



Effect of Fenugreek Seeds By-produced meal on Growth Performance, Feed Utilization, Body Composition and Some Physiological Traits for Common Carp (*Cyprinus Carpio*).
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ABSTRACT

This study was performed to investigate the effect of dietary supplementation with fenugreek seeds by-produced meal (FSM) on growth performance, feed utilization, body composition and some physiological traits of Common carp (*Cyprinus carpio*) with an average 10.6 ± 0.15 g weights and 8.78 ± 0.14 cm length. A 12-week feeding trial was conducted in 12 aquariums (75 – 60 – 45cm in diameters). Four experimental diets were formulated to containing 0% (Commercial diet), 10, 20 and 30% FSM by-produced. The results revealed that fenugreek seeds meal supplementation significantly enhanced the fish growth over the control group. Also, condition factor and survival rate were significantly increased with increasing FSM percentage in the diets up to 20%. Feed conversion ratio was significantly improved with increasing FSM up to 20% inclusion level. Fish feeding diets containing 20% FSM had significant increase in PER, whereas there was no significant difference in feed intake. Body protein and dry matter content was significantly increased with increasing FSM percentage in the diets until 20%, while the higher value of fat content was recorded with diet contained 10% fenugreek seeds. The higher significant plasma protein and lysozyme and lower plasma glucose was obtained in diet containing 20%. FSM. Fish fed control diet gave the higher value of plasma glucose and the lower value lysozyme, while Fish fed 30 % FSM provided the lower value plasma protein. It could be concluded that the dietary addition of 20% of fenugreek seeds by-produced meal enhanced the growth performance of Common carp.

Key words: Common carp, fenugreek seeds by-produced, growth performance.

Introduction

Carp species are widely cultivated freshwater fish family in worldwide. They are third only to tilapia and mullet as the most widely farmed fish in Egypt (**GAFRD, 2018**). Carp species have become important species in fish culture systems because of their tolerance of wide differences in pond temperature and water quality, ease of management, reproduction, high growth rates, high conversion of artificial feed and resistance to disease (**Syeed et al., 2018**). Common carp, *Cyprinus carpio*, is one of the most widely cultured carp species.

Several feed supplements have been used to improve growth performance and health status in various fish. Recently, research has increased on the supplementation of medicinal plants in trial to produce organic fishes. Fenugreek (*Trigonella foenum-graecum*) seed meal (FSM) has been reported to produce various beneficial effects, including growth promotion, immunostimulation and antimicrobial effects in fish. It is rich in protein (20-30%) high in lysine and tryptophan; carbohydrates (45-60%). Fenugreek seeds contain high phytonutrients, minerals, and vitamin contents. They have high amount of non-starch polysaccharide (NSP) fibers. Major NSP's are tannins, saponins, hemicelluloses, mucilages and galactomannans. Non-starch polysaccharides enhance bowel movement, support in smooth digestion and also help in decreasing LDLcholesterol level in the blood through binding. NSPs also capture toxic substances present in food and act as a shield for the colon mucosal layers against cancers. Fenugreek seeds also contain amino acid 4-hydroxyisoleucine, which boosts insulin secretion. Other essential phytochemicals present in fenugreek seeds (including trigonelline, gitogenin, yamogenin diosgenincholine and trigogenin) play important therapeutic roles, such as being anti-anorexic, anti-oxidant, anti-carcinogenic, antihyperlipidemic, anti-inflammatory, and antidiabetic uses. (**Blumenthal et al., 2000 and Riaz et al 2020**)

The use of FSM from 5 to 15 g/kg in the diets of common carp *Cyprinus carpio* and Striped Catfish led to significantly improved growth compared to a control diet (FSM free) (**Roohi et al., 2015 and Syeed et al., 2018**). Similarly, incorporation of FSM at levels of 1% in tilapia diets have effects a positive on growth performance, nutrient utilization and physiological parameters in Nile tilapia (**Mostafa et al., 2009 and Tonsy et al., 2011**). Also the inclusion of FSM at 50 g/kg also improved the growth of gilthead seabream *Sparus aurata* as well as some immunological responses (**Bahi et al., 2017 and Guardiola et al., 2017**). **Moustafa et al (2020)** found that the optimum growth performance, feed

conversion, immune response and antioxidant capacity compared other groups could be reached with the inclusion of (3%) FSM in the diet of Nile tilapia. **Abbas et al., 2019** revealed that the inclusion of 5 % of crude fenugreek seeds or 3 % of an alcoholic fenugreek seed extract as a feed additive enhances fish growth and immunity of Nile tilapia fish. **Sheikhlal et al., 2017** stated that the inclusion of FSM at 180 g/kg to replacement of FSM of African catfish improved the growth compared to a control diet However no previous study has been conducted on FSM by-produced as a partial replacement for diet protein.

The aim of this study was to evaluate the feasibility of including FSM by-produced in the diets of Common carp, *Cyprinus carpio* on the growth performance, feed utilization, whole- body composition and physiological traits.

Materials and Methods

Fish experimental:

Common carp (*Cyprinus carpio*) fingerlings with an average 10.6 ± 0.15 g weights and 8.78 ± 0.14 cm length was obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa. They kept for 2 weeks in indoor tank as an acclimation period to the laboratory conditions. Fish fed on a commercial diet containing 30% crude protein.

Fenugreek seeds meal

Fenugreek seeds meal (FSM) was obtained from local market; it's prepared as by-product of oil extraction from fenugreek seeds by mechanical extraction as traditional method

Feed preparation

Four experimental diets were prepared in the laboratory. A control diet consisted from standard commercial diet without any treatment. The second, third and fourth diets containing fenugreek seeds meal (FSM by-produced) at a concentration of 10, 20 and 30% of the ration respectively, were mixed with the commercial diet. The ingredients of each diet were separately blended with additional 100 ml of water to make a paste. The pastes were separately passed through a grinder, and pelleted in a modified paste extruder to form the tested diets. The pellets were dried in a drying oven (Fisher oven 13 – 261 – 28A) at 85°C for 24 hours and stored in plastic bags and finally kept in a refrigerator at -2°C for further use. Experimental diets were formulated to meet the nutritional requirement of fish (NRC, 1993). The proximate chemical composition of the experimental diets is shown in table (1).

Table (1): Proximate chemical analysis (% on dry matter basis) of the experimental diets containing different levels of FSM by-produced meal.

Items	Control (Commercial diet)	10%	20%	30%
Dry Matter	92.33	92.22	92.01	92.05
Crude Protein	25.32	25.588	25.856	26.124
Crude Fat	8.01	7.343	6.676	6.009
Ash	5.62	5.758	5.896	6.034
Fiber	7.68	7.712	7.744	7.776
¹NFE	53.37	53.599	53.828	54.057
²G.E.(Kcal/ 100g)	437.8	433.9	430.1	426.0

¹NFE (nitrogen free extract) = 100 – (protein % + lipid % + ash % + fiber %)

²GE (gross energy) was calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

Feeding experiment

After 15 days of acclimation period in the stock culture tanks, clinically healthy (*Cyprinus carpio*) were divided into four equal groups at a rate of 10 fish/aquarium (75 – 60 – 45cm in diameters). Each aquarium was filled with dechlorinated tap water supplied with continuous aeration via air-stones using aquarium air pumps and a natural photo-period. About half of the water was changed daily in all experimental aquaria. Fecal matters were siphoned out once daily. The biomass of fish in each aquarium was measured at the beginning of experiment and after each sampling; thereby the daily ration was adjusted. Dead fish were daily recorded and removed. Fish were fed with their respective diets at the rate of 3% of their body weight per day for the period of the experiment. The daily ration was subdivided into two feeds.

At the end of the experimental period (3 months), the following parameters will be measured:

Chemical analysis of diets and fish

The tested diets and whole-fish body from each group at the beginning and at the end of the experiment will be analyzed according to the methods of (AOAC, 1990 and NRC, 1993).

Growth performance

Weight Gain (WG) = $W_2 - W_1$.

Where: W_1 = Initial body weight (g) and W_2 = Final body weight (g).

Specific Growth Rate (%) (SGR) = $[(\ln W_1 - \ln W_0) \div T] \times 100$.

Where: \ln = Natural log, W_0 = Initial body weight (g), W_1 = Final body weight (g) and T = Time (day).

Feed utilization parameters

Feed Conversion Ratio (FCR) = feed intake (g) / body weight gain (g).

Protein Efficiency Ratio (PER) = gain in weight (g) / protein intake in feed (g).

Condition factor (K)

$K = \text{weight} / \text{length}^3 \times 100$

Fish survival (%) = 100 (final fish number/initial fish number).

Physiological traits

Plasma was obtained by centrifugation of the blood at 3000 rpm for 15 min and the non haemolyzed plasma was stored in a deep freezer at -20°C till analysis. Total serum protein was determined following the protocol of Lowry (Lowry *et al.*, 1951) using standard protein estimation kit. Glucose concentration was measured according to Trinder (1969) using Boehring Mannheim kits. Lysozyme activity of fish plasma was determined by turbidometric assays as described by Caruso *et al.* (2002).

Statistical analysis

Statistical analysis was performed using the Analysis of variance (ANOVA) two way classification and Duncan's multiple Range Test, (Duncan, 1955) to determine differences between treatments means at significance rate of $P < 0.05$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis System (SAS) program (SAS, 2000).

Results and Discussion

Growth performance and feed utilization:

Fenugreek is "generally recognized as safe" (GRAS) as a flavoring by the U.S. Food and Drug Administration. Recent research demonstrated that FSM is a good source of compounds with a positive impact in human health.

Chemical analysis of seeds by-produced meal.

The chemical analysis of seeds by-produced meal was illustrated in (Fig, 1). In this connection Tonsy *et al.*, (2011) found that proximate analysis of Fenugreek indicated high crude protein (26.2%), crude fiber

(4.8%), fat (8.1%), ash (3.12) contents. Also **Mostafa et al (2009)**, reported that the ash and crude lipids contents for Fenugreek meal were 4.5% and 6.22%, respectively, while the crude protein and fiber contents were 29.11% and 9.31 %, respectively. FSM was found to be rich in protein, fat, total carbohydrates and minerals such as calcium, phosphorous, iron, zinc and magnesium (**Gupta et al., 1996** and **Sheikhlar et al., 2017**).

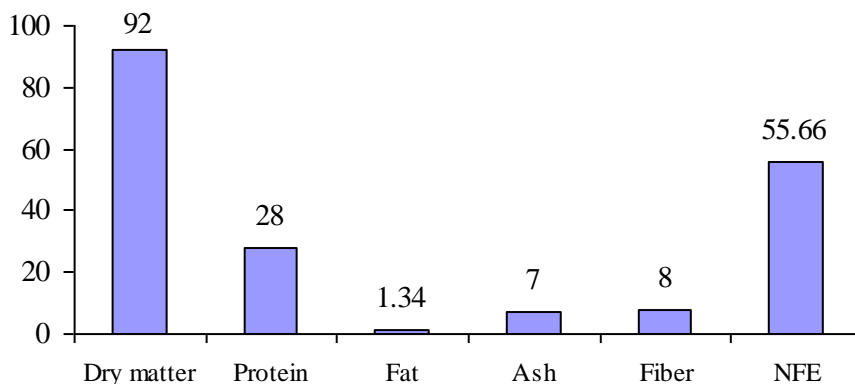


Fig (1): Chemical composition of FSM by-produced meal.

Growth performance

The growth performance and survival rates of the fish at the end of the experimental period are presented in Table 2. At the end of feeding trial, body weight gain was significantly high in all fish groups than in the control group. The body gain, condition factor and specific growth rate showed significant increase in the fed supplemented diets groups compared with control group. The positive growth promoting effects of FSM may be due to their chemical and physical properties. In this connection Syeed *et al.*, 2018 found that fenugreek seeds also improves the digestive enzyme activity and low metabolic needs, he add that fenugreek seed as an additive significantly improved weight gain and specific growth ratio as well as reduced mortalities of Common carp in the treated groups as compared to the control. Furthermore Roohi *et al.* (2015) studied fenugreek effect on common carp (*Cyprinus carpio*) and reported significant higher weight gain specific growth rate, and condition factor in treated than control, he add that no mortality was recorded during the feeding trial. Also these results are completely agreement wth those obtained by Basha *et al.*, 2018 who stated that Fish *O. niloticus* fed on FSM showed significant increase ($P < 0.05$) of all growth

parameters (ABW, WG, SGR, and L). Moustafa *et al.*, (2020) found that fenugreek supplemented diets could significantly increase the growth performance parameters of Nile tilapia as compared to the non-supplemented groups ($P < 0.05$).

Table (2): Growth performance and survival rates for Common carp fingerlings fed on different levels of FSM by-produced meal.

Item	Control	10%	20%	30%
Initial Weight	10.36±0.46 ^a	10.54±0.23 ^a	10.72±0.22 ^a	10.78±0.34 ^a
Initial length	8.8 ± 0.42 ^a	8.73±0.33 ^a	8.67±0.09 ^a	8.93±0.19 ^a
Final Weight	28.68±0.23 ^b	32.07±0.43 ^a	34.4±0.23 ^a	32.5±1.5 ^a
Final length	12.54±0.09 ^a	12.67 ±0.14 ^a	12.97±0.03 ^a	12.68 ±0.15 ^a
Weight gain	18.32±0.0.23 ^c	21.52±0.21 ^b	23.68±0.01 ^a	21.72±1.14 ^b
SGR	1.13±0.04 ^b	1.24±0.01 ^a	1.3±0.02 ^a	1.23±0.01 ^a
Condition Factor	1.46±0.04 ^b	1.58±0.03 ^a	1.58±0.02 ^a	1.49±0.02 ^a
Survival Rate	90 ^c	95 ^b	100 ^a	100 ^a

The same letter in the same row is not significantly different at $P < 0.05$.

In the current study fish survival in all fed supplemented diets groups was significantly high and ranged from 95 to 100% compared with control (90%), the increase in fish survival among the treated groups as compared to the control could be the result of immune system activation against various pathogens as well as opportunistic bacterial invaders (Syed *et al.*, 2018). Similar results were obtained by Sheikhar *et al.*, 2011 who reported that adding FSM to diet up to 30 % as substituted amounts with fishmeal showed significant optimum growth performance by African catfish. Also Tonsy *et al.*, 2011 studied the effects of diets supplemented with six different medical plants for Nile tilapia fry they found that supplementation level of 1 % FSM revealed significantly ($P \leq 0.05$) the highest growth performance parameters. Mehboob *et al.*, 2018 showed that Striped Catfish fry fed on diet containing FSM showed the highest final weight, weight gain, final body length and survival rate in comparison to control diet. On other hand Abdelhamid and Soliman, 2012 stated that no significant differences in final fish weights, weight

gain. Specific growth rate and survival rate of Nile tilapia fry (0.28 g) due to the dietary inclusion of fenugreek.

Feed utilization

Feed utilization of Common carp fed on different levels of FSM by-produced meal are shown in Table 3. Feed intake (FI) increased significantly, ($P < 0.05$), while FCR improved significantly in diets supplemented with different levels of FSM by-produced (Table 3). These results may be due to, the fenugreek seeds have a bitter taste (**Billaud and Adrian, 2001**), which might increase feed palatability and thus feed intake in fish. This is supported by **Syed et al., 2018** and **Roohi et al., 2015** they suggested that PER and FCR were significantly ($p < 0.05$) improved in Common carp groups fed with fenugreek based diet compared to the control. Similar results were recorded by **Tonsy et al., 2011**, **Basha et al., 2018** and **Moustafa et al., 2020** they found that found that fenugreek supplemented diets could increase PER and decrease FCR of Nile tilapia as compared to the non-supplemented groups ($P < 0.05$), feed consumption was higher in the FSM-fed Nile tilapia throughout the experimental period and the control group exhibited the lowest feed intake. In the present study fish fed 10 and 20% FSM by-produced gave significant increase in protein intake and PER than control diet. These results are in agreement with those obtained by **Sheikhlar et al., 2011** who reported that adding FSM to diet up to 20 % as substituted amounts with fish meal considering high nutritive value of FSM in compared to fish meal for African catfish. **Tonsy et al., 2011** indicated that supplementation level of 2 % medicinal plant revealed significantly the best feed and nutrient utilization parameters but feed intake was not significantly difference by all medicinal plant levels for mono sex Nile tilapia. More nearly results were obtained by **Antache et al., 2013** who stated that the best FCR and higher values PER were found by fenugreek (*Trigonella foenum graecum*), as compared to control diet at *Oreochromis niloticus*, with an average initial weight of 125.41 ± 34.33 g/fish. **Abdelhamid and Soliman, 2012** confirmed that Fenugreek Seeds had significantly (and proportional to the increase in its addition level) improved the feed utilization in form of protein productive value and energy retention.

Table (3): Feed utilization of Common carp fed on different levels of FSM by-produced meal.

Item	Control	10%	20%	30%
Feed Intake	44.82±0.21 ^a	47.28±0.81 ^a	46.74±1.21 ^a	47.43±1.38 ^a
FCR	2.45±0.25 ^a	2.2±0.21 ^b	1.97±0.08 ^c	2.19±0.18 ^b
Protein Intake	11.35±0.11 ^b	12.1±0.23 ^a	12.09±0.51 ^a	12.39±0.81 ^a
PER	1.62±0.05 ^c	1.78±0.18 ^b	1.96±0.11 ^a	1.75±0.23 ^b

The same letter in the same row is not significantly different at $P < 0.05$.

Body composition

Table 5. shows the whole-body composition of Common carp at the end of the experiment. No significant difference was observed in moisture contents for Common carp fed diets contains different levels of FSM by-produced. A significant increase in crude protein was noticed in the groups fed supplemented diets with FSM by-produced compared with the control one. A significant reduction in body fat contents was recorded in the fed supplemented diets groups compared with the control group, while ash content was not significantly differences between all diets groups or control diet. This improve in body composition may be due to the enhancement of fish health by FSM fed. Similar results were finding by **Mehboob *et al.*, 2018** revealed significant differences among FSM and control groups for crude protein contents while non-significant differences observed in crude fat, moisture, ash and fiber contents of Striped Catfish fry.. Also **Abdelhamid and Soliman, 2012** confirmed that fenugreek Seeds (at 2% addition level) had significantly increased Nile tilapia carcass protein percent. **Tonsy *et al.*, 2011** indicated that supplementation level of 1% different six medical plants for mono six Nile tilapia revealed significantly the highest CP %, EE % and energy content (Kcal /100g). On the other side, who added that the analysis of variance for all medicinal plant levels was not significantly differed in DM %. On other hand **Sheikhlar *et al.*, 2017** reported that adding FSM to diet up to 260 g/kg as substituted amounts with fish meal did not showed any significant difference on carcass composition of African catfish. Similarly **Mostafa *et al.*, 2009** showed that dry matter, crude protein, fat and ash in Nile tilapia body did not be affected by different FSM levels.

Table (4): Proximate chemical composition (% on dry matter basis) of Common carp fed diets containing different levels of FSM by-produced meal at the end of the experiment period.

Item	Start	Control	10%	20%	30%
Dry matter	19.55	25.54±0.7 ^b	27.12±0.1 ^a	28.88±0.1 ^a	25.15±0.3 ^{ab}
Crude Protein	62.36	64.01±1.0 ^b	65.95±1.0 ^{ab}	67.89±1.2 ^a	64.54±1.1 ^b
Ether Extract	17.61	18.09±0.1 ^a	16.59±1.3 ^b	17.29±1.2 ^a	17.31±0.3 ^a
Ash	16.83	15.89±0.3 ^a	15.51±0.2 ^a	14.69±0.1 ^a	15.53±0.1 ^a

The same letter in the same row is not significantly different at P < 0.05.

Physiological traits closed

The most portion of serum synthesizes in the liver and it can be used as an indicator of liver dysfunction. Also the determination of glucose concentration in blood serum is widely used as an indicator of stress in fish. Changes in levels of glucose in blood can be due to malnutrition or an injured kidney (**Jacobson-Kram & Keller 2001**). Lysozyme have important roles in the nonspecific immune defense system and it plays a major role in mediating protection against bacterial invasion (**Saurabh and Sahoo 2008**). Plasma glucose, plasma protein and lysozyme of Common carp as affected by different levels of FSM by-produced meal are shown in Table 5. The higher significant plasma protein (8.65) and Lysozyme (0.85) and lower plasma glucose (80.83) was obtained in diet containing 20%. FSM by-produced. Fish fed control diet gave the higher value of plasma glucose (114.13) and the lower value Lysozyme (0.53), while Fish fed 30 % FSM by-produced provided the lower value plasma protein (3.93). These results are in agreement with the results of **Syed et al., 2018** who reported that significantly higher ($p < 0.05$) serum protein and globulin levels were observed in treated fish groups over the control. Moreover, feeding fenugreek based diet resulted in a reduction in serum glucose in treated Common carp fish. Additionally, **Roohi et al. (2015)** studied fenugreek effect on common carp (*Cyprinus carpio*) and demonstrated that protein in the FSM groups increased significantly in comparison to the control group ($P < 0.05$). Furthermore, glucose levels were lower insignificant in fish fed on FSM compared to the control group ($P > 0.05$). The results of present study are in a partial agreement with results of **Mostafa et al., 2009** they stated that increased of plasma protein, while plasma glucose was no significant difference in the groups

fed supplemented diets with FSM compared with the control one for *O.niloticus*. **Basha et al., 2018** showed that decreased of Plasma glucose, while Plasma protein was no significant difference in the groups fed supplemented diets with FSM compared with the control one for *O.niloticus*. In respective to lysozyme activity the results of current study are in supported by **Moustafa et al., 2020** they found that fish fed fenugreek supplemented diets showed improved immune (lysozyme, immunoglobulin, and respiratory burst activity) and antioxidant parameters (superoxide dismutase and glutathione peroxidase and malondialdehyde). Moreover, **Sheikhlar et al ., 2011** found that lysozyme in catfish was only significantly increased ($p < 0.05$) in the FSM 180g/kg treatment, while plasma protein was no significant difference among the different treatments.

Table (5): Plasma glucose, plasma protein and Lysozyme of Common carp fed on ration containing different levels of fenugreek seeds by-produced meal.

Item	Control	10%	20%	30%
Plasma glucose	114.13±2.5 ^a	91.07±2.5 ^b	80.83±3.2 ^c	104±2.4 ^b
Plasma protein	4.73±0.57 ^{bc}	5.83±0.21 ^b	8.65±0.62 ^a	3.93±0.27 ^c
Lysozyme	0.53±0.09 ^b	0.65±0.08 ^{ab}	0.85±0.04 ^a	0.65±0.06 ^{ab}

The same letter in the same row is not significantly different at $P < 0.05$.

Conclusion

It could be concluded that, the use of fenugreek seeds meal in aquaculture can promote growth, feed utilization, body composition and physiological traits of fish. These benefits are important in aquaculture because it favor environmentally friendly organic production.

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تأثير كسب الحلبه على معدل النمو واستعمال الغذاء وتركيب الجسم وبعض الصفات الفيسيولوجية لأسماك المبروك العادى.

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الملخص العربى

قد أجريت هذه الدراسة لمعرفة مدى تأثير المكملات الغذائية من كسب الحلبه على النمو والاستفادة من الغذاء وتركيب الجسم فى اصباغيات أسماك المبروك العادى بمتوسط (١٠,٦ جم \pm ١٥,٠) وذات طول (٨,٧٨ سم \pm ١٤,٠). تم اجراء التجربة لمدة ١٢ اسبوع واستخدم ١٢ حوض زجاجى (ابعاد كل حوض ٧٥ \times ٦٠ \times ٤٥ سم) وتم عمل ثلاث مكررات لكل معاملة. تم تكوين أربعة علائق تجريبية تحتوي على ٠% (عليقه تجاريه)، ١٠، ٢٠ و ٣٠% من كسب الحلبه . جميع العلائق تحتوي على ٢٥ الى ٢٦ % بروتين خام , ٤٢٦ الى ٤٣٧ كيلو كالورى طاقة كلية لكل ١٠٠ جم .

اظهرت النتائج ان اضافة كسب الحلبه يحسن معنويا اداء النمو عن العليقه التجاريه, معامل الحالة و معدل البقاء زاد معنويا بزيادة مستوى كسب الحلبه فى العليقه حتى مستوى ٢٠%, ايضا معدل التحويل الغذائى تحسن معنويا بزيادة مسحوق كسب الحلبه فى العليقه حتى مستوى ٢٠%. الأسماك المغذاه كسب حلبه ٢٠% حسنت معنويا كفاءة البروتين ولكن لم يكن هناك اختلاف معنوى فى الغذاء المستهلك محتوى الجسم من البروتين والماده الجافه زاد معنويا بزيادة مسحوق كسب الحلبه فى العليقه حتى مستوى ٢٠%, ولكن اعلى محتوى جسم من الدهن كان فى العليقه المحتويه ١٠% كسب الحلبه فى العليقه, بينما محتوى الجسم من الرماد لم يتاثر معنويا باختلاف العليقه. ايضا المستوى ٢٠% كسب حلبه أعطى أعلى قيمه من بروتين البلازما واليزوزيم وأقل قيمة من جلوكوز البلازما , بينما العليقه التجاريه أظت أعلى قيمه من جلوكوز البلازما وأقل قيمه من اليزوزيم والعليقه المحتويه ٣٠% كسب حلبه اعطت اقل قيمه من بروتين البلازما. يمكننا التوصية بان اضافة ٢٠% من مسحوق كسب الحلبه يحسن من اداء النمو , الاستفادة من الغذاء , تركيب الجسم والحالة الفيسيولوجية لأسماك المبروك العادى.