

## Culture of Chinese carps using anaerobic fermented cow manure (Slurry) and comparison of survival and growth factors versus traditional culture

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Received: July 2011 Accepted: March 2012

### Abstract

The objective of this project is to compare growth and survival rate in Chinese carps reared under traditional culture conditions versus those reared in ponds treated with slurry (anaerobic fermented of cow manure). This experiment was conducted using two treatments; one treatment using slurry and the other using cow manure plus chemical fertilizer as the control with three replicates for each. Chinese carp were stocked at the density of 2375 individuals/ha ( Silver carp 60%, Bighead 15%, common carp 17% and grass carp 8%). In this investigation common carp and grass carp were fed with formulated diets and fresh grass, respectively. The survival rate in the slurry treatment was higher than that in traditional treatment. Survival rates were 98, 100, 84 and 52 percent for silver carp, bighead, common carp and grass carp, respectively in the slurry treated ponds and 96.0, 98.3, 82.8 and 20.0% percent in the control ponds. The results showed that survival rate and yields were higher in the slurry treated ponds than that in the control. The increasing percent of yield were 13.5, 2.6, 18.4 and 85.3 in silver carp, bighead, common carp and grass carp, respectively. The survival rate for grass carp was two times higher in the slurry treatment than control. Zooplankton abundance in slurry ponds was higher than that in control, but blue-green algae density in slurry treated ponds was less than (over 50%). In general the results indicate that slurry with higher nutritional content is more effective on the survival and growth rate of fishes and also is more efficient in the proliferation of plankton in particular zooplankton. Slurry also reduces the use of chemical fertilizers.

**Keywords:** Slurry, Chinese carp, Cow manure

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## Introduction

The Chinese carp species, silver carp, *Hypophthalmichthys molitrix*, bighead, *H.nobilis*, grass carp, *Ctenopharyngodon idella* are prevalent in fish farms among the other aquatics mainly in three Iranian provinces i.e Guilan, Mazandaran and Golestan in the coast of Caspian Sea.

To increase the survival and quality of larvae, fingerlings and fry, the presence of live food such as rotifera, other zooplankton and phytoplankton species, is essential especially in the first 2-3 weeks of their development. Hence, the use of fertilizers, which is cost effective, is the most effectual way to increase live food production in ponds. By feeding the organic manure (cow dung) to biogas plants a substance called is produced during the process of digestion. Slurry used in fish farms increases live food production which nurtures fingerlings and helps in feeding fish in grow out ponds. Slurry helps in fertilizing fish ponds and is effective from the economic point of view.

Nowadays the success of aquaculture activity lies in the use of suitable and cost-effective feeds. This also holds good in the case of Chinese carps like silver carp and bighead which are highly dependent on primary and secondary production. At present chemical fertilizers are used extensively for the production of phytoplankton and zooplankton species. These fertilizers not only have a negative impact on the environment but also are not suitable for

species like grass carp. The excessive use of such fertilizers promotes phytoplankton blooms, which are classified under undesirable species.

Lobzens et al. (1989), Lavens et al. (1991), and Watanabe et al. (1998) have studied the effect of live food specially rotifers on larvae of *Cyprinus carpio* . Their studies indicate that feeding the larvae in their first 30 days of development with live food had significant effects on their growth and development.

The use of biogas for fish farming has begun since 1970 (Kangmin and Qiuhua, 2000). China has a long history of pond fertilization using chicken, duck, pig and cow manure. By adopting the traditional fish farming the added compost to the ponds may increase the proliferation of natural organisms which serve as natural food for fish from juvenile to adult. However, this kind of practice is getting criticism from the point of view of fish health and hygiene. To overcome this, scientists have encouraged anaerobic digestion of manure. Slurry produced during this process of digestion is used to fertilize fish ponds. The results indicated that net fish yield reached 12t/ha in comparison to a much lesser yield (3.4 h/t) using chicken compost (Kangmin and Qiuhua, 2000). Kangmin and Quihua (2000) studied the survival rate of fingerlings cultured both by the traditional and the slurry method. It was found that the survival rate of the fingerlings of different species of carps in the slurry method was between 76-96 % whereas in

the traditional method the survival rate of the same fingerlings had a record of not more than 14-36 %.

This study mainly aims at treating carp ponds with slurry to increase the survival rate of fingerlings in the growout stage, comparing with the traditional method used for farming Chinese carps and develop a protocol for the use of suitable proportions of slurry to obtain suitable growth and survival rate of Chinese carps in the grow out stage.

### Materials and methods

Preparation of slurry and ponds, introducing larvae and larval culture. Preparation of slurry began on 9th April 2009 in fiberglass tubes. For this purpose cow dung was fed into a biogas system for digestion. Six ponds (each pond 700 m<sup>2</sup>) were selected in the Siahkhal Hatchery and were filled to their capacity with water

after the application of base fertilizer up to May 2009.

Due to the accumulation of sludge, the bottom of the ponds were not plowed and tilled. Carp fingerlings were introduced into the ponds after one week since no empty ponds were available in the hatchery. The fingerlings were stocked at a rate of 2375 individuals per ha and the species combination consisted of silver carp 60% (60 g), grass carp 15% (15 g), common carp 17% (30 g), and bighead 8% (60 g).

Pond numbers 1, 3 and 5 were randomly selected for slurry treatment and pond numbers 2, 4 and 6 were used as control. Commonly used formulated feed components (Table 1) were used for the culture of carp species in this study. In addition to formulated diets, fresh forage was also given to grass carp a few days after the beginning of their culture.

**Table 1 : formulated feed components for common carp**

1	Meal bean, Cotton seeds, Sun flower seeds, etc.	40-50%
2	Cereals (Barely, Corn, etc.)	25 %
3	Fish meal or blood flour, Silk worm pupa	10%
4	cereal, Rice, Corn and remaining flour mill	20%
5	Peas, Vetch, Lentils and Beans	5 %

The growth rate was calculated with the following formula:

$$DWG \text{ (g/day)} = (W_t - W_0)$$

$W_0$  = Primary Weight,  $W_t$  = Final Weight,  $t$  = Experiment Duration

$$DLG \text{ (m/day)} = (L_t - L_0)$$

$L_0$  = Primary Weight,  $L_t$  = Secondary Length

$$Sgr = 100(\ln(W_t) - \ln(W_0))/t \text{ (Bangal, 1978)}$$

$W_t$  = Final Weight,  $W_0$  = Primary Weight,  $t$  = Duration of Experiment

### *Chemical analysis of slurry and untreated cow dung*

Comparison of inorganic substances in slurry and cow dung manure revealed that calcium, phosphorus and potassium content of slurry were 1.26, 2.25, and 366 times higher than that in cow dung respectively. The inorganic content in ash

residue of slurry was 1.08 times higher when compared to cow dung compost (Table 2). In fact, protein and lipid contents were 1.45 and 1.19 times more than the raw manure.

**Table2: Chemical composition of cattle dung before and after digestion**

sample	Chemical composition (dry weight %)					
	potassium	phosphor	calcium	Raw lipid	Raw ash	Raw protein
<b>Slurry ( cattle dung after anaerobic digestion)</b>	2.11	0.80	2.24	1.71	54.72	15.13
<b>Raw cattle dung</b>	0.58	0.48	1.77	1.43	50.43	10.40

### *Physical and chemical parameters of the fish ponds- Sampling of Phytoplankton and Biometry:*

Physical and chemical parameters of temperature, nitrate ( $\text{NO}_3$ ), ammonium ( $\text{NH}_4$ ), orthophosphate ( $\text{PO}_4$ ), total hardness and carbon dioxide ( $\text{CO}_2$ ) were measured in the ponds once every four night but parameters like dissolved oxygen (DO), and pH were measured almost on a daily basis (APHA, 1989). Phytoplankton sampling also was performed every week. Identification of phytoplankton and zooplankton species was carried out following identification keys (Edmonson, 1959; Prescott, 1970; Boney, 1989). Biometry of fingerings was performed once. Due to drought conditions the water

level in the ponds could not be reduced. Hence, biometry of many fished was not performed. However, after completing the project biometry of all the fishes was conducted.

### *Examination of stomach and intestine*

Stomach and intestine contents of the 4 carp species in both treatments were examined under a dissecting microscope.

### *Carcass analysis for protein and lipid*

Measurement of protein and lipid in fish carcass was done using micro Kjeldahl and Soxhlet methods, respectively according to official methods of analysis (AOAC, 1998).

### *Statistical analysis*

The results were analyzed by the non-parametric Kruskal- Wallis test (as the data were severely non-normal) suggest significant differences in length of carps between the control and slurry treated ponds ( $P < 0.05$ ).

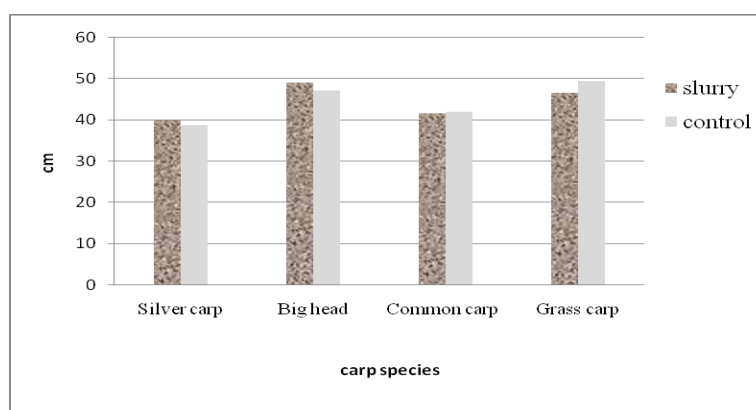
## Results

### Results of growth rate studies of fish

At the end of the rearing period a comparison of mean length of the carps in the control and slurry treatment was made. Mean length for silver carp (39.82 mm) and bighead (49.11 mm) was higher in the slurry treatment was higher while that for common carp (41.97 mm) and grass carp (49.57 mm) was higher in the control treatment (Table 3, Fig.1).

**Table 3:** Results descriptive statistics of Chinese carp length in ponds and different treatments

Treatment	Descriptive Statistics	Silver carp	common carp	Big head	grass carp
control	Min	34	34	39	43
	Max	46	50	53	59
	Average $\pm$ SD	38.8 $\pm$ 2.01 b	41.7 $\pm$ 2.01 a	47.3 $\pm$ 3.1 b	46.2 $\pm$ 3.51 a
slurry	Min	33	35	40	38
	Max	45	50	56	54
	Average $\pm$ SD	39.8 $\pm$ 2.09 b	41.6 $\pm$ 2.91 a	49.1 $\pm$ 3.95 a	49.5 $\pm$ 4.43 b



**Fig. 1:** Comparison of mean final length in Chinese carps in slurry and control treatments

Mean weight for silver carp, bighead; common carp and grass carp in the control group at the time of harvest was 589.4 g, 8.6 g, 1223.1142 g and 1612.5 g, respectively. However, the mean weight of

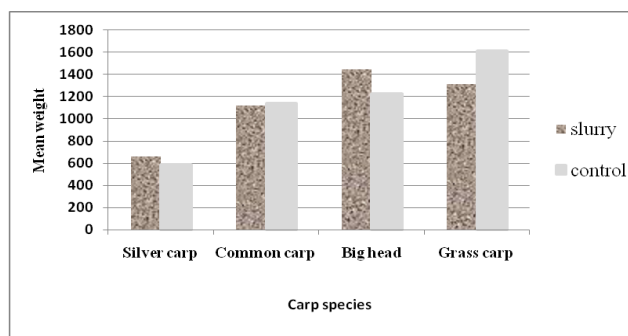
silver carp (659.7 g), bighead (1119.26 g) and common carp (1450.4 g) in the slurry treated group was higher when compared to the control group (Table 4 , Fig. 2) except in the case of grass carp (1113.8 g).

This is mainly due to their lower survival rate in the control group. The results showed that survival rate and yield were

higher in slurry than control. The increasing percent of yield were 13.5, 2.6, 18.4 and 85.3.

**Table4:** Results descriptive statistics of Chinese carp weight in ponds and different treatments

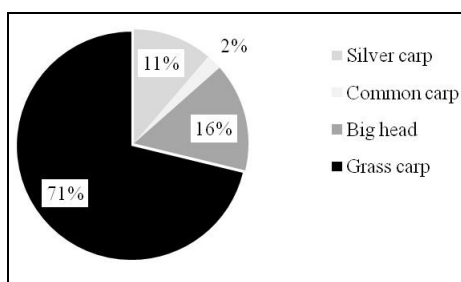
Treatment	Descriptive Statistics	Silver carp	common carp	Big head	grass carp
control	Min	378	624	713	899
	Max	449	1764	1670	2700
	Average $\pm$ SD	589.5 $\pm$ 100.33 b	1113.9 $\pm$ 309.37 a	1222.9 $\pm$ 228.17 b	1603.7 $\pm$ 480.58 b
slurry	Min	380	659	840	652
	Max	967	1708	2096	1929
	Average $\pm$ SD	659.6 $\pm$ 104.49 b	1125.3 $\pm$ 241.26 a	1448.2 $\pm$ 324.28 a	1295.3 $\pm$ 299.2 a



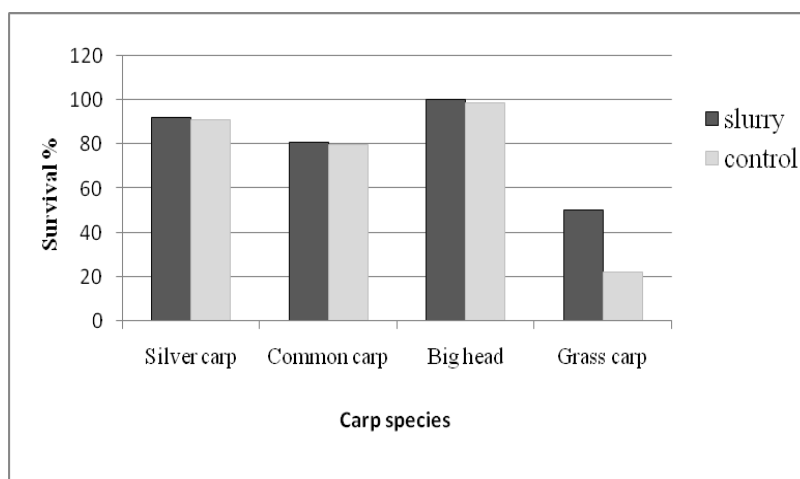
**Fig. 2:** Comparison of mean weight in Chinese Carps in slurry and control treatments

In Silver carp, Big head, common carp and grass carp respectively (fig.3). The effect of biogas slurry on the survival coefficient of carps was also studied. The survival coefficients of silver carp, big head, common carp and grass carp were 84, 100, 97 and 52 %, respectively in the slurry treatment, while that in control group were 96, 82.8, 98.3 and 20%, respectively (Fig.

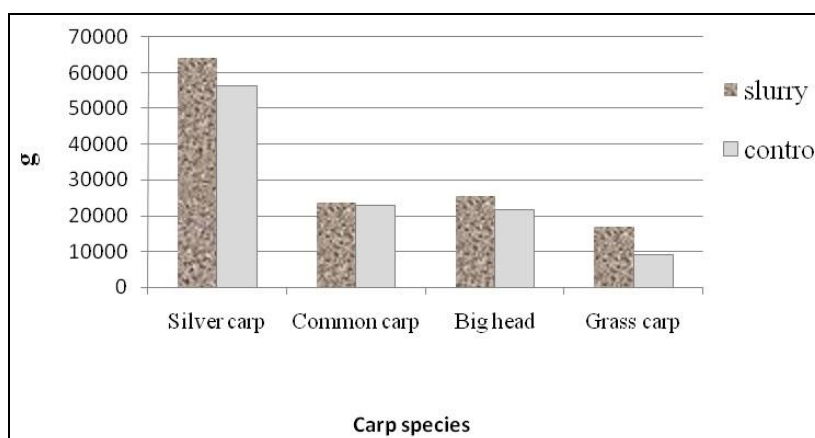
4). Higher harvest per ha was obtained for carps in the slurry treatment (1.858 tons) compared to that (1.573 tons) in control group (Fig.5). The estimated specific growth rates (SGR) for silver carp, bighead, common carp and grass carp were 1.558, 2.065, 2.35 and 2.9 % respectively in the slurry treatment (Fig. 6).



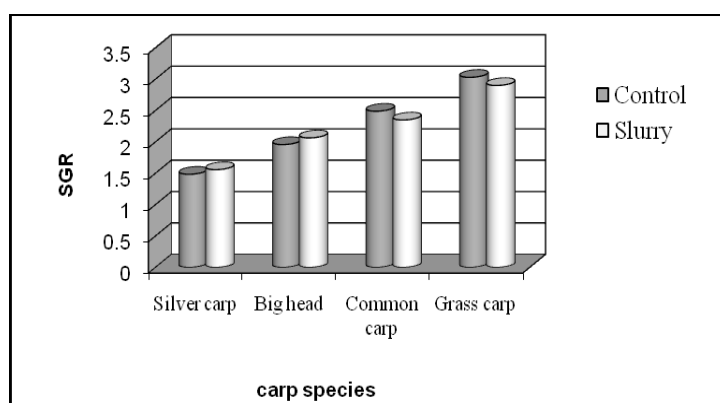
**Fig. 3: Comparison of weight gain (%) in Chinese carps in slurry and control treatments**



**Fig. 4: Comparison of survival % in Chinese carps in slurry and control**



**Fig. 5: Total production in Chinese carps in slurry and control treatments**



**Fig. 6: Comparison of SGR in carp species in control and slurry treatments**

To account for the daily weight gain in both slurry treatment and control the daily weight gain (DWG) was calculated at the end of the rearing period. The results indicated a higher DWG for silver carp (4.893) and bighead (9.029) in the slurry treatment, while DWG for common carp and grass carp were higher in the control group. Results on the daily length gain (DLG) of silver carp and bighead were higher in slurry treatment at the end of the rearing period, whereas DLG for common carp showed a very slight difference in slurry and control groups. DLG values for grass carp were higher in control group and this may be due to their low survival rates in the slurry treatment.

#### *Carcass analysis*

Comparison of total body protein content in silver carp in both the slurry treatment and control revealed higher carcass protein content (19%) in the slurry treatment than in the control (18.40 %).

The same comparison was even made for total body lipid content in silver carp which was again higher in the slurry treatment (1.09 %) than in the control (0.94 %). In bighead carcass protein and lipid contents were estimated to be higher in the slurry treatment when compared to control (Table 5).

Carcass protein and lipid content in common carp was slightly higher in the control (Table 5) (Fig. 3.24 and 3.25). And finally in grass carp the protein and lipid content were higher in the slurry treatment.



**Table 5: carcass (dry weight %) analysis in Chinese carps in slurry and control treatment stomach content analysis:**

% component	grass carp (control)	grass carp (slurry)	common carp (control)	common carp (slurry)	big head (control)	big head (slurry)	silver carp (control)	silver carp (slurry)
protein	16.27	16.46	17.91	17.55	17.77	18.09	18.40	19.0
lipid	1.46	2.06	2.62	1.75	0.45	0.71	0.94	1.09
water	80.80	81.31	77.78	80.75	80.80	80.72	80.33	80.12
ash	1.1	1.05	1.08	0.99	1.18	1.06	1.13	1.13

The results of stomach content in silver carp in the control indicated a higher consumption of *Oscillatoria* compared to other algae and a small amount of green algae (*Tetradraedron* and *Ankistrodesmus*), diatoms (*Synedra*, *Diatoma*, *Nitzschia*, *Navicula*, *Gomphonema*, *Achnanthes*), and dinoflagellates (*Gymnodinium*) were seen. At the anterior part of the intestine a significant amount of *Oscillatoria* and a small amount of *Gymnodinium*, *Synedra*, and *Ankistrodesmus* was seen.

Analysis of stomach content in silver carp in the slurry treatment demonstrated a significant amount of diatoms like *Synedra*, *Cymbella*, *Gomphonema*, *Nitzschia*, *Cyclotella*, *Achnanthes*, *Navicula*, *Diatoma*, *Cocconeis* and *Rhopalodia*, whereas

in the anterior portion of the intestine only a small amount of diatoms were found.

Among green algae *Scenedesmus*, *Tetradraedron*, and *Ankistrodesmus* species were dominant, whereas among dinoflagellates, *Gymnodinium* species was found in the stomach content. A very small amount of *oscillatoria* was seen in the stomach content of silver carp treated with slurry. *Euglena* spp and *Phacus* belonging to *Euglenophyceae* were reported in the stomach contents of silver carp. Examination of stomach content of bighead in the control showed the presence of rotifers (*Brachionus* spp and *Cephalodella* spp) and *Copepoda* (*Cyclops* spp), whereas in carps in the slurry loaded ponds a significant amount of rotifers (*Brachionus*, *Cephalodella*,

*Aschomorpha*, *Monostyla*, *Philodina*, *Anuraeopsis*) and Nauplius of copepoda and Cyclops were observed. It was observed that the culture ponds treated with slurry produced more zooplanktons compared to the control ponds.

*Relationship of Weight-length in silver carp control and slurry treated ponds*

After performing t-test, a significant difference (Table 6) between length and weight of silver carp, bighead and grass carp in both the slurry loaded and control ponds was confirmed ( $P < 0.005$ ). However, no significant difference was observed in the length and weight of common carp ( $P < 0.005$ ).

**Table 6: statistical analysis on mean length and mean weight in Chinese carps in slurry and control treatment**

species	parameter	pond						treatment	
								slurry	control
Silver carp	length	a	c	b	c	b	c	a	b
	weight	a	c	b	c	b	c	a	b
Common carp	length	b	a	bc	bc	b	c	a	a
	weight	b	a	b	b	ab	b	a	a
big head	length	bc	c	ab	bc	a	bc	a	b
	weight								
grass carp	length	abc	abc	c	ab	bc	a	a	b
	weight	bc	abc	c	b	c	a	a	b

*Physical and chemical analysis*

The result of Physical and chemical analysis was shown in the table 7.

**Table 7: Physical and chemical water quality analysis in slurry and control ponds**

Physical and chemical factors	Date treatment	27.4.2008	16.6.2008	2.7.2008	20.7.2008	29.7.2008	5.8.2008	19.8.2008	8.9.2008	23.9.2008	7.10.2008
(mg/L) O <sub>2</sub>	control	5.2	6.08	5.98	6.03	4.53	6.47	6.37	5.13	5.5	8.59
	slurry	5.9	6.01	6.11	5.37	3.97	6.57	5.76	5.37	3.73	7.02
nitrite mg/L	control	0.002	0.011	0.005	0.004	0.002	0.001	0.001	0.001	0.003	0.002
	slurry	0.002	0.006	0.002	0.002	0.002	0.004	0.002	0.002	0.002	0.002
Nitrate ( mg/L )	control	0.244	0.017	0.055	0.061	0.041	0.048	0.029	0.012	0.014	0.019
	slurry	0.016	0.036	0.027	0.049	0.042	0.085	0.038	0.011	0.031	0.018
Amonia ( mg/L )	control	0.27	0.274	0.309	0.294	0.188	0.306	0.2	0.118	0.324	0.423
	slurry	0.296	0.278	0.275	0.293	0.135	0.308	0.153	0.294	0.325	0.435
Total N ( mg/L )	control	0.97	1.373	0.609	0.911	-	0.592	0.504	0.722	0.762	0.513
	slurry	0.71	1.381	0.532	1.015	-	0.678	0.530	0.888	0.644	0.711
pH	control	7.84	7.86	7.83	8.2	7.83	7.98	8.19	7.85	7.91	8.11
	slurry	7.97	7.77	8.07	86.7	8	8.02	7.94	7.93	8.07	8.07
Phosphat e ( mg/L )	control	0.240	0.140	0.047	0.047	0.094	0.029	0.039	0.027	0.022	0.025
	slurry	0.233	0.174	0.056	0.052	0.107	0.03	0.044	0.022	0.022	0.024
Total P ( mg/L )	control	0.3	0.183	0.134	0.193	0.117	0.115	0.146	0.087	0.14	0.13
	slurry	0.307	0.226	0.157	0.204	0.166	0.153	0.159	0.17	0.123	0.388
EC ( us/cm)	control	1548	1572	1414	990.7	982.7	988	795.7	650.7	553	395
	slurry	1550	1597	1421	1258	1112	1107	956.7	722.3	573	447
Transpar	control	78.3	71.7	31	28	28.3	30.7	30.3	27.7	32.7	34.3

Conductivity ( cm )	Slurry	68.3	70	26.7	29.7	25.7	27.3	28.3	28.3	38.3	34.3
Turbidity F.T.U)	control	17.3	19	30.7	63	41	20.3	38	31.7	37.3	33.3
	Slurry	20.3	19.7	39.3	56	49	26.3	55.3	51.3	32	36.3
Temperature °C	control	26.3	27	25.2	30	30	30	28.8	26.1	26.4	23.6
	Slurry	26.2	26.7	25.2	30	30.1	30	29.1	26.1	26.4	23.6
HCO <sub>3</sub> ( mg/L )	control	142.3	152.5	162.7	136.2	158.6	159.6	138.3	126.1	144.3	150.5
	Slurry	144.3	151.5	156.6	160.6	170.8	150.5	152.5	122	151.5	142.3

## Discussion

### *Analyses of Physical and chemical parameters of water in culture ponds*

Results indicated higher total nitrogen (TN) and total phosphorus (TP) in slurry loaded ponds compared to the control ponds. These results were in agreement with Kangmin and Quihua (2000) who reported TN and TP were 59.8 times and 42.8 times, respectively higher in slurry prepared from the bio-digestion of pig manure. These findings were also true in the comparison of slurry and raw chicken manure.

According to Yuan (1985) most of the energy of ponds loaded with chicken manure turned into methane gas rather than TN and TP and the effective nitrogen and phosphorus in TN and TP were higher in slurry than those in chicken manure.

Moulik (1990) compared the effectiveness of applying manure after bio-digestion (slurry) to raw cow dung for plant growth and found that approximately 10 percent of the total nitrogen content in fresh dung is readily available for plant growth. Since, a major portion of it has first to be biologically transformed in the soil and is only then gradually released for plant use. When fresh cow dung dries, approximately 30 to 50 percent of the nitrogen escapes within 10 days. While, in the case of slurry the nitrogen escaping within the same period amounts to only 10 to 15 percent. Temperature and dissolved oxygen rates were almost similar in slurry loaded ponds and control. However, increase in survival rate and high densities of phytoplankton and zooplankton have a negative impact on oxygen availability in slurry loaded ponds. Electrical conductivity (EC) was noted to be higher in slurry loaded ponds. Due to high rates of respiration in slurry loaded ponds with abundant plankton and

high densities of fish, carbon dioxide accumulates more in these ponds compared to the control. Turbidity recorded was also higher in slurry loaded ponds due to high profusion of plankton which is also an indication of high fertility of these ponds as compared to the control.

*Biometry, growth and survival in carps*

Data extracted from all the six treatments revealed increase in survival rates and production of common carps in slurry loaded ponds. The production rate of silver carp, common carp, bighead and grass carp were 13.5, 2.6, 18.4, 85.3 times, respectively higher in comparison to the control. Their survival rate was also found to be higher than that in the control. It is very interesting to note that grass carp has a much lower survival rate in ponds loaded with fertilizers than in slurry treated ponds. This species has proven to be very sensitive when cultured using chemical fertilizers and many of them seem to die in their first weeks of development.

The average weight in silver carp and bighead in slurry loaded ponds was 12.0 and 18.6 percent, respectively higher than that in the control. The common carp showed 2 percent lower weight gain in the slurry loaded ponds as compared to the control. With regard to the very low survival rates of grass carp in the control ponds, the average weight for this species in the control pond was 18 percent higher than that recorded in the slurry treated ponds.

Statistically significant differences were observed in average weight and length of silver carp and bighead in the slurry loaded and control ponds. However, no significant differences were detected in average weight and length of common carp in the two groups studied. Significant differences observed in the average weight and length of grass carp in the slurry loaded and control ponds was due to the decreased survival rate in grass carp.

It has to be noted that aeration of the small ponds increased the turbidity of water. In fact one the main reasons why the silver carp didn't exhibit an ideal growth rate was aeration of small ponds which lead to murky and muddy pond water.

For further studies with slurry it is recommended that slurry is applied to bigger ponds where the pond beds have been improved with a combination of good aeration techniques. This will surely increase the rate of survival and yield. Another advantage of using big ponds for culturing is that if murkiness of water occurs it will not spread to other areas. In the present study when the aeration of ponds was stopped in the month of October, high abundance of plankton was observed which proves slurry to be a good substitute for raw manure or chemical fertilizers.

Muddy pond water and a short culture period (May-October) were some of the reasons that inhibited the growth of silver carp. Growth rate of silver carp was

not considered very ideal in both slurry and control ponds. Better results in terms of growth rate of cultured fishes can be achieved in bigger ponds or by using paddle aerators in the small ponds. In the present study the ponds used for fish culture didn't exceed 700 meters making them inefficient and unsuitable for grow out ponds. These ponds were also not suitable for fish culture as the walls and bottoms needed to be renovated. About 0.5 meter of the depth of these ponds was reduced due to sludge accumulation.

Muddy waters too can seriously reduce fish production. Muddy water chronically reduces the amount of sun light penetration into water which in turn reduces the amount of aquatic food produced by pond's food chain. This ultimately reduces the amount of fish that can be supported. In countries where slurry is used for fish culture, paddle like aerators are used too for proper aeration of ponds. However, in this study due to the high cost involved these aerators were not used. The use of suitable aerators and big ponds can prevent the muddiness of the water ponds to some extent.

Kangmin and Qiuhua (2000) produced 12.12 tons of carp by treating the pond with slurry prepared from the anaerobic digestion of manure, whereas only 3.4 tons of carp was harvested from a pond treated with chicken manure. These researchers also studied survival rate in carps using slurry and chicken manure up to the fingerling stage and found that survival rate in slurry treated ponds varied

from 76 percent to 96 percent for different carp species, while only 14 to 36 percent survival rate was obtained for the same species with chicken manure. They also reported highest survival rates in ponds treated with slurry. Kangmin and Qiuhua (2000) demonstrated that production in slurry treated ponds was 1.49 times of that in ponds treated with raw chicken manure. Survival rates for silver carp, bighead and grass carp were higher in slurry treated ponds.

Kangmin and Qiuhua (2000) compared the survival rate of silver carp, bighead and grass carp by using slurry and raw chicken manure. They found that the survival rate in silver carp was 96% and 15%, in bighead 96% and 25% and in grass carp 86% and 36 %, using slurry and raw chicken manure, respectively. They also stated that the slurry and the deposited materials in it can be used as compost in agricultural fields. Application of slurry in fish culture incorporates many benefits like increasing the production of zooplankton and the improvement of hygiene in these ponds which results in the enrichment of ponds which in turn has a positive impact on growth rate of fishes and reduces the production of methane gas ( Kangmin and Qiuhua , 2000).

Hence, using live food in the preliminary stages of culture and then weaning to formulated diets can be effective in decreasing mortality in fish, increasing the survival and growth rate, improving return coefficients and reducing production costs. Application of slurry

during early stages of rearing is highly recommended. Watanabe et al. (1983); Lobzns et al. (1989) and Lavens et al. (1991) emphasized the role of live food like Rotifers for larvae of common carp in their first 30 days of development. These studies have documented the preference for fermented cow dung to raw cow dung in culture systems.

#### *Chemical analysis of cow dung and slurry*

Cow dung is a local natural resource that can be converted into slurry that makes it a wonderful liquid manure, and bio gas. The relationship between the amount of carbon and nitrogen present in organic materials is expressed in terms of the Carbon/Nitrogen (C/N) ratio. A C/N ratio ranging from 20 to 30 is considered optimum for anaerobic digestion. If the C/N ratio is very high, the nitrogen will be consumed rapidly by methanogens for meeting their protein requirements and will no longer react on the left over carbon content of the material. As a result, gas production will be low. On the other hand, if the C/N ratio is very low, nitrogen will be liberated and accumulated in the form of ammonia (NH<sub>4</sub>). NH<sub>4</sub> will increase the pH value of the content in the digester. pH values of higher than 8.5 will start exerting toxic effects on the methanogenic population. Cattle dung has an average C/N of 24, which makes it an ideal material for anaerobic digestion (Karki and Dixit, 1984). Based on these observations, the present study used cattle dung to prepare slurry.

Researchers at Shenyang Agricultural University found that the protein content of the slurry increases from 16.62 % to 46.09 % after anaerobic fermentation (Kangmin and Qiuhua, 2000). Before the process of fermentation the content of amino acids relatively increases. Vitamin B content also increases by 7.2 times. The hydrolyzed enzyme is higher in slurry with the protein activating protein enzyme (53.82 mg Tyrosine). The starch activating enzyme (2678.25mg) and the glucose activating enzyme, (1775.14mg) are found in slurry. Some growth and cell fusion hormones were also measured in slurry. It is understood that application of slurry provides the essential content of N, P and K to plants and also promotes the growth of plants (phytoplankton). Slurry can be introduced as a fertilizing liquid containing several dissolved nutrients.

Dreges deposited and settled in bio digesters, with a nitrogen and phosphorus content of about 30-50% and considered to be rich in organic matter and humus are used as an indicator of soil fertility (Kangmin and Qiuhua, 2000). Due to the mentioned features in slurry, it can be used as an enriching liquid to increase the fertility of fish ponds.

Anaerobic digestion consists of a series of reactions by a group of bacteria on organic matter. During the process of cartelization, polymers such as cellulose, hemicellulose, pectin, and starch are hydrolyzed to oligomers or monomers, which are then metabolized by fermentative bacteria with the production

of hydrogen (H<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), and volatile organic acids such as acetate, propionate, and butyrate. syntrophic acetogens convert the organic acids other than acetate to methanogenic precursors (H<sub>2</sub>, CO<sub>2</sub>, and acetate). Finally methane was produced as metabolic byproduct in anoxic conditions by methanogenic bacteria (Wilkie and Collieran, 1987).

The final byproducts of the anaerobic digestion, methane and carbon dioxide, are odorless. Sometimes when wastes are stored for microbial degradation an imbalance occurs. This usually happens when methane is not produced fast enough to prevent accumulation of products during acid fermentation. This is an imbalance process where some volatile malodorous products are formed. It is only during balanced conditions when the volatiles are converted to methane and carbon dioxide.

Choosing a proper enriched substance (material) would play an important role in increasing the fertility in fingerling culture ponds. A good composition of the enriched substance will stimulate production of live food like rotifers for larvae of kutum which in turn increases their survival rate. At the same time turbidity of culture ponds increases due to increase in fertility.

In this study, application of slurry has proven to decrease the transparency of ponds during the culture period compared to that in the control. This is mainly due to its enriching features that stimulate bacteria, phytoplankton and zooplankton production making it an ideal manure compared to raw cattle dung. The

produced bacteria are responsible for decomposition of organic matter and in turn liberate inorganic matter into the culture ponds. Zooplankton communities in the culture ponds flourish by feeding on the bacteria and phytoplankton. The high abundance of the zooplankton makes the ponds look green in color which gradually turns to brown. This makes the water appear murky which reduces sun light penetration and in turn inhibits the growth of aquatic plants in the deeper areas (Baradaran Tahouri, 1994). Among the zooplanktons, rotifer, copepods and cladocerans are seen the most. Unfortunately, there is no standard method to estimate the abundance of different live food under similar conditions (spatial and temporal).

Hard waters, in which calcium content is more than the permissible limit, require higher levels of phosphorus. This mainly because the calcium and phosphorus content in water compete with each other. Chemical analysis of slurry and raw cattle dung revealed that the calcium and phosphorus content in slurry is 1.26 times and 1.66 times, respectively that in raw cattle dung. This was clearly evident in the higher production of phytoplankton and zooplankton in ponds treated with slurry. The use of Ca(CO<sub>3</sub>)<sub>2</sub> is limited. If alkalinity of the rearing pond exceeds 50 mg/l. The buffering role of dissolved calcium in water, expressed as ppm calcium carbonate, is its alkalinity. In a pond with higher alkalinity, the pH shift is reduced during the fish culture period. As pH increases, ammonia in the toxic non



ionized form too Increases. Kutum larvae continuously exposed to more than 0.02 pm of the un-ionized form may exhibit reduced growth and increased susceptibility to disease. Hence higher survival rates recorded in ponds loaded with slurry may be explained by this efficient role of slurry. Nutrient like (total N and total P) are important factors influencing phytoplankton abundance. In fact in culture ponds adding materials supplying large amounts of nitrogen is necessary for rapid and thorough decomposition of organic matter. Although potassium does not play an important role in fish culture, its content is found to be 3.6 times higher in slurry than in raw cattle dung. Another advantage of the application of slurry to granular fertilizers is its lower density making it an ideal manure to mix thoroughly on the entire water surface of rearing ponds Unlike granular fertilizers, slurry does not settle immediately to the bottom of the kutum rearing pond and is effective in increasing the fertility of these ponds.

Chemical analysis of slurry and raw cattle dung revealed that protein, lipid, ash, calcium, phosphorus and potassium content in slurry were comparatively higher than that in raw cattle dung. Presence of methanotrophic bacteria like *Methylococcus capsulatus* influence the anaerobic fermentation of cattle dung and finally a rich-nutrient substance, slurry, is produced. Similar results were reported by Willoughby (1999) who pointed out that protein production is reliant upon the bacteria living in natural gas.

These bacteria are methanotrophic bacteria which along with other heterotrophic marine bacteria are added to water (45 C °) saturated with methane and ammonium. At the end of the process the product produced contains 70% protein, 10% lipid, 10% hydrocarbon and 7% minerals. Moreover under certain circumstances when nutrient content of microorganisms in water is low, organic particles attach themselves to microorganisms in water resulting in an increase in the protein content of these organisms (Schroeder, 1980).

Hence, application of slurry in larval culture ponds has triggered methanotroph bacteria production which in turn is a good food source for zooplanktons. Similarly zooplanktons are also eaten by larvae at a different trophic level (Kangmin and Quihuna, 2000).

Comparison of oxygen consumption made between fresh chicken manure and slurry (Kangmin and Quihuna, 2000) indicated that each kg of chicken manure consumes 5,000 mg of oxygen in 15 hours while 570 kg of slurry, the same heat equivalent to chicken manure only consumes 67.76 mg of oxygen in 15 hours. These findings are similar to the results obtained in this case study and emphasize the application of slurry to cattle dung for rehabilitation of stocks. .

#### *Chemical analysis of stomach and intestine contents of Chinese carps*

Results of stomach contents in silver carp revealed a higher abundance of blue-green

algae belonging to the genus *Oscillatoria* in the control than in the slurry treatment, whereas diatoms and green algae were higher in the slurry fed ponds.

Berday et al. (2005) quantified the weight of food consumed per weight of fish produced in silver carp and reported that silver carp can consume a biomass of 6.6 g per every kg of its weight and/or  $1.6 \times 10^{11}$  cells per every kg of its weight. Consumption of zooplanktons is comparatively lesser and it is about  $2 \times 10^7$  per every kg of the fish weight. Feeding pattern and habits of filter feeders depend largely on the size, abundance, and toxicity of cyanobacteria and temperature of water.

According to Kim et al. (2008), silver carp becomes a non-selective grazer when a huge population of cyanobacteria is available. They report that shape and size of the food play an important role on the food selectivity of silver carp. Sometimes due to the large population of algae, feeding on green algae becomes inevitable. However, they prefer grazing on diatoms rather than on green algae, cyanobacteria and euglena. The presence of silver carp in ponds significantly limits the density of chlorophyll bearing planktons above 40 micron in size. Silver carp rarely grazes on larger algae like *Oscillatoria agardhii*, *Ababaena flos-aquae* and *Melosira distors*.

*Carcass analysis of Chinese carps in slurry loaded and control ponds*

The protein and lipid content of silver carps were estimated to be slightly higher in slurry fed ponds than in the control. In bighead the lipid content was measured to be 1.5 times higher in slurry loaded ponds than that in the control. The protein content was higher in fish in slurry fed ponds. Abundance of zooplankton like rotifers, cladocera and copepods were higher in the stomach content of bighead in slurry fed ponds. This high abundance was mainly attributed to the rich-nutrient present in slurry ponds which induced the density of metanobacteria which served as a rich food source for zooplankton community. These zooplanktons are good palatable food for larva in the culture ponds and increase their growth and survival rate (Kangmin and Qiuhua, 2000).

In common carp a slight difference was observed in the protein and lipid content of fish in both the treatments, being slightly higher in the control especially in the case of lipids. This slight increase can be attributed to lesser amounts of formulated food given to slurry fed ponds. In grass carp the protein and lipid content estimated were higher in slurry than in control ponds. Lipid content was 1.4 times higher in fish in slurry fed ponds compared to that of fish in the control ponds.

## Recommendations

On the basis of the results obtained it is evident that slurry makes a potential substitute for conventional chemical fertilizers and also for raw cattle dung

which pose a pollution treat to water resources. Slurry prevents the development of insidious algae booms and keeps the phytoplankton and zooplankton population in equilibrium.

- Studying the process of preparing slurry in laboratory conditions (*in vitro*) under controlled temperature and pH levels will aid in its application in different climate conditions of the country.

- Waste generated from different activities like poultry, canned factories, agriculture etc. can be put into a biogas digester for slurry production under hygiene conditions and its nutrient rich value for fertilization of fish-farming can be studied under different circumstances.

- Application of slurry for producing and releasing other boney fishes like pike perch, roach, common bream and sturgeon fishes can be taken up in future research mainly to study the effect of slurry on increasing the survival and growth rate in larvae of the mentioned species.

### Acknowledgment

We are very thankful to our colleagues and appreciate their constant support and encouragement. We would like to specially thank Dr. Motallebi, Director of the Iranian Fisheries Research Organization (IFRO), Dr. Sharif Rouhani, Research Deputy of IFRO, and Dr. Matinfar, Scientific Advisor of this project. Our special thanks are also due to Dr. Khanipour, former Director of Inland waters Aquaculture Research Center and

his Research Deputy, Mr. Khodaparast. We are very grateful to Mr. Tolouie, former Manager of Shahid Beheshti Sturgeon Hatchery and his staff, especially Mr. Shakurian, and Mr. Rasouli as well as Mr. Darvishi, Manager of the Shahid Ansari Hatchery and Mr. Ghanaat Parast.

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