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Impact of Artificial Feed on Survival and Growth of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum) during Exogenous Feeding in Raceways of Kathmandu, Nepal

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ABSTRACT

Artificial feed including low cost, alternative, and nonconventional animal protein in replacement to costly shrimp meal were formulated and their impact on survival and growth of rainbow trout, Oncorhynchus mykiss (Walbaum), during exogenous feeding in raceways were compared. Three feed formulations of silkworm pupae (SWP), silkworm moth (SWM), and synthetic amino acids (SAA) were evaluated against shrimp meal (SML) acting as control through feed efficiency indicators. Four diets (three formulated and one control) were fed to the free swimming fries, fries, and fingerlings for 150 days (5 months). There was significant difference in survival (P<0.01) and growth (P<0.05) of above mentioned stages. Survivability with SML diet fed stages showed superiority (P<0.01) among other feed formulations, SWP came next, SWM less, and SAA least. Growth with SWP diet fed stages exhibited superiority (P<0.05) amongst all the diets and SAA lowest whereas SML and SWM lied in between. Therefore, absolute growth rate, specific growth rate, relative growth rate, and feed efficiency were highest due to SWP, higher due to SML, low due to SWM, and lowest due to SAA. However, condition factor was highest due to SWP, higher due to both SAA and SML, and lowest due to SWM. Unlike other feed indicators, feed conversion ratio exhibited highest due to SAA, higher due to SWM, low due to SML, and lowest due to SWP. The highest growth period was observed during April to May and lowest during December to January in all the diets. Cost analyses revealed SWM containing diet cheapest with low production cost, SWP cheaper with lowest, SAA cheap with highest, and SML costly with high production cost. The study confirmed natural protein SWP, SML, and SWM were superior to SAA. Results indicated cost effective SWP containing diet could be used as better alternative to completely replaced SML without compromising survival and growth.

***Key words**: Formulated artificial feed, survival and growth, rainbow trout, exogenous feeding, raceways*

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) 1792, Anonymous[3] depend on quality and quantity of artificial feed, Rai *et al.*[17] that convert 0.07-0.10g hatchlings into 200-300g table fish in 16-18 months in raceways of Kathmandu, Nepal, Anonymous[2].

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Hatchlings of rainbow trout start exogenous feeding when their yolk-sacs are completely absorbed, Pradhan *et al.*[16]. Hatchery-grown hatchlings are exclusively dependent on artificial feed for their survival and growth, Bardach *et al.*[4]. Thus, an increase in rainbow trout production requires corresponding increases in nutrition, feeding, and feedstuffs through artificial feed. Artificial feed comprises proteins (animal and plant) 40-50%, Robinson and Li[19], carbohydrates 15-25%, Hasan[10], lipids 10-15%, Robinson and Li[19], minerals 1%, Hasan[10], and vitamins 1%, Hasan[10]. It is the single largest operating cost in rainbow trout culture in the world including Nepal. Artificial feed alone is 76% of the total variable cost and 40% of the total production cost of rainbow trout farming, Nepal *et al.* [14], and is one of the major constraints after seed supply to limit expansion of rainbow trout culture in Nepal. Its production cost for rainbow trout is high as it contains high quantity of protein diet, Rai *et al.*[17].

Table-1: Proximate analyses of feed ingredients for free swimming fries, fries, and fingerlings

Ingredients	Crude proteins (%)	Crude fibres (%)	Crude fats (%)	Ash (%)	Moisture (%)	Remarks
Silkworm pupae	57.21	2.39	31.29	4.01	6.65	Animal protein/powder
Silkworm moth	54.55	4.84	29.05	4.74	11.84	Animal protein/powder
Lysine	100.00	-	-	-	-	Synthetic amino acid/powder
Methionine	100.00	-	-	-	-	Synthetic amino acid/powder
Shrimp meal	64.80	3.25	3.81	10.09	10.59	Animal protein/powder
Soybean (roasted)	37.00	6.00	21.00	5.10	8.70	Plant protein/powder
Wheat	12.90	9.90	3.80	7.00	12.40	Energy supplement/ flour

The protein component of rainbow trout is the single most expensive portion and important dietary nutrient. Among animal and plant proteins, animal protein is the main dietary component used in formulated diet of rainbow trout, since its introduction in Nepal, Roy *et al.*[20] as it contains essential amino acids. The animal protein is the most costly item which when decreases in cost will sustain rainbow trout culture. Fishmeal (FML) has become one of the main animal protein supplements in the rainbow trout feed in Nepal due to its high nutrient density (20-35% protein) and digestibility, Nepal *et al.*[14]. It contains high level of protein and appreciable quantities of fat and minerals. Its protein has high biological value because of its richness in essential amino acids especially lysine and sulphur-containing methionine and cysteine. However, it is highly costly. Dried trash fish (DTF) is another main source of animal protein but its bad smell and poor milling quality limits its use, Roy *et al.*[20]. Shrimp meal (SML) is still another main protein supplements for rainbow trout feed in Nepal, Nepal *et al.*[14] but it is costly. Hence, FML and SML increase production cost in rainbow trout farming, Anonymous[1], Roy *et al.*[20] and Pradhan[16]. The dietary animal protein requirement for rainbow trout ranges from 30-35%. Therefore, most of the studies conducted in Nepal have been focused on finding alternate and nonconventional source of animal protein supplements in rainbow trout feed which should be locally available at relatively cheaper rate without affecting survival, growth, production, and quality.

Very limited numbers of feed ingredients are available to choose for the formulation of balanced diet. A review of literature on investigations into the use of plant feedstuffs in rainbow trout feed indicated that it was possible to utilize processed soybean meal at high level (up to 60%) without impairing survival, growth, and environment, Bista *et al.*[6]. The mixture of different levels of defatted soybean meal, corn gluten meal, and meat meal

could replace up to 90% of the FML, if combination of these ingredients produce the same profile of amino acids comparable to FML, Juadee and Watanabe[12]. Grain and byproducts are insufficient as these can't fulfill whole requirement of rainbow trout feed. One of the promising alternatives to the FML and SML is silkworm pupae (SWP), a waste product of silk industry.

SWP could be used as a cheaper, alternative, and top class nonconventional protein and energy feed for rainbow trout after proper processing at reasonable cost. Silkworm moth (SWM), *Bombyx mori* which die after spawning could also be used as another cheapest, alternative, and unconventional protein and energy feed for rainbow trout after proper processing at reasonable cost. Synthetic amino acids (SAA) could further be supplemented in the rainbow trout diet as an alternative and nonconventional protein feed to animal based protein source.

Table-2: Composition of feed ingredients (%) in the formulated diets of free swimming fries, fries, and fingerlings according to Pearson's square method

Ingredients	SWP	SWM	SAA	SML/control	Remarks
Silkworm pupae	55				Powder
Silkworm moth		60			Powder
Lysine			21		Powder
Methionine			7		Powder
Shrimp				50	Powder
Soybean	30	30	30	25	Roasted/powder
Wheat	13	8	40	23	Flour
Mineral premixes	1	1	1	1	Additives/Technovit M
Vitamin premixes	0.99	0.99	0.99	0.99	Additives/Technovit F
Vitamin C	0.01	0.01	0.01	0.01	Additives/Technovit C
Total	100.00	100.00	100.00	100.00	

Artificial feed was formulated using low cost, alternative and nonconventional animal protein ingredients of SWP and SWM, and nonconventional synthetic protein ingredients of SAA. The present paper describes the preliminary findings of the impact of SWP, SWM, and SAA available in Nepal in the formulated diets of rainbow trout on survival and growth during exogenous feeding and evaluates the formulated diets against SML acting as control through feed efficiency indicators.

MATERIALS AND METHODS

The study was conducted for one year from June, 2010 to May, 2011 in farmer's raceways at Kakani, Nuwakot district, Kathmandu, Nepal situated at latitude 27°48' N, longitude 85°15' E and altitude 1550m from main sea level (msl). Broods were stocked in June 2010, artificial breeding was done in November 2010, and free swimming fries (FSFs) for the research experiment were obtained in December 2010. Artificial feed was formulated using low cost, alternative, and nonconventional animal protein ingredients of SWP and SWM, and synthetic protein of SAA having lysine and methionine (3:1). The three formulated diets of SWP, SWM, and SAA were fed to free swimming fries (FSFs), fries, and fingerlings during exogenous feeding period and evaluated against the diet containing shrimp meal (SML) acting as control through feed efficiency indicators of absolute growth rate (AGR), specific growth rate (SGR), relative growth rate (RGR), feed efficiency (FE), condition factor (CF), and feed conversion ratio (FCR) along with cost analyses (CA) shown in Table-5. Compositions of test formulations are given in Table-2. Calculations of nutrition percentage (Pearson's square method) of the formulated diets based on above composition (Table-2) was also expressed (Table-3). Proximate analyses of each ingredients (Table-1) and formulated diets (Table-4) were done with reference to crude protein, crude lipid, crude fibre, ash and moisture by Kjeldahl protein analysis method, Soxhlet extraction method, organic residue left method, Moful furnace method, and loss in weight method respectively at Food Research Laboratory, Kathmandu, Nepal and Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, Siliguri, West Bengal, India.

Table-3: Calculation of nutrition percentage of the formulated diets based on above composition (Table-2) for free swimming fries, fries, and fingerlings

Formulated diets	Crude proteins (%)	Crude fibres (%)	Crude fats (%)	Ash (%)	Moisture (%)	Remarks
SWP	45.25	4.41	24.00	4.79	6.88	Animal protein
SWM	44.86	5.49	24.30	4.93	10.70	Animal protein
SAA	44.26	3.96	7.82	4.33	7.57	Synthetic protein
SML/control	44.62	5.41	8.03	7.57	10.33	Animal protein

FSFs of rainbow trout 0.025±0.0007g and 1.65±0.08cm were stocked at the density of 250m^{-2} for each feed formulation in quadruplicate nursing cum feeding cum rearing cages (1.0m×1.0m×1.0m) placed in raceway. In this way, 4000 FSFs (1000 in each set and 250 in each replica) were stocked. They were fed 45% crude protein (CP) for the research period of 150 days (5 months) from Tuesday, 7th December, 2010 to Friday, 6th May, 2011. To do this, they were supplied respective feed up to the satiation at the interval of 1 hour for 12 times during day time @ 15% of their live body weight for 30 days (1 month). FSFs which were grown into fries were given respective feed up to the satiation at the interval of 1.2 hours for 10 times during day time @ 12% of their live body weight for 60 days (2 months). Fries which were grown into fingerlings were provided respective feed up to the satiation at the interval of 1.5 hours for 8 times during day time @ 10% of their live body weight for 60 days (2 months). Data for survival and growth were obtained at every 15 days (two weeks) interval up to 5 months by taking samples of 10 for each (Ricker, 1975). Data of survival and growth were subjected to ezANOVA for interaction among feed formulations (treatments).

Table-4: Proximate analysis of prepared formulated diets for free swimming fries, fries, and fingerlings

Particulars	SWP	SWM	SAA	SML/control	Remarks
Crude proteins (%)	42.68	46.01	48.47	38.27	Crumble feed
Crude fibres	2.80	3.11	1.86	2.65	Crumble feed
Crude lipids	17.01	20.85	6.48	8.62	Crumble feed
Ash	5.65	7.13	5.06	11.67	Crumble feed
Moisture	10.35	3.66	11.54	8.97	Crumble feed

RESULTS AND DISCUSSION

FSFs, fries, and fingerlings of rainbow trout were fed three different feed formulations of SWP (27.555±0.15g), SWM (5.396±0.21g), and SAA (2.778±0.20g) along with a control diet containing SML (13.348±0.28g). Fingerlings obtained due to SWP, SWM, SAA, and SML were 898, 793, 221, and 916 in number respectively. Hence, survival (SR) of the above mentioned stages revealed SML fed stages to have 91.60±0.98% during 150 days (5 months) of nursing, feeding and rearing being significantly superior ($P < 0.01$) among all the formulated diets. SR of SML diet fed was comparable to SWP diet fed with 89.80±1.18% and SWM diet fed with 79.30±2.33% (Figure-1). However, stages fed with SAA diet had least SR of 22.10±9.76% among all the feed treatments (Table-5).

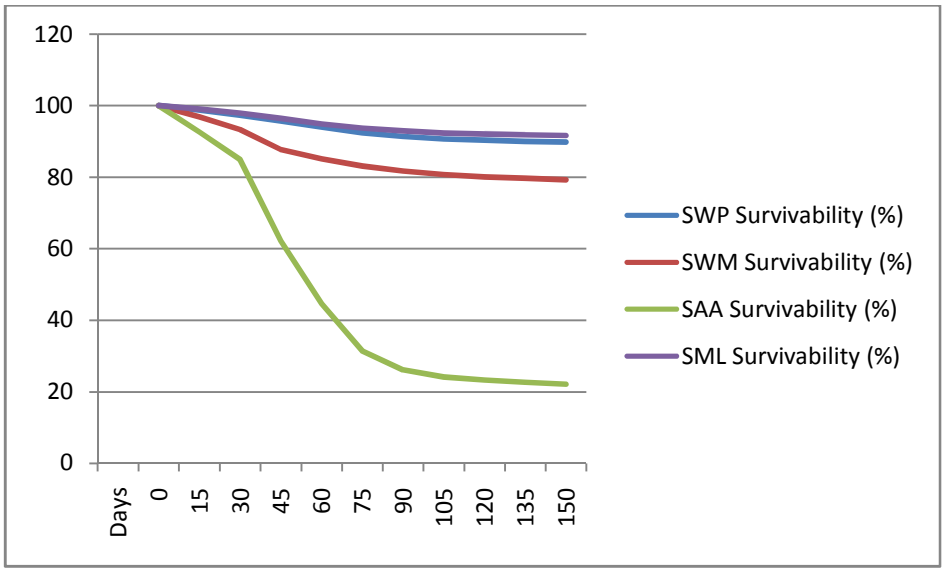

Fig. 1: Survivability of FSFs, fries, and fingerlings in 150 days (5 months)

Fingerlings obtained due to SWP, SWM, SAA, and SML were 13.792±0.08g and 9.70±0.08cm, 1.582±0.06g and 5.90±0.06cm, 0.598±0.04g and 3.44±0.07cm, and 5.446±0.09g and 8.52±0.07cm with the growth (GR) of 13.767±0.08g and 8.05±0.08cm, 1.557±0.06g and 4.25±0.06cm, 0.573±0.04g and 1.79±0.07cm, and 5.421±0.09g and 6.87±0.07cm respectively. There was significant difference ($P< 0.05$) among formulated diets on the GR of above mentioned stages against feed efficiency indicators of absolute growth rate (AGR), specific growth rate (SGR), relative growth rate (RGR), feed efficiency (FE), condition factor (CF), and feed conversion ratio (FCR) along with highest growth period (HGP) and cost analyses (CR).

The highest AGR was obtained with SWP diet fed stages (0.092±0.0005g day^{-1} and 2.754±0.016g $month^{-1}$) followed by SML diet fed stages (0.036±0.005g day^{-1} and 1.084±0.017g $month^{-1}$) and SWM diet fed stages (0.010±0.0005g day^{-1} and 0.311±0.012g $month^{-1}$). The lowest AGR was obtained with SAA diet fed stages (0.004±0.0004g day^{-1} and 0.115±0.009g $month^{-1}$). The highest SGR was due to SWP (9.178±0.053% daily-wise and 275.34±1.60% monthly-wise) and lowest due to SAA (0.382±0.023% daily-wise and 11.46±0.46% monthly-wise) and with SML (3.614±0.56% daily-wise and 108.42±1.75% monthly-wise) and SWM (1.038±0.041% daily-wise and 31.14±1.22% monthly-wise) in between. The RGR was highest due to SWP (3.671±0.021% daily-wise and 110.14±0.064% monthly-wise), higher due to SML (1.446±0.023% daily-wise and 43.37±0.70% monthly-wise), low due to SWM (0.415±0.016% daily-wise and 12.45±0.49% monthly-wise), and lowest due to SAA (0.153±0.012% daily-wise and 4.58±0.35% monthly-wise). Hence, FE was highest due to SWP (49.96±0.011%) and lowest due to SAA (20.61±0.062%) and with SML (40.70±1.444%) and SWM (28.85±0.018%) in between. Therefore, AGR, SGR, RGR, and FE were highest due to SWP, lowest due to SAA while due to SML and SWM in between. However, CF was highest due to SWP (1.85±0.15), higher due to SAA (1.40±0.09), low due to SML (1.39±0.014), and lowest due to SWM (1.33±0.012). unlike other feed indicators, FCR with SAA diet fed stages exhibited highest conversion rate of 4.852±0.0146 and was significantly different ($P<0.05$) with that of SWP being lowest conversion rate of 2.002±0.0003, however, conversion rate of 2.466±0.0831 and 4.852±0.0146 respectively with SML and SWM were intermediate. The HGP was observed during April 19 to May 3, 2011 and lowest in the period of December 5 to December 19, 2010 whereas it was medium in the period of March 20 to April 3, 2011.

Cost analyses (CA) that included cost of feed and production cost of rainbow trout due to

feed revealed that SWP containing diet kg^{-1} was NRs 73.45 with production cost of rainbow trout kg^{-1} NRs 147.05, SWM containing diet NRs 49.45 with production cost NRs 171.40, SAA containing diet NRs 167.95 with production cost NRs 814.90, and SML containing diet NRs 193.45 with production cost NRs 477.05. Hence, CA revealed SWM containing diet cheapest with low production cost, SWP cheaper with lowest production cost, SAA cheap with highest production cost, and SML costly with high production cost. FSFs, fries, and fingerlings grew exponentially with the three feed formulations containing animal protein of SWP, SWM, and SML. The periodic GR of above mentioned stages was somewhat stagnant with feed comprised of SAA (Figure-2). Therefore, GR with SWP diet fed stages exhibited superiority ($P<0.05$) over other formulated diets against all feed efficiency indicators and that of SAA diet fed stages lowest whereas SML and SWM diets fed stages lied in between.

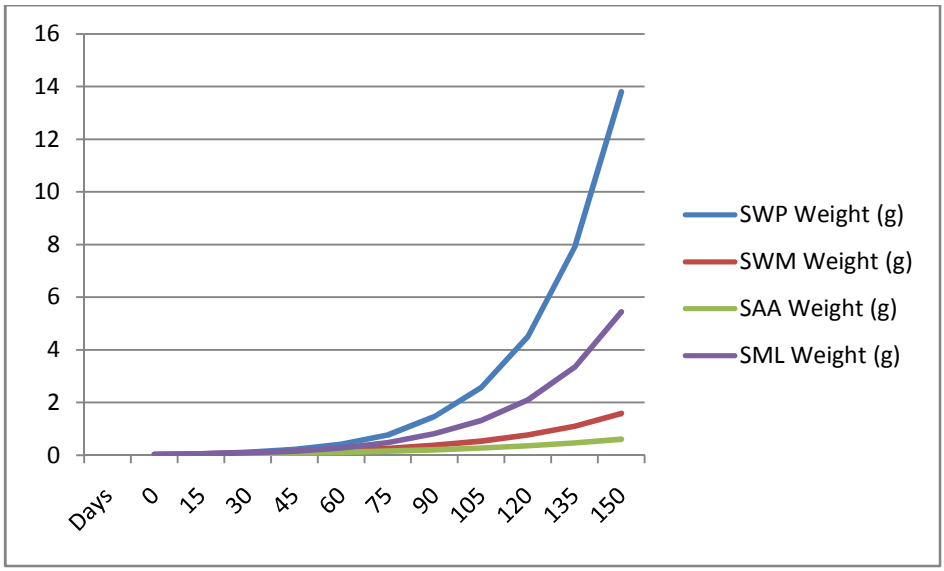

Fig. 2: Growth of FSFs, fries, and fingerlings in 150 days (5 months)

Rainbow trout fed FML diet containing 37% CP grew as fast as those fed 42% CP diets supplemented with SAA containing lysine, methionine, threonine, and tryptophan, Cheng *et al*. [9]. Further, reduction (2.7%) of dietary digestible CP from 27.0 to 23.3% with SAA had no negative impact on growth performance of Nile tilapia, Botaro *et al*.[7]. Again, dietary crude protein (CP) could be reduced from 41.26 to 35.52% in the diets of *L. vannamei* as long as SAA were supplemented, Huai et al[11]. However, SR and GR were poorest due to the diet of SAA which may be due to the absence of animal protein with required amino acid profile and insufficient lipid due to required fatty acid profile.

Table-5: Survival and growth of FSFs, fries, and fingerlings of rainbow trout due to 3 formulated diets (SWP, SWM, and SAA) with control (SML) during the period of 150 days (5 months)

S.No.	Particulars	SWP	SWM	SAA	SML/CON
1.	Animal or synthetic proteins (kg^{-1}diet)	0.55	0.60	0.28	0.50
2.	Crude proteins (%)	42.68	46.01	48.47	38.27
3.	Cost (NRs) (kg^{-1} feed)	73.45	49.45	167.95	193.45
4.	Cost (NRs) (kg^{-1} trout production)	147.05	171.40	814.90	477.05
5.	FSFs (number) stocked	1000	1000	1000	1000
6.	FSFs (g) stocked	0.025±0.0007	0.025±0.0007	0.025±0.0007	0.025±0.0007
7.	FSFs (cm)stocked	1.65±0.08	1.65±0.08	1.65±0.08	1.65±0.08
8.	Formulated diet (g)	27.555±0.15	5.396±0.21	2.778±0.2	13.348±0.28
9.	Research period (days)	150	150	150	150

10.	Fingerlings (number) obtained	898	793	221	916
11.	Fingerlings (g) obtained	13.792±0.08	1.582±0.06	0.598±0.04	5.446±0.09
12.	Fingerlings (cm) obtained	9.70±0.08	5.90±0.06	3.44±0.07	8.52±0.07
13.	Survival (%)	89.80±1.18	79.30±2.33	22.10±9.76	91.60±0.98
14.	Growth (g)	13.767±0.08	1.557±0.06	0.573±0.04	5.421±0.09
15.	Growth (cm)	8.05±0.08	4.25±0.06	1.79±0.07	6.87±0.07
16.	AGR (g day^{-1})	0.092±0.0005	0.01±0.0005	0.004±0.0004	0.036±0.005
17.	AGR (g $month^{-1}$)	2.754±0.016	0.311±0.012	0.115±0.009	1.084±0.017
18.	SGR (% day^{-1})	9.178±0.053	1.038±0.041	0.382±0.023	3.614±0.056
19.	SGR (% $month^{-1}$)	275.34±1.60	31.14±1.22	11.46±0.87	108.42±1.75
20.	RGR (% day^{-1})	3.671±0.021	0.415±0.016	0.153±0.012	1.446±0.023
21.	RGR (% $month^{-1}$)	110.14±0.64	12.45±0.49	4.58±0.35	43.37±0.70
22.	FE (%)	49.96±0.011	28.85±0.018	20.61±0.062	40.70±1.444
23.	CF (%)	1.85±0.15	1.33±0.12	1.40±0.09	1.39±0.14
24.	FCR (ratio)	2.002±0.0003	3.466±0.0022	4.852±0.0146	2.466±0.0831

SWP, although low cost ingredient, has more protein and lipid than SML, Bhuiyan *et al*.[5] and is rich in amino acid profile than FML, Solomon and Yusufu[21]. Hence, feed formulation with SWP had exhibited superiority in GR over all other feed formulations against all feed efficiency indicators (Table-5), however less SR than SML might be due to more fatty acid in SWP than it was required. Because SWP as diet for fingerlings of common carp and Indian major carp has proven its suitability as substitute of oil cake and rice bran, Chakrabarthy *et al*.[8] so it will be better substitute of SML. Because common carp fed with increasing level of SWP revealed progressive growth with highest growth in 30%, Cheng *et al*.[9] in comparison to diet containing 30% FML, Nandeesha *et al*.[13] hence, above mentioned stages of rainbow trout had shown highest growth in comparison to SML.

CONCLUSION

The study confirmed that natural and animal protein of SWP, SWM, and SML were superior to synthetic protein of SAA. However, SWM and SAA need further study along with different composition of CP of plant and animal. Finally, results indicated that cost effective SWP containing diet could be used as better alternative to completely replaced SML without compromising survival and growth of rainbow trout.

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