extra CO_2 was added during the study. In the other tanks, extra CO_2 was added at start-up in order for the total amount of CO_2 to be ca. 5 mg/l. After 14 days, the amount of CO_2 supplied to the water was increased in order for it to be ca. 10 mg/l. At this point in time, 4 more tanks (2 tanks for each diet) were restrained in order for these to continue at ca. 10 mg/l of CO_2 in the water for the remainder of the study. This procedure was repeated after further 14 days, thereby increasing the amount of CO_2 in the water to 15 mg/l, and then increasing the amount of CO_2 in the water to 20 mg/l after another 14 days. Each time, 4 and 4 tanks were restrained to continue throughout the study with 15 and 20 mg/l of CO_2 in the water, respectively. At this point in time, the fish was weighed in at ca. 8 g. After another 14 days, CO_2 in the water was increased to 35 mg/l in the last 4 tanks, and the described CO_2-regime was maintained for the remainder of the study.

As in Example 1, UV-radiated seawater was supplied to the freshwater for buffering thereof. 0.5 ‰ seawater was supplied to the freshwater throughout the entire study.

Table 3.1. Measured CO_2-level (lowest and highest; mg/l) and pH-value in the water.

Desired amount of CO_2 (mg/l) in the water	**After 30 days**		**After 49 days**	
	CO_2 (mg/l)	**pH**	**CO_2 (mg/l)**	**pH**
No addition	-	-	-	-
5	4.0-8.1	6.34-6.49	6.2-6.6	6.38-6.42
10	9.5-9.9	6.24-6.25	8.1-9.5	6.20-6.28
15	12.1-13.2	6.10-6.12	21.7-22.8	5.98-6.03
20	-		20.2-25.7	5.88-5.92

-: not measured

Table 3.2. Analysis of fodder composition. The values are provided on a dry substance basis.

	Control fodder, extruded (mm)			**Extruded fodder + 4 % of mineral mix (mm)**		
Analysis	1.5	2	3	1.5	2	3
Protein	49.4	50.9	50.9	49.4	50.5	50.6
Fat	23.5	23.4	22.0	24.7	23.4	22.6
Water	4.7	4.8	5.2	4.3	5.6	5.7
Total of minerals (ash)	11.1 [a]	11.1 [a]	11.0 [a]	13.8	10.6	10.6
P (%)	1.4	1.3	1.3	1.8	1.8	1.7
K (%)	0.78	1.1	1.1	0.98	1.3	1.3
Ca (%)	2.2	1.2	1.2	2.8	1.9	1.8
Mg (%)	0.16	0.16	0.17	0.19	0.19	0.19
Na (%)	0.68	0.56	0.56	0.98	0.81	0.80

[a] 4 % of Diamol added

Table 3.3. Weighing results in grams (g).

Desired level for total amount of CO_2 (mg/l) in the water	Time of testing (days after start)											
	56				99				182			
	NM		HM		NM		HM		NM		HM	
	Avg.	Sd.	Avg.	Sd.	Avg.	Sd.	Avg.	Sd.	Avg.	Sd.	Avg.	Sd.
0	6.6	0.2	8.6	0.8	18.1	4.6	23.9	1.6	50.7	3.6	64.8	9.5
5	7.7	0.6	9.0	0.3	26.3	4.3	24.7	0.1	57.7	8.0	65.2	2.4
10	7.9	0.3	9.0	1.2	25.4	5.6	28.1	3.7	61.0	1.9	67.0	4.4
15	7.2	0.6	8.9	0.1	20.8	3.1	29.3	3.2	57.0	2.2	71.9	0.4
20	7.7	0.1	8.9	0.0	23.9	4.6	28.2	4.2	57.9	2.9	71.3	4.3
35	7.3	0.2	8.8	0.0	19.5	0.0	23.0	1.8	51.8	0.6	62.0	6.2
Avg.	7.4	0.5	8.9	0.5	22.3	4.4	26.2	3.3	56.0	4.8	67.0	5.4

NM: normal level of minerals in the fodder
HM: high level of minerals in the fodder
Avg: average
Sd: standard deviation

Already after 56 days, a significant weight difference existed between the groups receiving control fodder (NM) and the groups receiving fodder with an increased mineral content (HM). Collectively for all groups, fish receiving a fodder with increased content of minerals weighed 19.5 % more than fish receiving control fodder. After 99 and 182 days, the corresponding difference was 17.5 and 19.7 %. In this study, there was no marked effect of the amount of CO_2 in the water. This may be due to the fish not growing very well in this study. Specific growth rate for the entire period was between

1.73 og 1.82 %/day for the NM-groups, and 1.84 - 1.93 %/day for the HM-groups.

Claims

1. A rearing fodder of extruded fodder type, comprising proteins, fats and carbohydrates, for freshwater fish, wherein the fish is larger than 2 grams, and wherein the freshwater has a content of CO_2 being above 5 milligrams/litre, wherein the fodder has an increased content of at least one of the minerals: phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) beyond what exists naturally in the used protein raw materials, fat raw materials and carbohydrate raw materials, characterised in that the collective amount of minerals in the extruded fodder is at least 10 %, on a dry substance basis, of the total weight of the fodder, and wherein one or more of the minerals phosphate (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) is/are added beyond what exist in the used protein raw materials, fat raw materials and carbohydrate raw materials.

2. The rearing fodder according to claim 1, characterised in that the amount of phosphate (P) is within the range 1.1 - 2.1 %, on a dry substance basis, of the total weight of the fodder.

3. The rearing fodder according to claim 1, characterised in that the amount of potassium (K) is within the range 0.7 - 1.1 %, on a dry substance basis, of the total weight of the fodder.

4. The rearing fodder according to claim 1, characterised in that the amount of

calcium (Ca) is within the range 1.3 - 3.0 %, on a dry substance basis, of the total weight of the fodder.

5. The rearing fodder according to claim 1, c h a r a c t e r i s e d i n that the amount of sodium (Na) is within the range 0.6 - 0.9 %, on a dry substance basis, of the total weight of the fodder.

6. The rearing fodder according to claim 1, c h a r a c t e r i s e d i n that the amount of magnesium (Mg) is within the range of 0.17 - 0.21 %, on a dry substance basis, of the total weight of the fodder.

7. Use of an extruded rearing fodder according to any one of claims 1 to 6 for feeding of freshwater fish, wherein the fish is larger than 2 grams, and the water has a content of CO_2 being above 5 milligrams/litre.

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