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Effect of Adding Alfalfa Leaves Powder on Growth and Health Performance of Common Carp *Cyprinus carpio* L.

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ABSTRACT

This study has been done for evaluating the growth performance and health performance of young common carp *Cyprinus carpio* (average initial body weight 49.7653 gm) isoenergetic with isonitrogenous diets which contain six levels (0, 5, 10, gm of two types of alfalfa powder, have been used for a period of 105 days. By adding of alfalfa powder with 5 g Gask Alfalfa in the diets enhances ($P < 0.05$) in fish nutrient utilization with growth performance had compared with control diet (no alfalfa powder). The adding if the two type of alfalfa powder improves health performance as compared to the control in mean of Condition factor.

Keywords: Alfalfa leaves, growth performance, health parameters, common carp

INTRODUCTION

In aquatic feeds discovering alternate feed resources increased with having significant role like traditional ingredients which become less available and costlier (El-Sayed, 1999; Naylor, et el. 2000). in aqua feeds adding plant protein sources based on some factors such as nutritional benefits, protein quality, processing conditions, types and economic feasibility (El-Sayed, 1999).

Alfalfa contains protein per hectare more than other oilseed crops or grains; so it is very usable to pasture of livestock and hay production (Monteros and Bouton, 2009). Because of fibers, minerals, proteins richness and vitamins.

Alfalfa is a valuable animal feed which is add in the diet of livestock, particularly in ruminants. Its quality is measured by two main components: protein content with protein digestibility (Hill, *et al*., 1988).nutritional value of Alfalfa has been known by protein content that based on the share of leaves in dry matter yield that correlated with protein content directly (Julier, *et al*., 2001; Katic, *et al*., 2005).

There are a few knowledges about use of alfalfa powders in aqua feeds. The recent study was done to evaluate the use of alfalfa leaves powder in normal diets for common carp.

MATERIALS AND METHODS

Experimental fish: The experiment was done 84 days on 75 common carp *C. carpio* L. brought from local fish pondsin Daqoq/ Kirkuk/ Iraq. Fish weight varied between (48.144 -52.257 g). Fish distributed among experimental plastic tanks with mean initial weight 49.7653 g. laboratory pre-acclimation and feeding with commercial pellets (Percentage of chemical composition are clarified in Table 1 and 2) were for 21 days prior to the real feeding trials.

Table 1. Chemical composition of the different diet by NRC. (1993).

Ingredients	Crude Protein %	Crude Fat %	Dry Matter %	Crude Fiber %	Energy Kcal/ kg
Animal protein Concentrate	40	5	92.9	2.2	2107
Yellow corn	8.9	3.6	89	2.2	3400
Soybean meal	48	1.1	89	7	2230
Barely	11	1.9	89	5.5	2640
Wheat bran	15.7	4	89	11	1300

Table 2. experimental diet Composition.

Ingredients	Percentage (%)
Yellow corn	15%
Wheat bran	18%
Soya bean meal 48%	40%
Concentration protein	10%
Barley	15%
Vitamins + Minerals Mix	2%
Total	100
Calculated chemical composition	
Crud protein	28.06
Metabolizable energy (kcal/kg feed)	2242.7
%Arginine	0.2394
%Lysine	0.25375
%Methionine + cysteine	0.12872
%Threonine	0.017
%Tryptophan	0.029

Experimental system: in this trial for five treatments Fifteen plastic tank (100 L) had used each with having three replicates, used water quality parameters seen in (Table 3). Proper continuous aeration added to each tank by using Chinese's air compressors, Hailea ACO-318 (power: 45 watt, air flow: 70L/min), Hailea ACO-328 (power: 55 watt, air flow: 82L/min), Resun ACO-010 (power: 200 watt, air flow: 0.135 m3/min) and eleven small aquarium air pumps, Luckiness 828 (power: 5 watt, air flow: 3.5L/min). five fish stocked in each replicate. Then randomly replicates were putted for reducing differences among treatments. siphoning method as a daily cleaning applied for removing remained feces and feeds from the system. The experimental trial represents five treatments with three replicates; each with five fish per replicate as bellow:

T1: Commercial diet without any supplement, T2: adding 5 g Gask Alfalfa/ kg diet, T3: adding 10 g Gask Alfalfa/ kg diet, T4: adding 5 g Iranian Alfalfa/ kg diet, T5: adding 10 g Iranian Alfalfa/ kg diet.

Table 3. Water quality parameters within the tanks.

Parameters	Values
Temperature (°C)	28±1
Dissolved Oxygen (mg/L)	4±1
Hardness (ppm)	330±10
pH	7±0.5
Total Dissolved Solids (TDS) (ppm)	230±10

Alfalfa farming: Alfalfa seeds bought from commercial local markets in Sulaimani; Iranian and Gask alfalfa. Planting done in July/ 2017 till September/ 2017 for obtain needed amount of alfalfa for fish feeding experiment. After harvesting the alfalfa were cutting and sunlight drying to make the powder.

Diet formulation: Experimental diets contain standard ingredients found in Sulaimani City markets, enriched with alfalfa powder. The items were mixed to obtain dough. Next for pelleting electrical mincer had used by Kenwood Multi-processors. Room temperature drying applied for a four days with crushing to gain fine particles. In two-time per a day at10:00 a.m. and 3:00 p.m feeding applied with 3% of body weight. In every tank bimonthly fishes were weighed together. Then levels of feeding were recalculated depending on new weights. For 12 weeks the feeding trial was continued.

Feed utilization parameters and Growth: Fish weighed (g) together for all replicate every two weeks. Feed consumption of each replicate will readjust by the obtained biomass at every two weeks.

Weight gain (g/fish) = Mean of weight (g) at the end of the period, weight (g) at the starting of the experimental period.

Weight gain (g/fish) = $W_2 – W_1$

Where:

W_2: Fish weight (g) at the end of experimental period

W_1: Fish weight (g) at the starting of the experimental period

(DWG) (g/day) weight gain daily = Weight Gain at experimental period = $W_2 – W_1$ / T

T: time between W2 and W1 (84 days)

Relative growth rate (RGR %) = Weight Gain / Initial weight × 100 = $W_2 – W_1$/ W_1 × 100 (Brown, 1957).

(SGR) Specific growth rate = (Final body weight- Initial body weight] /experimental period) ×100 = [(In W_2 – In W_1) / T] × 100 (Lagler, 1956).

Feed conversion ratio (FCR) = Total feed fed (g) / total wet weight gain (g) (Uten, 1978).

Feed efficiency ratio (FER) = Total weight gain (g) / Total feed fed (g) (Uten, 1978).

Protein efficiency ratio (PER) = Total wet weight gain (g/fish) / amount of protein fed (g/fish) (Uten, 1978).

Health (Biological) parameters:

All fish specimens will be dissected and the abdominal cavity was opened to weigh each organ alone, and they calculated as follows (Lagler, 1956).

Intestine weight index % = Intestine weight (g)/body weight (g) × 100

Intestine length index % = Intestine length (g)/body length (g) × 100

Condition factor= Fish weight / Body length^3

Statistical analysis:

The trial was conducted by using (ANOVA) with completely randomized design (CRD) with general linear models (GLM) procedure of XLSTAT 2016 Version 02.28451. Duncan's test had been applied for comparing among treatments means.

RESULTS AND DISCUSSION

According the results which is shown in table (4) the 5 g Gask Alfalfa/ kg diet had notably higher than other treatments followed T5 with 10 g Iranian Alfalfa/ kg diet in weight gain and Relative Growth Rate. There are no notable differences recorded in each of Specific Growth Rate and daily Growth Rate

Table 4. Effect of adding Gask and Iranian Alfalfa leaves powder in different levels on common carp *Cyprinus carpio* L. growth performance.

Treatments	Weight Gain	Daily Growth Rate	Specific Growth Rate	Relative Growth Rate
T1 Control	83.465 cd ± 6.97	0.994 a ± 0.08	0.152 a ± 0	34.137 b ± 2.58
T2 5 g Gask Alfalfa/ kg diet	135.925 a ± 11.64	1.618 a ± 0.49	0.218 a ± 0.05	55.152 a ± 16.68
T3 10 g Gask Alfalfa/ kg diet	89.656 c ± 3.13	1.067 a ± 0.03	0.157 a ± 0	35.520 b ± 1.89
T4 5 g Iranian Alfalfa/ kg diet	73.810 e ± 5.91	0.879 a ± 0.07	0.132 a ± 0.01	29.177 c ± 2.66
T5 10 g Iranian Alfalfa/ kg diet	90.005 b ± 10.48	1.071 a ± 0.12	0.161 a ± 0.01	36.703 b ± 5.16

Mean values and several superscripts within a column vary significantly (P≤0.05).

Adding of 25% and higher alfalfa meal reduced goldfish growth. The impact of alfalfa on goldfish growth did not reported yet; however, for some other fish species have different results. The dietary alfalfas level impact on growth had been measured as 7% for *Diploduspuntazzo* (Chatzifotis *et al*., 2006), 5% for *Oreochromis aureus* (Yousif *et al*., 1994), and 10% for *Oreochromis niloticus* (Ali *et al*., 2003) and these goes parallel with the present results that decreased growth performance.

Table (5) showed the effect of adding Gask and Iranian Alfalfa leaves powder in different levels on common carp *C. carpio* L. of Food Conversion Ratio in which the T4 with 5 g Iranian Alfalfa/ kg diet was clearly higher than other treatments. Protein efficiency Ratio in the T2 with 5 g Gask Alfalfa/ kg diet was notably higher than other treatments. There is not any visible difference in Food Efficiency Ratio among treatments.

Alfalfas negative impact on feed utilization and fish growth results on some reasons in their diet such us high cellulose level or antinutritional components. Diets enriched with alfalfa can effect and include cellulose content elevation, When the cellulose levels ranged from 4-10%. raised level of dietary cellulose had been demonstrating to have impact fish growth (Al-Asgah and Ali, 1996), adversely nutrient digestibility (Kirchgessener *et al*., 1986) also Alfalfa include some antinutritional factors such as saponins, phytoestrogensor, protease inhibitors, and antivitamins (Francis *et al*., 2001).

Table 5. Effect of adding Gask and Iranian Alfalfa leaves powder in different levels on common carp *Cyprinus carpio* L. feed utilization.

Treatments	Food Conversion Ratio	Food Efficiency Ratio	Protein Efficiency Ratio
T1 Control	8.128 b ± 0.49	0.124 a ± 0	2.975 c ± 0.24
T2 5 g Gask Alfalfa/ kg diet	6.969 c ± 1.76	0.178 a ± 0.04	4.844 a ± 1.48
T3 10 g Gask Alfalfa/ kg diet	7.800 bc ± 0.31	0.129 a ± 0	3.195 b ± 0.11
T4 5 g Iranian Alfalfa/ kg diet	9.369 a ± 0.74	0.109 a ± 0	2.631 c ± 0.21
T5 10 g Iranian Alfalfa/ kg diet	7.947 bc ± 0.9	0.131 a ± 0.01	3.208 b ± 0.37

Mean values with several superscripts in a column vary significantly (P≤0.05).

It can say that Researchers not recommend to add alfalfa meals to somefish species diets. However, it used at 35% of the diet in *Oreochromis mossambicus* (Olvera-Novoa *et al*., 1990). And 25% of freshwater crayfish, *C. quadricarinatus* diet (Harpaz *et al*., 1998).

The different results during studies cause of n digestion physiology variation in fish species and in alfalfa processing technology. Goldfish has long digestive tract which allow efficient digestion extraction in plant ingredients, so by this fish alfalfa could be better utilized. Having more inclusions of alfalfa (40%) leads to higher FCR in goldfish, so recommending less utilizing at like these high levels.

Same results were mentioned at 20% in O. aureus (Yousif *et al*., 1994), 45% inclusion level in *O. mossambicus* (Olvera-Novoa *et al*., 1990), 10% in *O. niloticus* (Ali *et al*., 2003) and 21% in *D. puntazzo* (Chatzifotis *et al*., 2006) all of them has same results as in the present results.

the presence of high trypsin inhibitory activity results of *O. mossambicus* fed high concentration (N45%) of alfalfa having high level of alfalfa in the diet leads of an imbalance of the essential amino acids profile (Hood *et al*., 1981).

For clarifying this, lysine and methionine added to diets as supplementation, potentially limiting amino acids. Vary from trout and salmon (Yanar *et al*., 2008).

All results clarify that goldfish were able for utilizing zeaxanthin from alfalfa and lutein (Yanar *et al*., 2008). In Goldfish same observations have seen while feeding different natural carotenoid sources, like *C.vulgaris, H. pluvialis* , red yeast, Spirulina (Kiriratnikom *et al*., 2005), *X. dendrorhous* (Xu *et al*., 2006), and A. maxima (Gouveia *et al*., 2003; Gouveia and Rema, 2005).

Only other aquatic animal seen for utilizing dietary alfalfa for pigmentation was The juvenile freshwater crayfish (Harpaz *et al*., 1998). For the result it can said that inclusion of alfalfa powder over 5% in diets decreased (P <0.05) growth performance. Results indicate that in common carp feed utilization when compared to the control diet (No alfalfa powder). In the current study diets were used isoenergetic and isonitrogenous. Experimental diets chemical composition clue that that raising alfalfa level of meal. In all the diets Lysines level methionine level was the same and get the essential amino acids requirement of common carp (NRC, 1993).

Fish growth performance declining, very poor utilizations diet with raising levels of Alfalfa powder in the feed diet due to some factor like protease inhibitors, and anti-nutritional factors and crude fibers high level. physiological and nutritional role of the tannin content in alfalfa diet (Liener 1989) could have a critical role on nutrient utilization and growth depression, even it has little information about the impact of tannins on fish (Becker and Makkar 1999).

Diets had not have any the presence of protease inhibitors, deficiency of essential amino acids and other anti-nutritional substances in alfalfa meal might be causes of subsequently the absorption of amino acids with poor protein digestibility. Climbing crude fibers level in fish diet exert a negative impact on the nutrient digestibility (Kirchgessener 1986) and may cause fish growth depression (Al-Asgah and Ali 1996).

In the study of Olvera-Novoa *et al*. (1990) feed intake, Weight gain nitrogen deposition and specific growth rate have been better in *O. mossambicus* were while alfalfa protein inclusion level was low (15-20%). (cytoplastic) purified in leaves protein concentrate have great performance compare protein concentrate of crude (chloroplastic) in leaves. A decreased in growth rate and nutritional parameters observed by Yousif *et al*., (1994). In the tilapia (*O. aureus*) meal raising of dehydrated alfalfa powders levels as shown in the present results so they did not suggest using dehydrated powder of alfalfa in tilapia meal. Our results clarified that diet with inclusion different levels of alfalfa powder produced comparable results for manipulate diet however decreased growth while having higher levels include. Using Chinese blunt snout bream has alfalfa meals better utilization compare by using Tilapia may be results in species variation which has refer to have long intestine tract that permit the creating polysaccharide decomposing microflora who have high efficiency which probably positively impact on some apparently indigestible feed ingredients digestion (Viola and Arieli 1983).

In the diets using of alfalfa meal more than 5% decreased both the feed intake and the efficiency of utilization of nutrient. Having bad growth performance with having bad diets utilization at alfalfa meal level 20% in tilapia diets could be clarified in the context. Exogenous and endogenous factor roles at the same time effect on efficiency of transfer nutrients from feed to fish (Shearer1994).

Did not notable differences observed in Intestine weight and Length Index, T2 and T4 were higher significantly in Condition factor as demonstrate in Table (6).

Table 6. Impact of adding Gask and Iranian Alfalfa leaves powder in different levels on common carp *C. carpio* L. some biological indices.

Treatments	Intestine weight Index	Intestine Length Index	Condition factor
T1 Control	3.168 a ± 0.05	1.820 a ± 0.07	1.457 b ± 0.04
T2 5 g Gask Alfalfa/ kg diet	3.225 a ± 0.06	1.660 a ± 0.02	1.644 a ± 0.04
T3 10 g Gask Alfalfa/ kg diet	3.034 a ± 0.14	1.600 a ± 0.1	1.397 b ± 0
T4 5 g Iranian Alfalfa/ kg diet	3.221 a ± 0.04	1.829 a ± 0.09	1.499 ab ± 0.04
T5 10 g Iranian Alfalfa/ kg diet	2.816 a ± 0.22	1.573 a ± 0.21	1.156 c ± 0.08

Mean values with several superscripts in a column vary significantly (P≤0.05).

Adding of low levels of alfalfa in the meal enhances (P<0.05) utilization of nutrient with growth performance in fish while compared with control diet, and improving health performance as compared to the control by mean of biological parameters as the alfalfa leaves as feed additives for giving more time for fish to digest their feeds.

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تأثير إضافة مسحوق أوراق البرسيم في النمو والأداء الصحي لأسماك الكارب العادي *Cyprinus carpio* L.

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أجريت هذه التجربة لتقييم النمو والأداء الصحي لأسماك الكارب العادي (بمتوسط وزن 49.7653 جرام) باستخدام تراكيب علفية تحتوي على ثلاث مستويات (0 ، 5 ، 10 جرام) من مسحوق البرسيم بكلا النوعين المحلي والايراني و لمدة 105 يومًا. عزِّز إضافة مسحوق البرسيم مع 5 جرام من البرسيم المحلي أداء النمو (P<0.05) واستهلاك العلف مقارنةً بالعليقة القياسية (بدون إضافة مسحوق البرسيم). عمل مسحوق البرسيم بكلا النوعين (المحلي والايراني) على تحسين الأداء الصحي مقارنةً بالقياسية في متوسط عامل الحالة.