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STATUS AND DEVELOPMENT TREND OF AQUACULTURE AND FISHERIES IN NEPAL

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ABSTRACT
Fisheries sector of Nepal constitutes a crucial component of the nation's natural wealth, buoyed by its diverse geographical features and ample water resources. While traditional capture fisheries have historically played a significant role in fish production and employment, maintaining production levels presents challenges necessitating thorough reassessment. The landlocked geography compels a focus on inland finfish farming in the aquaculture subsector, supported by governmental initiatives such as the Fish Mission and Prime Minister Agriculture Modernization Project. The Terai belt, with its favorable climate, serves as a pivotal area for fish production, covering 88% of the total pond area. Nepal's total fish production stands at 108,385 mt, with capture fisheries contributing 19% and aquaculture 81%, collectively employing around 0.52 million individuals. Fish consumption in Nepal is on the rise, with per capita availability increasing from 330 g to 3780 g between 1981/82 and 2022/23. Efforts from both the government and private sector aim to commercialize the sector, addressing issues of food security, poverty reduction, and creation of income and employment opportunities. Despite challenges, targeted endeavors through governmental initiatives, private sector involvement, and sustainable practices are vital to unlocking the full economic, employment, and food security potential of fisheries sector.

Key words: Productivity, Production, Capture fishery, Aquaculture, Fish seed

INTRODUCTION
Nepal boasts rich geographical diversity and abundant water resources, making it a land of significant natural wealth. In addition to its diverse geography, Nepal boasts a substantial biodiversity profile, particularly in the realm of fish, harboring a total of 252 fish species (Shrestha 2019), constituting approximately 1.6% of the global freshwater aquaculture diversity. Within this diverse array, Nepal is home to 16 endemic fish species. Concurrently, the inhabitants of Nepal have an enduring engagement in capture fisheries, a historical practice deeply rooted in ancient times. This historical significance of fish in Nepal's diverse cultures and traditions is substantiated by archeological evidence and documentation, which underscore the presence of fishing gear and the cohabitation of indigenous communities (such as Majhi, Malah, Bote, Danuwar, etc.) in proximity to natural water bodies (Adhikari and Thapa 2016). Despite the historical significance of fish in Nepal, the modern era of fish farming commenced with the establishment of the Fisheries section under the Agriculture Council in 1956 AD.

Aquaculture has emerged as a rapidly advancing agricultural subsector in Nepal, driven by its landlocked geography, necessitating exclusive reliance on inland finfish farming. The diverse climatic conditions in Nepal allow for the cultivation of both warm and cold-water species, including indigenous and exotic Carps, Pangasius, Tilapia, catfish, and Rainbow trout. Despite the slow progress in institutional aquaculture development over the past seven decades, the sector witnessed significant growth in the previous decade (Kunwar and Adhikari 2017). Government initiatives such as the Fish Mission, One Village One Product, resource center establishment, and the Prime Minister Agriculture Modernization Project (PMAMP) played a crucial role in this advancement (Chaudhary and Jha 2018). The Terai belt of Nepal holds a prominent position in fish production, accounting for 88% of the overall pond area (Zhuang and Ghimire 2017). The leading districts in terms of fish production are Bara, Dhanusha, Saptari, Rupandehi, Siraha, Morang, Parsa, Rautahat, Sarlahi, and Chitwan (CFPCC 2019).

The primary objective of Nepal's fisheries development program is to enhance the production and productivity of fish by utilizing the diverse water resources available in the country. The program aims to commercialize the fisheries sector, contributing to food and nutrition security, poverty reduction, and increased income and employment opportunities. Private sector involvement, along with technical support for the conservation of fishery resources and the development of an eco-friendly fisheries sector, is crucial for the success of these initiatives. These programs operate within the framework defined by the Constitution of Nepal, which delegates responsibilities to local, provincial, and federal levels. There exist diverse institutional frameworks dedicated to advancing fisheries. The Central Fisheries Promotion and Conservation Center (CFPCC), along with its three subordinate offices under the central government, each equipped with specific mandates play a pivotal role in this regard. Additionally, fish development centers, Veterinary Hospitals, and Animal Service Expert Centers operate under provincial government jurisdiction, while livestock section under local government, contribute significantly to the execution of fisheries development programs. Fishery extension is mostly carried out by local government. Development of fish market and large investment is done by provincial government and Central government mostly works on policy, quarantine issue and is responsible for coordination with national and international organization. To bolster research initiatives in fisheries development, institutions like the Nepal Agricultural Research Council are actively engaged in overseeing various fisheries research activities. Furthermore, the human resources involved in fisheries activities are nurtured and developed through academic institutions such as the Agriculture and Forest University and Tribhuvan University. These universities play a key role in shaping the skill sets and knowledge base of individuals contributing to the fisheries sector in Nepal.

While fish consumption in Nepal lags behind poultry, pork, buffalo, and mutton, a growing awareness of the health benefits associated with fish consumption has fueled increased demand for aquaculture products (Rijal and Jha 2020). The government actively supports the establishment of commercial fish farms to boost employment and income in rural areas. Notably, many of these newly established farms are managed by returning youths with experience abroad, contributing to a reduction in youth migration and aligning with the broader goal of promoting economic development and sustainability in Nepal's aquaculture sector (Rijal and Jha 2020).

PRODUCTION AND PRODUCTIVITY TREND

Capture Fisheries

Nepal is rich in natural water resources, with rivers, lakes, reservoirs, swamps, and lowland irrigated paddy fields standing out as significant sources of fresh water (Figure 1). Rivers and lowland irrigated paddy fields emerge as the primary and most influential natural water resources. Moreover, lakes marginal swamps and reservoirs cover a smaller water surface area compared to other natural water resources. In addition to these water bodies, there are extensive network of 7,900 kilometers of irrigation canals throughout the country (Gurung 2014).

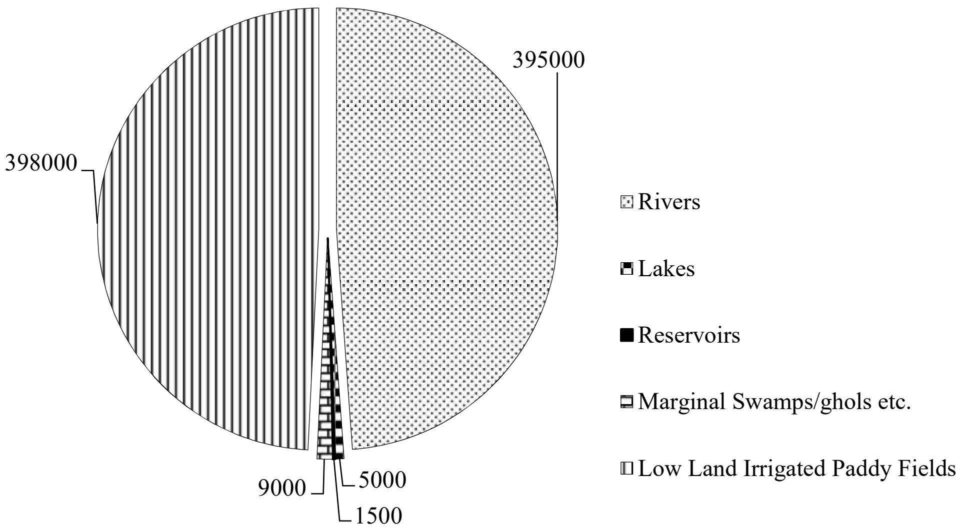

Figure 1: Natural water resources (ha) in Nepal

The capture fisheries sector is vital for its dual role in fish production and employment generation. Fish production from capture fisheries has remained stable at 21,000 tons over recent years. Noteworthy contributors to capture fish production include irrigated paddy fields, rivers, and swamps, while lakes and reservoirs play a minimal role (Figure 2). The preeminent contributors to the natural water area in Nepal are rivers and lowland irrigated paddy fields, collectively constituting 98% of the total natural water surface. Despite their expansive coverage, these areas demonstrate comparatively low productivity, yielding 18 kg of fish per hectare. In contrast, lakes and reservoirs, encompassing a smaller portion of the natural water landscape, exhibit higher productivity at 200 kg and 350 kg per hectare, respectively. Notably, marginal swamps, constituting a mere 1.1% of the total natural water body, stand out for their remarkably elevated productivity, producing 577 kg of fish per hectare. This data highlights the complex interplay between the extent of natural water areas and their corresponding productivity levels, providing valuable insights for fisheries management strategies in Nepal.

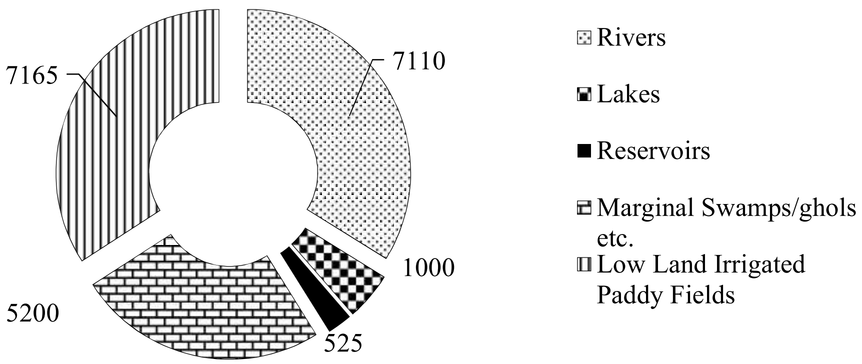

Figure 2: Fish capture (mt) from diverse natural aquatic sources.

While capture fisheries initially exhibited an increasing trend, it has remained almost constant since the year 2000, posing a significant challenge to maintain this capture at a standstill. Therefore, there is a need for a comprehensive reevaluation of the status and potential of capture fisheries, including an assessment of freshwater snail, crab, shrimp, water chestnut, and makhana collection and yield. It is also essential to estimate the communities and population that consume these products, along with assessing the farming perspectives of these fisheries commodities.

Aquaculture

Pond fish culture emerges as the predominant practice among various fish culture methods, experiencing rapid growth while other aquaculture activities have largely remained stagnant over the past decades. Since the inception of aquaculture development in Nepal, finfish culture has been a major focus, with a significant portion dedicated to major carps, particularly common carp and Chinese carps, utilized in carp poly-culture. This approach has made substantial contributions to aquaculture production in Nepal. The introduction of new technologies and species has seemingly led to an increase in aquaculture production in the country.

The introduction of Rainbow trout has brought a fresh perspective to cold-water aquaculture, marking a paradigm shift in Nepal's aquaculture landscape. Similarly, the introduction of Tilapia and Pangasius has opened up new avenues for promoting monoculture systems, offering significant production potential for aquaculture in Nepal. The innovation of the 'chhadi fish' pond aquaculture technology has proven to be a lucrative investment for fish farmers, enabling faster returns and increasing national production. In this technology, fry/hatchlings are densely stocked in ponds, and multiple harvests (at least 3-4 times a year) is done to capture fish that have reached sizes less than 50 g contributing 35% of total fish production (Pathak et al., 2023). Local consumers show a preference for smaller, single-piece fish from head to tail, referred to as 'chhadi' fish (Rijal and Jha 2020). The national fish production has witnessed a remarkable increase, surging more than four fold over the last 20 years, from 17,665 tons in 2002/03 to 97,385 tons in 2021/22 (Figure 3).

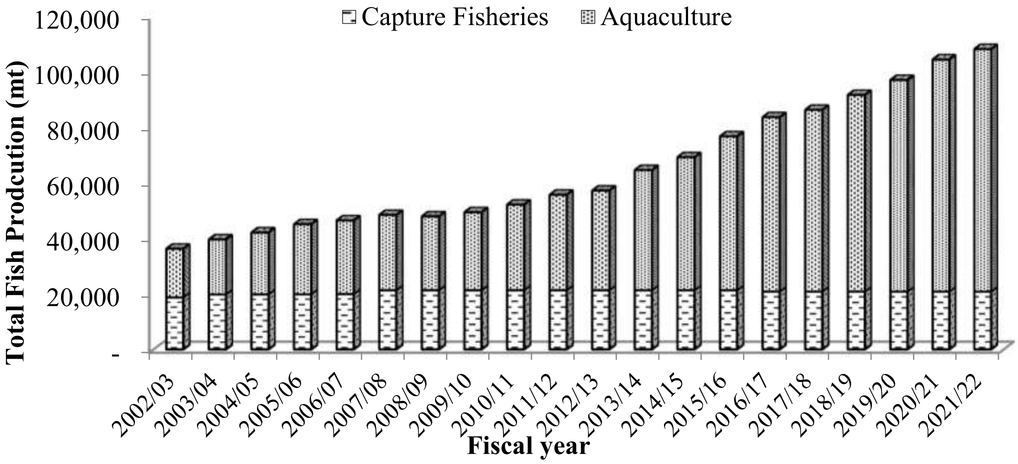

Figure 3: Fish production trend capture fisheries and aquaculture.

The state of aquaculture in Nepal is currently in a phase of growth, and although the fish production levels are comparatively lower than those of larger countries worldwide, recent advancements in the sector are highly promising. Pond aquaculture, particularly with common carps, Chinese, and

Indigenous Major Carps, significantly dominates the overall fish production, boasting an average productivity of 5.41 tons per hectare. Monoculture of common carp, tilapia, and especially catfish is also practiced in various locations across the country. The interest in aquaculture is growing swiftly among young farmers, reaching 55 districts out of 75 in 2017, a significant jump from 30 districts a decade ago (Chaudhary and Jha 2018). Remarkably, fish farming has expanded to 76 districts out of 77 in the current scenario. This surge in interest is especially notable following the successful implementation of rainbow trout farming technologies in the colder regions of hills and mountains.

Currently, the poly-culture technology of carp fish farming in ponds has been widely disseminated in the southern plain areas and mid-hill regions of the country, emerging as a viable and common aquaculture activity. However, mono culture of pangasius and tilapia are also gaining popularity contributing about 10% of fish production from pond. Pond culture alone contributed to 71.33% (77,320 mt) of the total fish production in 2021/22 (CFPCC 2021/22) (Table 1).

Following pond aquaculture, swamps represent the second-largest contributor to fish production, encompassing an area of 3,550 hectares and yielding 8,930 mt of fish in 2021/22. These swamps are predominantly concentrated in the mid-western and far-western Terai region of Nepal. To enhance the productivity of these swamps for various purposes, their restoration, maintenance, and management are imperative for the sustainability of natural resources and the well-being of marginalized communities relying on them for food, nutrition, livelihood, and employment opportunities.

Cage culture practices in lakes and reservoirs contributed approximately 313 mt of fish in the fiscal year 2021/22. The introduction of cage fish culture technology in Nepal dates back to 1972 in Lake Phewa, initially used for raising brood fish of common carp. Presently, the estimated fish culture coverage area has reached nearly 73,803 cubic meters with an average fish productivity of 4.24 kg/m^3. Cage fish cultivation primarily involves plankton-feeding fish, relying on naturally available phytoplankton, zooplankton, detritus, and some aquatic vegetation for growth. While external feed is generally not applied, there is a possibility that this practice may evolve in the future due to the potentially increased profitability of adopting feeding practices in cages.

Rice cum fish culture, a successful farming technique in countries like India, Indonesia, China, and Bangladesh, has gained limited attention in Nepal. The extent of land dedicated to Rice cum fish farming has experienced a substantial reduction, declining from 300 hectares in the fiscal year 2007/08 to a mere 49 hectares in 2021/22, accompanied by a decline in productivity as well. The notable reduction in area can be attributed to limited availability of fish seed in mid hills and increased pesticide usage in rice fields. However, to promote this integrated farming system, especially in the Terai and lower mid-hill regions, special long-term projects should be prioritized in the future.

The introduction of rainbow trout, a cold-water species, began in 1969 from India and later from England and Japan in 1988 (Rai 2010). After the successful breeding program of trout commercial trout farming initiated in Rasuwa and Nuwakot districts under collaborative efforts of Nepal Agricultural Research Council, Japan International Cooperative Agency, and the Directorate of Fisheries Development Program contributing a significant growth of trout farming in Nepal. Then, trout has become a distinctive and valuable fish species in the Nepali market, known for its taste and high nutritional value. The production of trout has gained popularity and witnessed a substantial increase, reaching 747 metric tons in the fiscal year 2021/22. This is a remarkable surge from a mere 192 metric tons a decade ago. Furthermore, integrating trout farms with restaurants has become a successful practice, particularly beneficial for small-scale farmers to sustain their businesses.

Table 1: Status of aquaculture production in 2021/22 (CFPCC 2021/22)

Particulars	Pond (no.)	Total Area (ha)	Fish Production (mt)	Productivity kg/ha
1. Aquaculture production			**87,385**	
1.1 Pond Fish culture(Carps)	49,862	14,137	77,320	5,419
1.1.1 Mountain	670	48.96	134	2,737
1.1.2 Hill	8,874	1,452.85	6,386	4,396
1.1.3 Terai	40,318	12,635.2	70,799	5,603
1.2 Marginal Swamps (ghols)		3,550	8,930	2,515
1.3 Rice cum fish culture		49	17	288
1.4 Cage fish culture (m^3)		73,803	313	
1.5 Enclosure fish culture		40	53	1,325
1.6 Trout Fish Culture in Raceway		5.3	747	140,943
1.7 Fish Production in Public Sector			5	
2. Capture Fisheries production			**21,000**	
3. Total Fish Production			**108,385**	

The productivity of pond fish was only 0.8 tons per hectare in 1981/82 but has seen a significant increase, reaching 5.41 tons per hectare by 2021/22. This notable increase in pond fish productivity is attributed by various factors, including improved availability of fry, effective fertilization practices, strategic feeding regimes, and enhanced management practices such as the introduction of aeration technology, effective control measures for fish diseases, comprehensive training programs, and adherence to Good Management Practices (GMP). Furthermore, the Government of Nepal consistently underscores the importance use of improved technology, advocating for the utilization of pellet machines to produce cost-effective, high-quality feed on farms and the incorporation of aerators to enhance water quality. These measures are intended to achieve higher productivity in pond fish culture.

The prevalent fish culture technique, carp poly-culture, is predominantly characterized by the dominance of Chinese Carps, Indian Carps, and Common carps in production. Within this context, Silver carp holds the highest contribution to production, accounting for 21% of the total, followed by Common carp at 18% and Naini at 16%. In contrast, Rainbow trout and Tilapia exhibit minimal contributions, each are making up around 1% of the overall production.

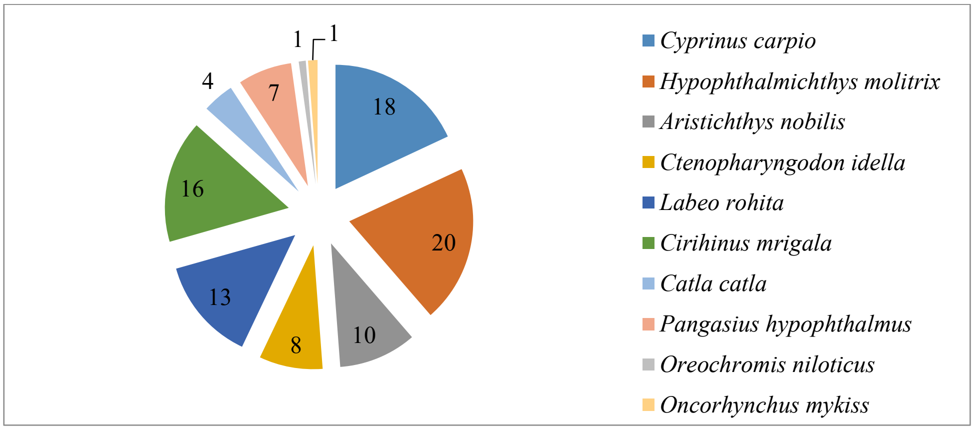

Figure 4: Percentage of species wise contribution in aquaculture.

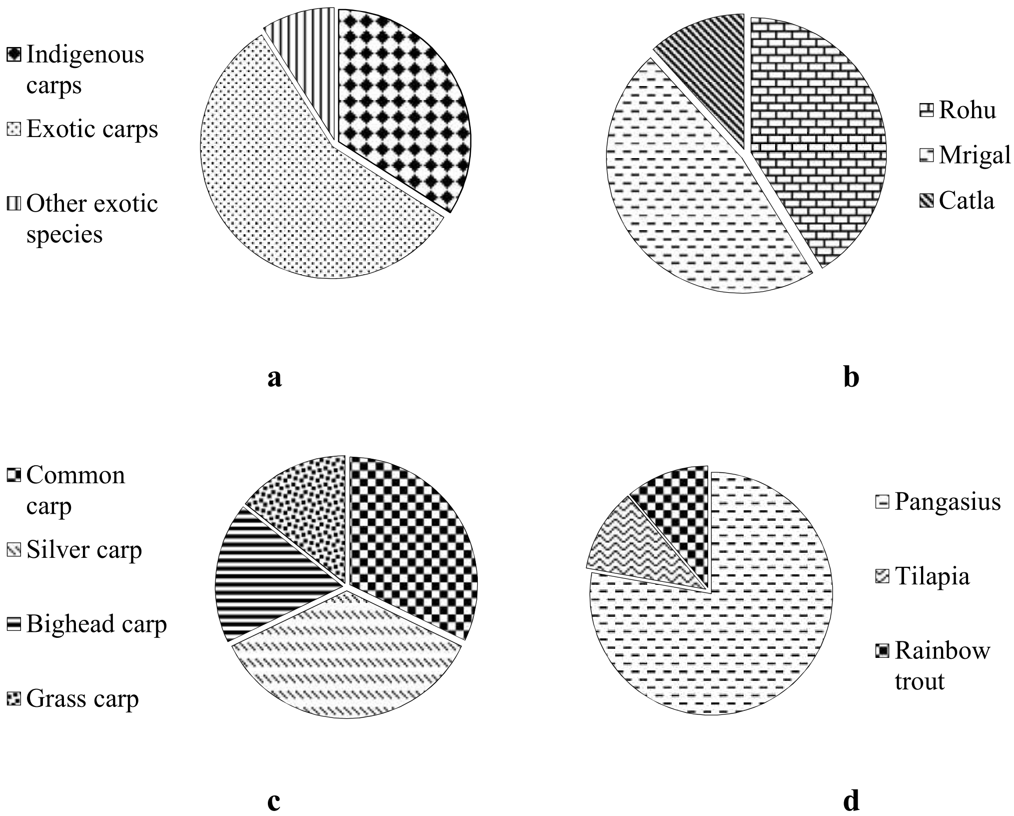

Figure 4: Species wise contribution in aquaculture production (**a.** Group wise contribution; **b.** Contribution of indigenous carps; **c.** Contribution of exotic carps; **d.** Contribution of other exotic species)

FISH SEED PRODUCTION

Seed is a critical input for aquaculture production, and ensuring the quality of seed is essential for enhancing the productivity of aqua farms (Kunwar and Adhikari 2017). In Nepal, fish seed is distributed in three forms: hatchlings (4-5 days old), fry (2-3 cm or more than 1 g), and fingerlings (more than 5 g body weight on average).

Table 2: Status of fish seed production in 2021/22 (CFPCC 2021/22)

A. Fish seed(Fry) Production/Distribution (No. in '000)	538,742
A[1] Public Sector	127,974
a. Hatchling*	332,600
b. Fry	15,172
c. Fingerling	14,826
A[2] Private Sector (Fry)	410,768

*Hatchling of public sector is distributed for fry production in private sector

In the context of Nepal, while self-sufficiency has been attained in the production of fish seed of carp species and rainbow trout, there persists a requirement for importing (around 90% of total demand) of Pangasius fingerling to sustain pangasius production. The fish seed sector in the country involves the participation of seven government institution and five research centers, 99 private hatcheries and 232 nurseries. The aggregate fish seed production in Nepal has reached 538 million, with private sector contributing around 76%, and public sector contributing the remaining 24%.

Over the past decade, seed supply by both private and public sector has experienced a substantial increase producing 5.7 million in 2001/02 to 538 million in 2021/22 (Table 3). This growth can be attributed to the government's emphasis on encouraging private sector participation in seed supply. Various supportive programs have been initiated to empower the private sector, including the establishment of fish seed resource centers under private ownership.

AQUACULTURE EXPANSION

The aquaculture sector in Nepal has evolved into a lucrative sub-sector of agriculture, providing favorable returns on investment. In the Terai region of Nepal, the consumer base for fish is expanding, driven by increased availability in the market. Aquaculture practices in the country encompass diverse areas, including pond culture, rainbow trout culture in raceways, rice fish farming, fish culture in swamps and enclosure, cage culture with innovative and modern techniques like aquaponic, re-circulating aquaculture.

Despite the diverse array of aquaculture practices, pond culture has gained the highest popularity, witnessing substantial growth from 5,987 hectares in 2002/03 to 14,137 hectares in 2021/22.This is closely followed by trout culture in raceways and cage culture, while rice-fish farming and enclosure fish farming are on a declining trend. Initially, the adoption of pond aquaculture was limited due to high initial investments and insufficient technologies and expertise, coupled with infrastructural challenges. However, the scenario changed with the initiation of a government subsidy program for pond construction, leading to a notable increase in the popularity of pond fish farming.

The Government of Nepal has continued its support for aquaculture businesses through various subsidy programs, contributing to the widespread adoption of pond fish aquaculture. The number and water surface area of fish cultivating ponds have seen a marked increase (Figure 4). The fiscal year 2015/16 witnessed the highest achievement in pond construction, reaching 734 hectares (CFPCC 2017). While pond fish culture remains dominant in the Terai belt, its expansion into hill regions has gained momentum, particularly after the government implemented a pond expansion program in mid-hill districts starting from the fiscal year 2011/12.

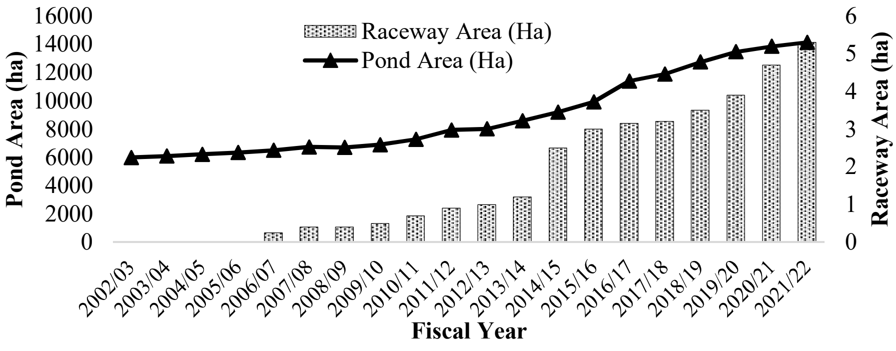

Figure 5: Pond and raceway area expansion trend.

The expansion of areas was primarily concentrated in the Terai region of Nepal, attributed to its favorable climate for warm water fish culture. Conversely, significant growth in raceway areas was observed in the middle hills of the central part of the country. Considering that the Terai region was a major production area, Madhesh province exhibits the highest number of ponds and water areas, followed by the Lumbini province. In contrast, Karnali Province demonstrates the least contribution to the aquaculture sector, contributing less than 1% in both pond area and raceways.

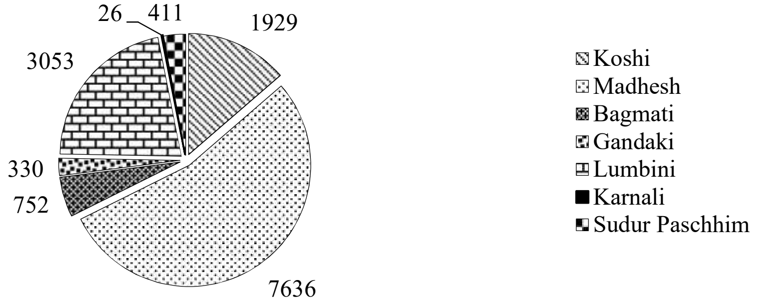

Figure 6: Contribution of different province in pond water surface area (ha)

ECONOMIC CONTRIBUTIONAND EMPLOYMENT GENERATION

In Nepal, the pervasive issue of unemployment, driving a significant annual migration of youths in search of jobs, poses a serious challenge and heavy reliance on remittance in the Nepali economy makes it vulnerable and unstable (ILO 2015). To address the outmigration problem and foster national development, the expansion of aquaculture emerges as a viable option to create jobs domestically and attract young talent within the nation. Given this context, the fisheries sub-sector holds potential as an alternative, offering employment opportunities in various fisheries and aquaculture-related activities.

The combined efforts of capture fisheries and aquaculture, including related activities such as harvesting, processing, marketing, and other industries associated with aquaculture, are generating substantial employment opportunities for a significant portion of the population. Currently, the aquaculture and fisheries sector contribute 0.44% to GDP and 1.83% to AGDP. Notably, the sector's contribution to the economy is on a consistent upward trajectory.

Capture fisheries contribution in employment generation

Natural water bodies, particularly rivers, swamps, and lakes, play a vital role in sustaining the economy of numerous fishing communities. Approximately twelve distinct ethnic communities are directly or indirectly involved in fisheries (Gurung 2005). These communities reside in close proximity to water resources, relying on fisheries and aquatic resources for their livelihoods across generations. Engaging around 365,596 individuals in capture fisheries (CFPCC 2022) with 60% being female, these communities actively participate in various aspects of the fishing industry (Rijal and Jha 2020). Female members contribute not only to the fishing activities but also play roles in the preparation of fishing gears, nets, and other equipment, as well as in the selling of fish in the market. While capture fisheries have been a traditional source of livelihood for many, the number of individuals relying on natural water bodies for their livelihoods through capture fisheries is gradually diminishing. This decline is attributed to the comparatively low income generated from capture fisheries, prompting individuals to seek alternative and more lucrative income-generating opportunities.

Aquaculture contribution in employment generation

Over the past few decades, aquaculture has evolved into a more intensive and diversified practice, offering faster returns on investment. Consequently, there has been a substantial increase in the participation of individuals in the aquaculture business, and the number of households engaged in the aquaculture sector has witnessed significant growth. In the last 20 years, these figures have doubled, with the number of people involved reaching 154,762, and the number of households engaged in aquaculture rising to 60,271 in fiscal year 2021/22.In the realm of aquaculture and related businesses, there is a predominant male presence, making up 68% of the workforce (Rijal and Jha 2020). This stands in contrast to the opposite scenario observed in capture fisheries.

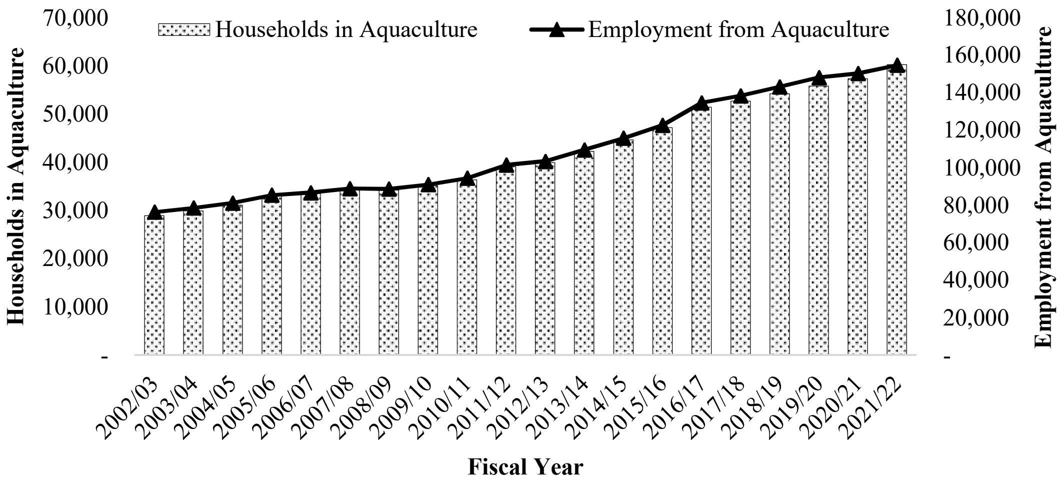

Figure 7: Trend of employment (no) from aquaculture and household (no) in aquaculture.

FISH MARKET AND MARKETING SYSTEM

Fish marketing system in Nepal is not well developed and marketing strategies exhibit variability across regions, lacking a singular approach. The various functions or services within fish marketing encompass collecting small quantities from numerous producers, grading, packing, transporting to distant city-based wholesale markets, and distributing to retailers. Small produce is directly dispatch to local markets by farmers themselves and for substantial productions, contractors typically handle marketing activities. Farmer organizations often operate through cooperatives, exemplified by the success of Harpan Phewa Matsya Sahakari in Kaski district and similar cooperatives in Nawalparasi, Rupandehi, and Kanchanpur districts (KBNPK 2010).

A recent trend in the emergence of live fish marketing systems is observed, reflected in the increasing number of live fish shops. The government supports this by providing financial assistance to establish fish marketing stalls and collection centers. Concentrated mainly in the capital and other major cities where demand is high, there are currently stalls in Nepal. Over the last decade, the prices of agricultural commodities, including fish, have seen a substantial increase. In 2001/02, fresh fish was priced at NPR 100 per kg, and was 300 per kg in 2018 (Chaudhary and Jha 2020) which has now reached an average of NPR 350 per kg. Despite the rise in price, fish remains a more affordable source of animal protein for lower and middle-class citizens compared to other meat products.

Fish prices vary across locations, with metropolitan and capital cities experiencing higher costs. The demand for fish fluctuates monthly, with a study revealing increased fish demand during winter and lower consumption reported in Asadh, Shrawan, and Bhadra (KBNPK 2010). While national production falls short of meeting the entire demand, Nepal imports a certain quantity of fish. India stands as the major fish exporter, followed by Vietnam, China, and Bangladesh, contributing to fish imports in Nepal.

Table 3: Price of fish in Kalimati market (Source: Kalimati Fruit and Vegetable Market Development Board, Date: 2022/10/03)

Fish Species	Minimum Price	Maximum Price	Average Price
Fresh Rohu	330	360	345
Fresh Pangasius	260	280	270
Fresh Chhadi	240	260	250

Table 4: Price of farm gate and consumer price in Chitwan (Source: Fishermen's Association Nepal, Chitwan, Date: 2022/10/03)

Fish Species	Farm gate price	Consumers price in market
Silver carp, Tilapia	280	380
Other Carps	330	430
Pangasius, Chhadi	250	300-350

Most fish markets in Nepal portray local, unregistered, and unmanaged characteristics, frequently lacking adequate sanitary maintenance. However, in urban areas, a few well-managed markets stand out. The principal challenges faced by fish markets in Nepal include issues related to the preservation of fish quality, inadequate waste management practices, lack of market accessories like collection center, chilling center, road connectivity to the production site and the use of unscientific and unsophisticated methods for fish transportation, and a limited emphasis on product diversification and value addition.

Post-harvest management

Post-harvest loss in the fish industry pertains to the disposal or sale of fish at a reduced value due to either a decline in quality or market-related factors (Dhakal et al. 2020). Fish, being a highly perishable agricultural commodity, should be preserved promptly following harvest. However, Nepal is at an early stage in post-harvest management and most of the harvested fish are sold within a day in local market with a significantly low amount of post-harvest loss accounting only 3-5%.

In Nepal, typical post-harvest management practices include grading of fish based on size and species at the harvest site. These graded fish are then transferred to local markets by local vendors. For those fish destined for distant markets, middleman collect them at a centralized collection center. Subsequently, the fish are graded and packed in Styrofoam with a 2:1 ratio of fish to ice. Various types of vehicles are employed to transport the packaged fish to distant markets. Additionally, given the popularity of selling live fish in the country, live fish are collected and transported to distant markets in tanks equipped with aeration facilities. The primary obstacles in post-harvest management include insufficient awareness regarding its significance, a dearth of resources and infrastructure, elevated costs associated with post-harvest management, insufficient training, and gaps in existing policies.

Value addition of fishery products is gaining popularity in Nepal. The primary motivation behind this shift towards value-added products is to enhance the product's price, make it convenient or ready-to-consume, and secure better prices during periods of surplus production. The predominant methods of value addition in Nepal have traditionally been drying and smoking. However, contemporary techniques such as freezing and vacuum packaging, pickling, and filleting are becoming increasingly popular in the current scenario.

Import and export of fish and fishery products

Nepal is at an early stage in post-harvest management, resulting in approximately a 10% loss. Typical post-harvest management practices in Nepal involve grading based on fish size and species, followed by preservation in a ratio of 2:1 for fish and ice. Post-harvest loss in fish refers to fish that is either discarded or sold at a relatively low price because of quality deterioration or owing to market dynamics.

The import of fish in Nepal shows decreasing trend, with less than 4% of the total fish consumption in the fiscal year 2021/22 attributed to imports from various countries. This marks a substantial decline from the approximately 20% observed a decade ago. The primary contributors to the import volume are fresh rohu and pangasius, followed by notable quantities of dried fish and pangasius fillets. The import portfolio also encompasses comparatively smaller quantities of species such as salmon, shrimp, scallops, mussels, octopus, and marine arachnids. The export value is notably lower compared to the import figures in Nepal's fishery trade. A substantial portion of the exports is directed towards India, primarily comprising fresh fish sourced from ponds located in close proximity to the border.

Table 5: Import and Export value of fish and fishery product in Nepal (Source: Costume Department)

Fiscal Year	Import (thousand, NPR)	Export (thousand, NPR)
2017/18	1,853,570	1,911
2018/19	1,894,018	1,459
2019/20	1,765,136	334
2020/21	1,698,061	0
2021/22	1,346,580	1

MAJOR ISSUES OF THE SECTOR

Despite its vast scope and potential, the sector has not experienced the anticipated growth due to various challenges. Issues related to policies, technical aspects, human resources, and organizational matters persist. Essential institutions such as Central and provincial Fish labs, Rainbow Trout and Aquarium Fish Development Centers are notably absent. Additionally, Karnali province lacks a dedicated Fish Research and Development Center.

The absence of a specific Aquaculture and Fishery Act to regulate the sector is a significant drawback. The current regulation under the Animal Health and Livestock Service Act 2055 does not adequately address the concerns of the fishery sector. The sector also grapples with the unavailability of sufficient and quality inputs such as seeds, feed, and machinery. The lack of a well-established marketing system contributes to high losses and safety issues.

Furthermore, fish farmers face challenges, including high electricity charges and the unmet demand for Krishi Meters in Aquaculture. Access to insurance and concessional loans is limited, and the sector remains highly vulnerable to climate change. Nevertheless, the most pressing challenge remains the scarcity of human resources, as only 312 technicians cater to the sector under central, provincial, and local government jurisdictions. This shortage of personnel makes it difficult for farmers to access technical services, compounded by a low ratio of extension workers to farmers, standing at approximately 1:192.

CONCLUSION AND RECOMMENDATION

Aquaculture stands as a thriving sector within Nepal's food industry, boasting an annual growth rate of approximately 10% in recent decade, the highest among SAARC nations. Recognizing its significance and potential, both federal and provincial governments are increasingly directing attention towards aquaculture. This focus suggests a potential substantial increase in fish production within the country. The escalating demand for fish has created market opportunities, attracting investments in commercial fish farms. However, newly established farms require technical support to enhance competitiveness in local, regional, and global markets.

The current state of technical expertise among human resources in aquaculture is insufficient to represent the advancements of the 21st century due to limited exposure to study and training programs. Specialized hands-on training and studies in fields such as fish breeding, disease management, nutrition, genetics, and water quality are essential aspects that need attention from relevant authorities in the near future. Establishing a robust coordination mechanism among development, research, and educational institutions is imperative for effective and efficient implementation of aquaculture and fisheries programs.

Pond aquaculture currently dominates the fish farming landscape and is a prioritized practice. However, marginal swamps, covering an area of 12,500 hectares, should not be neglected. Only 30% of these swamps are currently utilized for aquaculture, highlighting the need for proper planning and management to optimize fish production. This will not only generate employment opportunities but also provide income to many landless individuals. Nepal's abundant natural water resources, including lakes, reservoirs, and swamps, make the nation highly potential for culture-based fisheries, an area that is still underutilized for fish production.

The favorable water resources and climatic conditions in Nepal also support cold-water fisheries, particularly in trout farming. Promoting trout culture requires minimizing production costs to attract more farmers in the future, making trout accessible to middle-class consumers. Similarly, for sustainable development of fishery sector, our indigenous fish breed should be conserved and promoted. Fish like Asala, Sahar, Katle having unique taste and features should be promoted for commercial culture developing package of production. For sustainable utilization and promotion of the sector, effective implementation of Fishery Development Policy, 2079 is crucial. All the institution involved in the development of this sector, should plan and implement program according to the objective envisioned by Policy.

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SYNERGETIC EFFECTS OF STINGING NETTLE (*Urtica parviflora*) WITH MULTI-ENZYME INCORPORATED SHRIMP BASED FEED ON GROWTH AND CORTISOL LEVEL OF RAINBOW TROUT (*Oncorhynchus mykiss*) FINGERLINGS

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ABSTRACT
The study, conducted over 90 days (October 18 to January 17, 2021), investigated the impact of stinging nettle, also known as sisnu (*Urtica parviflora*) and multi-enzyme supplements on Rainbow trout fingerlings. Five treatments were tested in a complete randomized design (CRD), with varying levels of sisnu meal and enzymes added to a shrimp-based diet. Results showed that T4 (1.5% sisnu meal + 0.05% multi-enzyme) had significantly higher mean extrapolated net fish yield (85.1±2.3t/ha/cycle), daily weight gain (1.16±0.03g/fish/day), specific growth rate (1.90±0.04%/day), and lower feed conversion ratio (1.7±0.05) compared to other treatments. Cortisol levels was significantly lower in T4 (0.0447±0.001ng/mL) as compared to T1, T2, and T3 but higher than T5. The mean profit of T4 (441±10 NRs/kg) and benefit-cost ratio (1.79±0.03) was significantly higher than other treatments. The study suggests that incorporating 1.5% sisnu meal and multi-enzyme supplements can enhance shrimp-based feed efficacy for trout growth. Further exploration of unconventional feed sources with enzyme supplementation is recommended for optimal results at advanced stages.

Key words: Rainbow trout, Stinging nettle, Multi-enzyme, Cortisol level, Benefit-cost ratio.

INTRODUCTION
Rainbow trout (*Onchorhynchus mykiss*) was introduced in Nepal in 1988 to meet many needs including use of vast cold-water resources for aquaculture in mountain region of the country and import substitution of high valued fish and alike commodities. Since then, the trout farming has been expanded to 38 districts of Nepal (CFPCC 2022) with a slow growth pace which is indicated by the national production of 0.045 metric tons in 1998 to 668 metric tons in 2021/22 (CFPCC 2022). The lack of improved feed, as well as the farmers' inability to obtain trout feed at a reasonable price are important obstacles in rainbow trout culture in Nepal (Gurung et al. 2017). One of the major challenges for wider up scaling of trout culture throughout the country is supply of quality feed.

Fishmeal and shrimp meal are an essential component in the diet of trout. In view of the increasing cost and relative scarcity of these feed stuffs, a considerable research effort has been expanded in the country on evaluating the suitability of plant ingredients as complete or partial replacement of the fishmeal components in trout diet. Soybean meal, the promising alternative to shrimp meal has about two-thirds of phytate (Inositol hexakisphosphate) which interacts with trace minerals and protein, reducing their availability (NRC 1993). Influence of dietary factors is recognized as cofounding factor to health status and survival of fishes in intensive systems (Blazer 1992; NRC 2011). Use of dietary natural additives and feed supplements on the immune system, and growth function is a new approach (Ringo and Song 2016; Saeidi Asl et al. 2017).

Among the various herbal supplements, stinging nettle locally called as sisnu is one of new thrust in aquaculture. *Urtica parviflora*, one of common Nepalese sisnu species is gaining importance for its cost-effectiveness, availability, easy to process & potent immune-stimulatory functions (Gulcin et al. 2004; Turker and Yıldırım 2015). Due to these attributes of this herb, make it a great, affordable, and well recognized dietary addition (Vicco et al. 2018). The impacts of locally available stinging nettle on the growth and immunity of fish including the rainbow trout, has been the subject of extensive research (Awad and Austin 2010; Saeidi Asl et al. 2017). Exogenous enzyme supplementation improves endogenous enzyme activity, decomposes anti-nutrients, improves feed consumption, and promotes growth (Goda et al. 2012; Carvalho et al. 2017; Komar and Yaser 2019). Exogenous enzyme supplementation in feed not only reduces environmental impact by recycling feed intake (Verdegem 2013) but also improves animal digestion by converting feed into tiny molecules (Bedford 2000). Many studies have also demonstrated that exogenous multi-enzyme mixture with reasonable adjustment could significantly enhance fish growth and enhance feed efficiency (Ghomi et al. 2012). Investigations on interactions between phytochemical plants and commercial multi-enzymes with aim of assessing the potential of their combined effects on growth performance of fish are not enough. Therefore, this study was conducted to assess the possibility of supplementation of *U. parviflora* and commercial multi-enzyme as growth promoters in the diet of rainbow trout fingerlings.

MATERIAL AND METHODS

Experimental site and design

The experiment was conducted for 90 days (October 18, 2020 to January 17, 2021) at National Fishery Research Centre (NFRC) Godawari, Lalitpur. Fingerlings of rainbow trout with an average weight of 23.1±0.3g were procured from Sindhu Trout Farm, Helambu-3, Sindhupalchok. Fingerlings were acclimatized to the experimental rearing condition for one week and fed with control diet (CP 30%). After acclimatization, fingerlings were stocked at density 83 fish/m^2 in 15 sub raceways having surface area 1.6 m^2. Five treatments viz T1-Shrimp based feed (Control), T2 (control+0.05% multi-enzyme), T3 (control+0.05% multienzyme+0.75 % sisnu meal), T4 (control+0.05% multienzyme+1.5% sisnu meal), T5 (control+0.05% multienzyme+2.25% sisnu meal) were established following a completely randomized design with three replicates.

Experimental diet preparation

Fresh young tender leaves of wild stinging nettle were collected from Rainbow Trout Fisheries Research Station, Dhunche, Rasuwa surroundings. Authentication and identification of the plant was carried out at the National Herbarium and Plant Laboratories (NHPL), Godawari. The leaves were cleaned, cut into small pieces and air dried under shade to prevent the loss of vitamins and other volatile nutrients. The final powder was stored in plastic bag, air tied and stored in refrigerator at 4°C until use. The proximate analysis of stinging nettle was carried out before including in the research diet. To prepare experimental diet, all feed ingredients (Table 1) were grinded thoroughly and sieved to pass through 0.5 mm mesh size. The feed ingredients including multi-enzyme for the respective feed treatments were mixed together in a blender and pelletized in a pellet machine. The composition of multi-enzyme is given in Table 2. The proximate composition of the experimental diets was determined following standard methods of AOAC (1990) in National Food Research Centre, Khumaltar, Nepal (Table 3).

Table 1: Percent composition of ingredients in the formulation of the test diets for Rainbow trout fingerlings

	Treatments				
Ingredient	**T1**	**T2**	**T3**	**T4**	**T5**
Jwala (small shrimp)	54	54	54	54	54
Soybean full roasted	35	35	34.2	34	33.7
Wheat flour	5	5	5	4.5	4
Soybean oil	6	6	6	6	6
Additives					
Sisnu leaf powder	0	0	0.75	1.5	2.25
Multi-enzyme	0	0.05	0.05	0.05	0.05
Additional					
Vitamin premix	1	1	1	1	1
Mineral mix	1	1	1	1	1
Vitamin E	0.25	0.25	0.25	0.25	0.25
Rena-c premix	0.1	0.1	0.1	0.1	0.1
Salt	1	1	1	1	1
Yeast	1	1	1	1	1

Table 2: Composition of multi-enzyme included in the experimental diet of Rainbow trout fingerlings

Enzyme	**Per kg composition**
Amylase	3,00,000 IU
Protease	3,00,000 IU
Lipase	2,00,000 IU
Alpha Galactosidase	1,00,000 IU
Beta Galactosidase	1,00,000 IU
Xylanase	10,00,000 IU
Cellulase	2,00,000 IU
Pectinase	2,00,000 IU
Glucanase	1,00,000 IU
Phytase	2,00,000 IU

Table 3: Proximate analysis of formulated feed for Rainbow trout fingerlings

	Experimental feed treatments				
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
Moisture %	3.66	3.47	3.89	3.95	3.96
Crude fat %	12.54	12.52	12.49	12.43	12.39
Total ash %	17.84	17.79	17.88	17.81	18.06
Crude protein %	36.32	36.32	36.30	36.29	36.28
Crude fiber %	4.01	4.01	4.15	4.19	4.22

Feeding

Experimental fish were fed four times a day for 90 days, starting from 8:00 h to 16:00 h at 4-hour interval, with their respective diets at the rate of 4% of the total fish biomass until fish reach to 100 g individual size. Later, the feeding rate was decreased to 3.5% of the total fish biomass until harvest. Fecal matter was removed by siphoning on alternate day. The flow-through system had a water flow of 4-5 $L.min^{-1}$ through an inlet at one and an outlet at the opposite end of each tank.

Sampling protocol

At fortnightly, 10% fishes from each experimental unit were randomly sampled and weighed to evaluate the growth of fish and adjustment of daily feed volume. Five trout fingerlings were randomly collected from each experimental unit at the end of experiment and anesthetized with clove oil at 40mg/L (Inoue et al. 2003). Blood was drawn from the caudal vein of each sampled fish into 1ml sterilized hypo-dermal syringe. Blood sample was transferred to micro tube containing heparin as anti-coagulant and immediately used for the analysis of plasma cortisol as an indicator of stress. Plasma cortisol level in the blood was analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) test method and the analyses was performed in National Animal Health Research Centre, Khumaltar.

Determination of growth performance

Growth performance of the fish was measured in terms of daily weight gain (DWG), specific growth rate and other parameters calculated were survival rate (SR), feed conversion ratio (FCR) and net fish yield (NFY) protein efficiency ratio (PER) using following formula:

$$\text{Daily weight gain (g/fish/day)} = \frac{W_t - W_0}{\text{Culture period (days)}}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln W_t - \ln W_0}{\text{Culture Period (days)}} \times 100$$

Where,

W_0= Mean stocking weight of fish

W_t= Mean harvesting weight of fish

$$\text{Net fish yield (t/ha/cycle)} = \frac{\text{Total harvest weight (kg)} - \text{Total stocking weight}}{\text{Area of experimental unit} \times 1000}$$

$$\text{Feed conversion ratio} = \frac{\text{Weight of feed consumed (g)}}{\text{Body weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total weight gain (g)}}{\text{Total protein intake (g)}}$$

Economic analysis:

The economic analysis was calculated mainly based on farm gate price for the stocked and harvested fish and current local market prices for all other inputs in Nepal. Total variable cost included seed and feed cost only. Price for rainbow trout fingerlings at the start of the experiment was 25 NRs per piece whereas farm gate price of harvested trout was 1000 NRs per kg. Total variable cost, Gross per unit cost, profit, and Gross margin and Benefit cost (BC) ratio was calculated as:

$$\text{Gross margin} = \text{Total income} - \text{Total variable cost}$$

$$\text{Total income} = \text{Income from fish sale}$$

$$\text{Total variable cost} = \text{Seed cost} + \text{Feed cost}$$

$$\text{Gross per unit cost (NRs./kg)} = \frac{\text{Total variable cost}}{\text{Total harvested fish}}$$

$$\text{Net profit (NRs./kg)} = \frac{\text{Gross margin}}{\text{Total harvested fish}}$$

$$\text{Benefit cost (B: C) ratio} = \frac{\text{Gross margin}}{\text{Total variable cost}}$$

Water quality analysis

Fortnightly water quality parameters including temperature, dissolved oxygen and pH of the experimental units were measured with digital pen thermometers, portable Hanna Oxygen and portable Hanna pH meters, respectively. Water samples were collected from each experimental units at the same time and stored in the refrigerator. Total ammonium nitrogen (TAN), Nitrate (NO_3) and Nitrite (NO_2) in the sampled water was measured in the laboratory using exact Eco check Kit #48698K.

Harvesting

At the end of experiment, rainbow trout was harvested by draining and seining each experimental unit. Harvested fish was measured using electric balance. Fish was counted, and weighed in batch.

Statistical analysis

The differences between the group means of body weight gain, SGR, FCR, and PER, were tested by analysis of variance (ANOVA). Duncan's Multiple Range Tests (DMRT) was applied to determine the significance of differences between any two means. All statistical tests were performed using statistical package SPSS (Version 25.0). Comparisons were made at 5% probability.

RESULTS

Water quality

Water quality was within acceptable ranges throughout the experiment although water pH was nearly lower boarder line for aquaculture (Table 4). Mean values of water quality parameters in different diet group treatments ranged between: temperature 13.5-13.7°C, dissolved oxygen 7.9-8.1 mg/L, pH 6.7-6.8, TAN 0.019-0.021 mg/, nitrate 0.25-0.28 mg/L, and nitrite 0.15-0.17 mg/L. TAN concentration was significantly high ($P<0.05$) in T2, T3 and T4 compared to the mean in T5. Similarly, T2 and T4 exhibited high concentration of nitrate ($P<0.05$) than in T1, T3 and T5. The concentration of nitrite was significantly higher in T4 ($P<0.05$) than in T5 and non-significant with other treatments.

Table 4: Mean±SE value of water quality parameters during experimental period of 90 days

Water quality parameters	Treatments				
	T1	T2	T3	T4	T5
Temperature(°C)	13.5 ±0.01[a]	13.6±0.01[a]	13.7±0.01[a]	13.5±0.01[a]	13.6±0.01[a]
DO (mg/L)	8.1 ± 0.01[a]	7.9± 0.01[a]	8.0±0.01[a]	7.9±0.01[a]	8.0±0.01[a]
pH	6.8 ± 0.01[a]	6.8±0.01[a]	6.7±0.01[a]	6.8±0.01[a]	6.8±0.01[a]
TAN (mg/L)	0.020±0.001[ab]	0.021±0.001[b]	0.021±0.001[b]	0.021±0.001[b]	0.019±0.001[a]
Nitrate (mg/L)	0.26±0.01[ab]	0.28±0.01[c]	0.27±0.01[bc]	0.28±0.01[c]	0.25±0.01[a]
Nitrite (mg/L)	0.16±0.01[ab]	0.16±0.01[ab]	0.16±0.01[ab]	0.17±0.01[b]	0.15±0.01[a]

Growth parameters

Plotting of bi-weekly body weight gain of fish revealed that the fish in all the treatments followed linear growth trend (Figure 1).

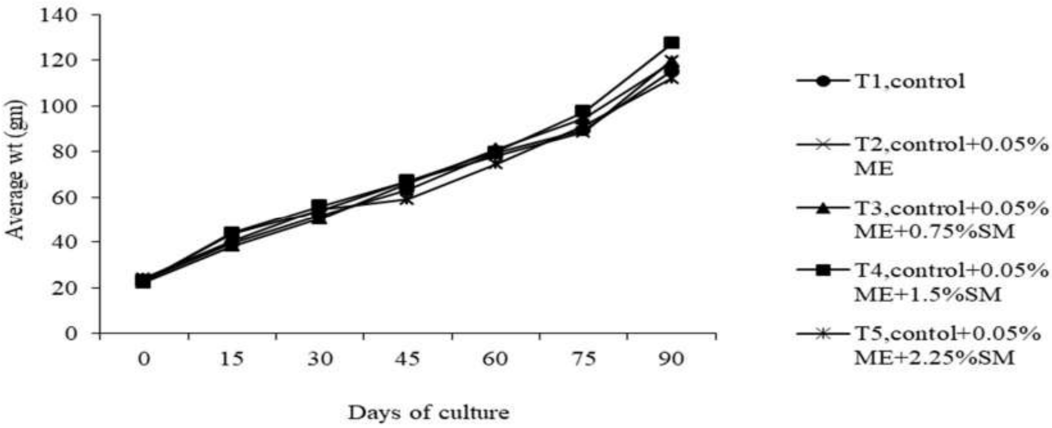

Figure 1: The bi-weekly growth trend of rainbow trout fingerling in different diet-treatments during the 90 days of experimental period

The final weight, weight gain, extrapolated net fish yield (NFY), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER), and survival rate of fish in different treatments are presented in Table.5. Results showed that there was no significant difference (P>0.05) in survival rate of fishes among the treatments at the end of experiment. The mean extrapolated net fish yield in T4 (85.1±2.3t/ha/cycle) was significantly higher (P<0.05) than other treatments. The mean daily weight gain (DWG) of T4 (1.16±0.03g/fish/day) was significantly higher (P<0.05) than all other treatments while the mean DWG of T2 (1.08±0.01g/fish/day) and T3 (1.07±0.01g/fish/day) were significantly higher than T5 (0.98±0.03g/fish/day) with no significant differences (P>0.05) with T1. The mean specific growth rate (SGR) of T4 (1.90±0.04) was significantly higher (P<0.05) than T1 and T5 while non-significant with T2 and T3. The mean SGR of T3 was significantly higher (P<0.05) than T5 with no significant differences than T2 and T1. The mean Feed conversion ratio (FCR) of T4 (1.7± 0.1) was significantly lower (P<0.05) than other treatments while the mean FCR of T2 and T3 were significantly lower (P<0.05) than T1 with no significant differences (P>0.05) with T5. Similarly, the mean protein efficiency ratio (PER) of T4 (1.85±0.08) was significantly higher (P<0.05) than other treatments.

Table 5: Mean±SE value of growth parameters of rainbow trout during experimental period of 90 days.

Growth parameters	Treatments				
	T1	T2	T3	T4	T5
Final weight (g/fish)	115.2±2.7[ab]	119.7±1.1[b]	119.0±0.8[ab]	127.3±1.3[c]	112.0±3.2[a]
Weight gain (g/fish)	92.0±2.2[ab]	96.8±1.2[b]	96.7±1.1[b]	104.3±2.3[c]	87.8±2.6[a]
Survival (%)	98.5±0.4[a]	97.7±0.4[a]	97.7±0.9[a]	97.7±0.4[a]	98.5±0.9[a]
Extrapolated net yield ton/ ha	75.1±2.2[a]	78.2±1.0[a]	78.1±1.4[a]	85.1±2.3[b]	73.0±2.2[a]
Daily weight gain (DWG)	1.02±0.02[ab]	1.08±0.01[b]	1.07±0.01[b]	1.16±0.03[c]	0.98±0.03[a]
Specific growth rate (SGR)	1.78±0.03[ab]	1.84±0.03[bc]	1.86±0.03[bc]	1.90±0.04[c]	1.70±0.01[a]
Food conversion ratio (FCR)	2.1±0.04[c]	1.9±0.02[b]	1.9±0.04[b]	1.7±0.05[a]	2.0±0.01[bc]
Protein efficiency ratio (PER)	1.33±0.03[a]	1.42±0.02[a]	1.42±0.03[a]	1.85±0.08[b]	1.34±0.01[a]

T1= control, T2 = control+0.05% multi-enzyme, T3 = control+0.05%multi-enzyme+0.75% sisnu powder,
T4 = control+0.05% multienzyme+1.5% sisnu powder, T5 = control+0.05% multi-enzyme+ 2.25% sisnu powder

Blood parameters

Plasma cortisol levels of fishes in different treatments during experimental period are given in Table 6. After 90 days of culture period, the cortisol level was significantly lower ($p<0.05$) in T4 (0.0447±0.001ng/mL) than T1 (0.0763±0.002ng/mL), T2 (0.0737± 0.001ng/mL), and T3 (0.0565±0.001ng/mL) but significantly higher ($p>0.05$) than T5 (0.0330±0.001ng/mL).

Table 6: Cortisol level of rainbow trout fed with different level of dietary *U. parviflora* in 90 days.

Cortisol (ng/mL) (Mean±SE)	Treatments				
	T1	T2	T3	T4	T5
	0.0763±0.002^{d}	0.0737±0.001^{d}	0.0565±0.001^{c}	0.0447±0.001^{b}	0.0330±0.001^{a}

Mean value with different superscript letters in same row are significantly different

Economic analysis

The economic analysis of different treatments is shown in Table 7. The mean gross per unit cost of T4 was significantly lower ($p>0.05$) than other treatments while the mean gross per unit cost of T2 was significantly lower than T5 with no significant differences with T1 and T3. Similarly, the mean profit, gross margin and BC ratio of T4 was significantly higher ($p<0.05$) than other treatments. The mean profit, gross margin and BC ratio of T2 was significantly higher ($p<0.05$) than T5 with no significant differences ($P>0.05$) with T1 and T3.

Table 7. Mean±S.E. value of economic analysis of different treatments in NRs. /1.6m^2 raceway during experimental period of 90 days

Variables	Treatments				
	T1	T2	T3	T4	T5
Seed	3325	3325	3325	3325	3325
Feed	6139±67^{a}	5992±32^{a}	6109±32^{a}	5994±159^{a}	61019±103^{a}
Total income	15097±414^{a}	15563±103^{a}	15466±235^{a}	16683±328^{b}	14553±322^{a}
Gross per unit cost (NRs/kg)	628±14^{bc}	599±5^{b}	610±8^{bc}	559±10^{a}	648±8^{c}
Net Profit (NRs/kg)	372±14^{ab}	401±5^{b}	390±8^{ab}	441±10^{c}	352±8^{a}
Gross margin (NRs)	5633±361^{ab}	6246±118^{b}	6033±213^{ab}	7364±290^{c}	5126±222^{a}
B:C ratio	1.59±0.04^{ab}	1.67±0.01^{b}	1.64±0.02^{ab}	1.79±0.03^{c}	1.54±0.02^{a}

DISCUSSION

The treatment group T4 has highest final weight 127.3±1.3 g than T5, T2, T1 and T3. Similar result was shown by Maharjan (2021) where 1.5% sisnu meal was recommended as optimal inclusion level in starter feed and increasing inclusion sisnu meal had shown decreasing growth parameters might be due to the increment of fiber content. T4 has highest extrapolated net fish yield 128±1.3 ton/ha/cycle among all treatments. Similar results were shown by (Bhusal et al. 2019; Maharjan 2021). The antioxidant properties could have mitigated physiological stress caused by management procedures and enabled the supplemented group to retain enhanced yield (Elabd et al. 2019). The T4 has highest daily weight gain (DWG) 1.16±0.03g/fish/day and Specific Growth Rate (SGR) 1.90±0.04%/day than T5, T1, T2 and T3. Similar result was obtained by Maharjan (2021). Several authors have reported that exogenous muti-enzymes eliminate the effects of anti-nutritional factors and improved the utilization of dietary energy and amino acids, resulting in improve growth performance of fish (Farhangi and Carter 2007; Soltan 2009). Similarly, T4 has lowest FCR 1.7±0.05 among all treatments. Similar research findings were also been observed by Awad et al. (2012) where fish groups receiving *Urtica dioca* supplementation diet had low FCR in trout fingerlings through enhancement only in pepsin activity. Supplementation of immuno-stimulants like sisnu meal in feed could have improved appetizing and digestion-stimulating properties, antimicrobial effects with the growth of beneficial bacteria as reported by Ngugi et al. (2015) in juvenile and adult Victoria Labeo (*Labeo victorianus*) changed with *Aeromonas hydrophilla*. T4 has highest PER (Protein Efficiency Ratio) 1.85±0.08 among all treatments. Similar result was obtained by Maharjan (2021) which could be due to enhanced feed palatability in sisnu meal enriched diet resulting better feed utilization as demonstrated by the FCR and growth rate of fish.

Plasma cortisol level decreased with increasing *U. parviflora* which was similar to the *U. dioica* fed diet of juvenile and adult fish of *Labeo victorianus* (Ngugi et al. 2015). And similar results were observed by Singh et al. (2020) in Rainbow trout. This might be caused by the antioxidant properties of *U. parviflora.*

Economic analysis in the present experiment was estimated between the treatments showed that the profit was found highest in T4 having combined 1.5% sisnu leaf powder and 0.05% multi-enzyme than other treatments, it might be due to higher total income obtain from higher fish yield. Also benefit cost (B:C) ratio was found highest in T4 having combined 1.5% sisnu leaf powder and 0.05% multi-enzyme than other treatments, it might be due to lowest per unit cost and highest total income. And the gross profit per kg production was obtained NRs 441/1.6 m^2 during experimental period of 90 days where selling price of the fish was NRs 1000/kg. Supportive result was reported by Bhandari and Parajuli (2016) from the survey data where the gross profit per kg production was obtained NRs 102 in average trout farm of 200m^2 with selling price NRs 750/kg. Moreover, the scale of production could have affected the production cost economics.

CONCLUSION

The result of this study demonstrated that the stinging nettle (*U. parviflora*) of 1.5 percent with 0.05 percent multi-enzyme meal, was shown to be the most promising diet in terms of growth, FCR, PER, Net yield, DWG, SGR and the stress parameter cortisol. The findings, immuno-stimulatory potential of dietary supplement stinging nettle, improved growth performance and lower cortisol levels provided additional insight to an enhancement in trout welfare.

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COMBINED EFFECT OF ACIDIFICATION AND PHYTASE SUPPLEMENTATION ON GROWTH PERFORMANCES AND SURVIVAL OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT
The study conducted at the Rainbow Trout Fishery Research Station aimed to assess the impact of citric acid and microbial phytase on growth of rainbow trout fingerling from August to November 2020 using a Completely Randomized Design with three replications. Treatments were: 0% citric acid and no FYT phytase feed (T1); 1% citric acid and 500 FYT phytase feed (T2), 1% citric acid and 1000 FYT phytase feed (T3); 3% of citric acid and 500 FYT phytase feed (T4) and 3% of citric acid with 1000 FYT phytase feed (T5). T5 (3% citric acid and 1000 FYT/kg microbial phytase) exhibited the highest daily growth rate, harvest weight, condition factor, and specific growth rate, while T4 (3% citric acid and 500 FYT/kg microbial phytase) performed better in terms of feed conversion ratio and survival rate. No significant differences were observed in water quality parameters. The study concluded that the combination of citric acid and microbial phytase positively influenced growth by lowering gut pH, enhancing phytate hydrolysis, exerting antimicrobial effects, slowing gastric emptying, and improving mineralization and nutrient absorption. These findings highlight the potential benefits of incorporating citric acid and microbial phytase in rainbow trout fingerlings' diets to enhance growth performance.

Key Words: Citric acid, Phytase, Condition factor, Specific growth rate

INTRODUCTION
Rainbow trout, one of the country's main cold-water fish species (Timalsina et al. 2017), contributed to cheap animal protein and food security in mid hills and high hills of Nepal. The widespread growth of trout farming faces a significant hurdle in the form of limited access to high-quality and economically viable feed (Gurung et al. 2014), emphasizing the need for innovative solutions for sustainable development. The limited supply of high-priced fishmeal, coupled with high demand and public concern about the environmental, social, and economic impacts necessitates the use of fishmeal alternatives in sustainable aquafeed (New and Wijkstrom 2002; Baruah et al. 2004; Tacon et al. 2011; Boonyaratpalin 2014; Tacon and Metian 2015; Turchini et al. 2019; Naylor et al. 2021,). The availability and cost-effectiveness of plant proteins make them a viable alternative. However, essential amino acid imbalances, low mineral bioavailability due to phytic acid binding, and the presence of anti-nutritional factors in alternative plant protein sources, (Swick and Ivey 1992; NRC 1993; Gatlin et al. 2007; Oliva-Teles et al. 2015) impose challenges in their extensive usage in aquafeed formulations. Phytate bound phosphorus (P) is unavailable for fish (Papatryphon et al. 1999), as fish lack endogenous phytase to hydrolyse inositol-phosphate linkages. Moreover, phytate has a strong binding affinity to cations, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, and K^{+}, forming insoluble salts and reduces the bioavailability of minerals. Furthermore, phytate also form insoluble complexes with proteins, thereby reducing protein digestibility (Cao et al. 2007; Kumar et al. 2012; Riche and

Garling Jr 2004). It has been confirmed that inclusion of exogenous enzymes phytase in aquafeeds can reduce the negative effects of phytate on digestion as well as reduce the nutrient discharge in the water (Rodehutscord and Pfeffer 1995; Cain and Garling 1995; Papatryphon and Soares 2001; Debnath et al. 2005a; Debnath et al. 2005b; Maas et al. 2018; Shafique et al. 2018; Yigit et al. 2018; Rodrigues et al. 2022). On the other hand, the extensive use of antibiotics in trout aquaculture without maintaining withdrawal time has threaten public health due to antibiotic residue bio-accumulation (Sharafati-Chaleshtori et al. 2013; Soltani et al. 2015) development of multidrug resistance bacteria and downstream effect of antibiotics (Timalsina et al. 2022), while excessive levels can even negatively affect fish growth and overall conditions. The ban on the use of antimicrobials as growth promoters through feed supplements and sub-therapeutic doses of antimicrobials issued by the Ministry of Livestock Development (MoLD) of Nepal and the regulatory procedures of the National Antimicrobial Resistance Containment Action Plan (Acharya and Wilshon 2019; DoHS 2016) have put pressure on aquaculture to screen alternatives to in-feed antibiotic usage. Organic acids in aquafeed research have been reported to increase feed hygiene (Lückstädt 2008), promote nutrient digestibility and feed utilization (Vielma et al. 1998; Vielma et al. 1999; Pandey and Satoh 2008; Ng and Koh 2017), modulate the beneficial gut microbiota (Jaafar et al. 2013), and reinforce health (Elala et al. 2015). More specifically, several studies investigated the effects of citric acid on rainbow trout globally in terms of growth, feed intake, specific growth rate (SGR), and feed conversion ratio (Pandey and Satoh 2008; Jaafar et al. 2013; Hernández et al. 2021), whereas a few preliminary research findings (Singh et al. 2020) in Nepal have shown encouraging insights for further optimization. In addition, rainbow trout having high gastric pH of nearly 4.0 (Sugiura and Ferraris 2004), fails to secrete effective acid (Koelz 1992) as the stomach of fish has only one type of cell secreting both HCL and pepsinogen called oxynticopeptic cells. Furthermore, low pH also enhances nutrient uptake (Boling et al. 2001) and phytate solubility (Jongbloed 1987). The present study was conducted for the evaluation of the combine effect of acidification and phytase supplementation on growth performances and survivability of rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Experimental site and design

The experiment was carried out at the Rainbow Trout Fishery Research Station, Dhunche, Rasuwa, which is situated in Bagmati Province of Nepal, and geographically it lies between 28°6’37” N latitude and 85°18’20” E longitude at an altitude of 1,850 meters above sea level. The experiment was conducted for 90 days from August 2020 to November 2020.

Treatment details

The experiment was carried out in a Completely Randomized Design (CRD). The experiment was consists of five treatments and each treatment had three replications: 0% citric acid and no FYT phytase feed (T1); 1% citric acid and 500 FYT phytase feed (T2), 1% citric acid and 1000 FYT phytase feed (T3); 3% of citric acid and 500 FYT phytase feed (T4) and 3% of citric acid with 1000 FYT phytase feed (T5). The randomization of treatments was done by creating random numbers in the MS Excel sheet using RAND ( ) command.

Feeding

Daily hand feeding was provided on the basis of morning pond water temperature monitored using a digital pen thermometer and feeding rate was adjusted based on the trout feeding guide developed by Tacon (1987). The total daily feed ration was divided into 2 equal portions delivered at 10:00 AM and 4:00 PM under ambient lighting in the first month and reduced to one delivered at 12:00 PM in later months.

Feed Formulation

All basic ingredients shrimp meal, soya full roasted, wheat grain, and rice grain, including Vitamin premix, mineral mix, yeast, and salt (additional) were ground thoroughly and sieved to pass through 0.5 mm mesh size, and mixed together with citric acid and Phytase, which were compared as additives in different concentrations as shown in Table 1.

Proximate analysis of diet and fish

Quadrant sampling was done at each lot of feed preparation during experiment period to draw representative diet sample for proximate analysis according to AOAC (1990) at the Food Research Division, Khumaltar.

Research set up

Raceways were scrubbed to clean, water levels maintained, and 1000 trout fingerlings were acclimatized for a week, in two raceways, feeding the control diet at the rate of 5% of the average body weight. Then after, 15 trout's were netted, quick dip bathed in 3% salt solution and reassigned to each individual 15 raceways having a water volume of 0.3m^3 (3m x 0.5m x 0.2m). Final harvesting of rainbow trout was done after 90 days by draining each raceway completely. Harvested fishes were measured using an electronic balance. Fishes were counted, total length measured and batch weight was recorded.

Table 1: Ingredients and composition, percentage of the experimental diet.

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Shrimp	45.3	45.3	45.3	45.3	45.3
Soya full roasted	35	35	35	35	35
Wheat grain	7.7	6.7	6.7	5.7	5.7
Rice bran	6	6	6	5	5
Additives					
*Vitamin Premix	2	2	2	2	2
**Mineral mix	2	2	2	2	2
Yeast	1	1	1	1	1
Salt	1	1	1	1	1
Citric acid (%)	0	1	1	3	3
Phytase (FYT/kg)	0	500	1000	500	1000

*Vitamin mixture/kg premix containing the following: 33000IU vitamin A, 3300IU, vitamin D3, 410IU vitamin E, 2660mg Vitamin BI, 133mg vitamin B2, 580mg vitamin B6, 41mg vitamin B12, 50mg biotin, 9330mg choline chloride, 4000mg vitamin C, 2660mg Inositol, 330mg para-amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid **Mineral mixture/kg premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5mg Cobalt.

Water quality analysis

Water temperature (°C) was measured at daily at out flow of each raceway using digital thermometer. Dissolved oxygen (DO) in (mg/L), and pH was measured fortnightly using Thermos-Electron Corporation Russell 060p and Thermo-Electron Corporation, U.S.A., Orion 5 star S.N. 005840 respectively.

Statistical analysis

The data were collected during the course of time and on the basis of individual fish observations, the population means for each character were computed. The analysis of variance was used to compare different growth parameters and water quality parameters using R-Stat version 4.1.1. The mean and standard errors was calculated for each treatment. The data entry was done through MS Excel 2016. The accepted level of significance was $p<0.05$.

The fish production and related parameters were analyzed using following formula:

$$\text{Daily weight gain (g/fish/day)} = \frac{W_t - W_0}{\text{Culture period (days)}}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln W_t - \ln W_0}{\text{Culture period (days)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Weight of feed consumed (g)}}{\text{Fish body weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{Condition factor} = \frac{\text{Weight (g)}}{\text{Length (cm)}^3} \times 100$$

$$\text{Thermal growth coefficient (TGC)} = \frac{1000 \times (W_t^{\frac{1}{3}} - W_0^{\frac{1}{3}})}{\text{Culture period (days)}} \times \text{Average water temperature (°C)}$$

$$\text{Daily growth coefficient (DGC)} = \frac{100 \times (W_t^{\frac{1}{3}} - W_0^{\frac{1}{3}})}{\text{Culture period (days)}}$$

Where,

W_t= Mean harvest weight (g/fish) and

W_0= Mean stocking weight (g/fish)

RESULTS AND DISCUSSION

Proximate analysis of experimental diets

Proximate analysis of locally prepared diets on dry matter basis is presented in Table 2. Result showed, there some degree of variation in crude protein, ether extract content; crude fiber, and total ash content (significant difference) among different treatments.

Table 2: Proximate composition of five diets used in the experiment period of 90 days

Treatments (Mean ± SE)	Constituents (Mean ± SE)				
	Crude Protein (CP) %	Ether extract (EE)%	Crude fiber (CF)%	Total Ash (TA)%	NFE (%)
T1(Control)	46.13±0.1[c]	8.88±0.2[a]	6.72±0.2[a]	12.91±0.1[b]	25.36[c]
T2	46.01±0.8[c]	8.99±0.2[a]	6.74±0.3[a]	13.52±0.1[a]	24.74[e]
T3	46.93±0.6[a]	8.45±0.1[a]	6.96±0.1[a]	12.73±0.3[bc]	24.93[d]
T4	46.48±0.1[b]	8.16±0.1[b]	6.08±0.6[b]	12.64±0.6[bc]	26.64[a]
T5	46.45±0.3[b]	8.37±0.3[b]	6.31±0.4[b]	12.35±0.1[c]	26.52[b]

Nitrogen Free Extract (NFE) = 100- (CP+EE+CF+TA).

Growth performance

The variation in different growth parameters during experimental period in different treatments are presented in Table 3 & 4.

Effect on growth parameters

Different concentrations of citric acid and microbial phytase showed a significant effect on the harvest weight (Table 3). Among various treatments, the highest harvest weight (435.9 ±43.0 g) was recorded at T5 followed by T4 (432.8±50.4 g), T3 (349.4±0.1 g) and T2 (319.2±34.0 g) respectively, while the lowest harvest weight (227.9±31.4 g) was recorded at control groups without citric acid and microbial phytase (T1). In the present study, addition of citric acid 3% supplemented with both 500 as well as 1000 FYT/kg phytase Phytase and 1% citric acid with 1000 FYT/kg phytase has improved the harvest weight showing positive interactions between them. Study has shown that the dietary acidification improves nutrients as well as mineral absorption and digestion by increasing the activity of digestive enzymes in fish gut (Hussain et al., 2011a). Furthermore, as mentioned by Lieb and Portz (2005), acidification creates an environment that is favorable for phytase to reduce the amount of phytate in digested food, thereby preventing the formation of phytate-protein–mineral complexes. Also, citric acid release the minerals from the phytic acid complex (Sugiura et al., 1998; Boling et al. 2000; Brenes et al. 2003). Citric acid is successfully used to improve growth performance and nutrients utilization due to its phytate hydrolyzing capability (Dai et al. 2018). A study by Forster et al. (1999) showed that dietary Phytase exhibited high final body weight in the rainbow trout, improved the nutritive value of the trout diet, and reduced dietary phosphorus output into the environment; regardless of their Phytase levels (500, 1500, or 4500 FTU/kg diet). Study carried out by Maas et al. (2018) has showed that sunflower meal based diet supplemented with 1000 FTU/kg phytase had an increased growth performance in Nile tilapia advance fingerlings. Hassan et al. (2009), Hassaan et al. (2013) and Rachmawati and Samidjan (2016) also reported using Phytase (1000 FTU/ kg) in (soybean meal based diet improved growth performance in Milk fish, Nile tilapia and Giant tiger prawn respectively. Also, Sugiura et al. (2001) has mentioned that dietary acidification with citric acid reduced the impact of phytase in high-ash diets, but it significantly boosted the enzyme's action in low-ash diets, which is supportive to present study treatments T3, T4 & T5 performance. Also, increasing the harvest weight in rainbow trout with a feed of 1000 FYT/kg phytase at 1% and 3 % citric acid as well as FYT 500 could be because the fish is able to perform metabolism process more efficiently than fish on other treatments. The utilization of energy by fish in the 1000 FTU dose is possible higher, resulting in greater growth than other treatments. According to Wagle and Pradhan (2013), optimal water temperature requirement for optimal growth of rainbow trout in flow through raceways is 16 to 18 degrees Celsius, which is attained throughout in our research study. The findings of Baruah et al. (2007b), Shah et al. (2016), and Hussain et al. (2011b) in *Labeo rohita* on soyabean-based and corn gluten meal-based diets, respectively, support the positive interaction of phytase and citric acid in increasing nutrient utilization, and, ultimately, increasing fish harvest weight. Similarly, Arsalan et al. (2021) in mrigal and Singh et al. (2020) in rainbow trout both confirm similar findings. Additionally, weight gain was reported better in fish fed dephytinized soy proteins than in fish fed with conventional diet by Vielma et al. (2002).

A significant effect was shown by different concentrations of citric acid and microbial phytase on the survival rate (Table 3). Among various treatments, T4 showed the best performance in terms of survival rate because the highest survival (64.4±4.4 %) rate was obtained at T4 followed by 55.6±2.2, 53.3±3.8 and 53.3±3.8% at T2, T3 and T5 respectively. Treatments T2, T3 and T5 did not show significant differences in survival rate. The lowest survival rate was obtained at T1 which was (48.9±3.8%). All the treatments treated with different concentrations of citric acid and microbial phytase recorded high survival rate as compared to treatment not treated with citric acid and microbial phytase. Availability of phosphorus increases the survival of fishes while lack of availability of phosphorus would decrease fish survival (Robinson et al., 2002). Further, citric acid reduces the pH in the gut which increases the phytate hydrolysis, kills the pathogens, and decreases the rate of gastric emptying (Singh et al., 2020). It has been reported that citric acid is being successfully used to improve resistance against diseases (Dai et al., 2018). The combination of dietary phytase and citric acid is a useful approach to the production of low-pollution feeds (Khajepour et al. 2012). Due to these reasons, survival rates were high in feeds treated with phytase and citric acid as compared to non-treated feeds. The result is supported by the findings of Nie et al. (2017) in crucian carp (*Carassius auratus*).

Effect on growth coefficients

Similarly, no significant effects were observed on daily growth rate, Thermal unit Growth Coefficient and Daily Growth Coefficient with different concentrations of citric acid and microbial phytase (Table 4). Different concentrations of citric acid and microbial phytase showed a significant effect on feed conversion ratio (Table 4). Among different concentrations of citric acid and microbial phytase, the maximum feed conversion ratio (2.29±0.06) was recorded on T1 followed by 2.09±0.08, 2.01 ±0.01 and 1.96±0.05 at T2, T3 and T5 respectively while the lowest feed conversion ratio (1.93±0.08) was recorded on T4. Improvement in feed conversion ratio was due to enhanced release of nutrients of plant based diets by breaking down the bonds between phytate-protein and phytate-minerals (Vielma et al. 1998; Hussain et al. 2011b). Further, phytase effectively increases phosphorus availability of soybean meal, increase protein digestibility in plant based meal and also improves the availability of other minerals and trace element (Cao et al., 2007). Supportive results were reported by Jakson et al. (2006), where feeding trials conducted on channel catfish fed with five diets (0, 500, 1000, 2000, or 4000 units of phytase/kg diet) had significantly lower feed conversion ratios (FCR) at 1,000 units of phytase/kg diet compared to the fish fed no supplemental phytase and the other treatments. In terms of protein efficiency ratio (PER), the significantly higher PER one was observed in T5 (0.79±0.01) followed by T4 (0.78±0.02), T3 (0.65±0.04), T2 (0.57±0.04) and T1 (0.26±0.12). The treatments T5 and T4 were not significantly different with T3 but were significantly different with T2 and T1. Similar findings were observed by Wang et al. (2009) and Singh et al. (2020) in rainbow trout; Baruah et al. (2007a and 2007b), Bano and Afzal (2017) in *L. rohita* and Taheri and Taherkhani (2015) in broiler. With the significant decrease in FCR, there was increment in PER with the increment in the dietary inclusion of citric acid in rainbow trout juveniles as seen in work of Supportive results in different fish species were reported by various authors where the addition of 1000 FTU phytase gives the highest protein efficiency (PER) value of Asian Seabass feed (Yudhiyanto et al. 2017); tilapia feed (Hassaan et al. 2013) and tiger grouper feed (Rachmawati and Hutabarat 2006). In context of specific growth rates, the significantly highest specific growth rate was observed at T5 which was 1.34±0.06%/day and T4 (1.16±0.06%/day) and T3 (1.15±0.06%/day) were similar. The lowest specific growth rate (1.01±0.06%day) was obtained at T1 which was not significantly different with T2 (1.04±0.06%/day). Among all treatments, T5 recorded the significantly higher condition factor (1.35±0.04) followed by T4 (1.06±0.03), T3 (1.03±0.08), T1 (1.03±0.04) and T2 (0.96±0.08). The value of condition factor is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. According to the condition factor standard T5 is fairly good condition fish than the other (Barnham and Baxter 2003). Similarly, supportive results by Mahmoud et al. (2019) showed a significant increase growth performance (final weight, weight gain, & specific growth rate) and feed utilization (feed conversion ratio, protein efficiency ratio & protein productive ratio) of Nile tilapia fingerlings fed on of 30 g/kg citric acid along with 1000 FTU/kg phytaseis. Moreover, Hussain et al. (2020), also reported similar results having the maximum weight gain, and the low FCR value of *C. mrigala* fingerlings on the guar meal based diet having 2.5% c,itric acid and 1000 FTU /kg Phytase level supplementation. In addition, Iqbal et al. (2021) also reported that supplementation of 30 g/kg citric acid along with 1000 FTU/kg phytase improved weight gain, specific growth rate, Fulton condition factor, and FCR performance in *L. rohita* fingerlings. These overall increased growth response may be attributed to the increased availability of nutrients and minerals might be due to the enzymatic breakdown of phytate-nutrient complexes (Shah et al. 2016). Further, citric acid may lower the intestinal pH, which favors the phytate nutrient complex solubility and nutrients absorption from the gastrointestinal tract resulting in improved growth performance of fish (Cross et al. 1990; Shah et al. 2016).

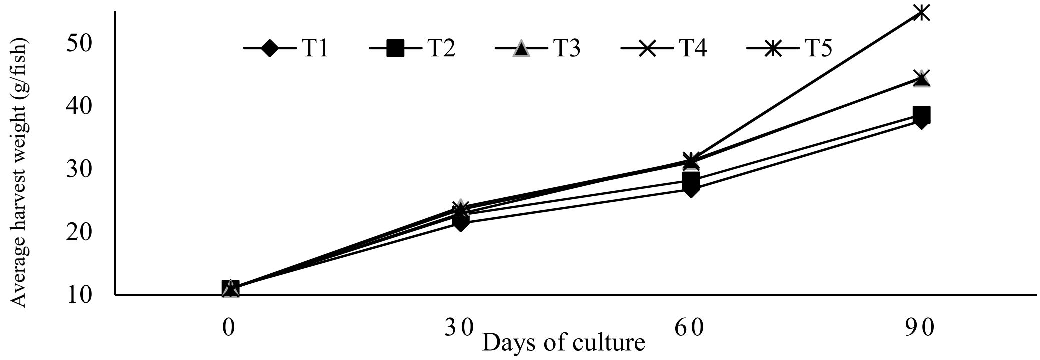

Figure 1. Monthly growth trends of fingerlings of each treatments during the experimental period of 90 days.

The monthly growth trend of rainbow trout fry shows fry grew steadily until 60 days of culture period and exponentially from then after up to 90 days. Similar result was observed by Pandit and Nakamura (2010) in Nile tilapia fry.

Table 3: Mean value of growth parameters of rainbow trout during experimental period of 90 days. All data presented are for 0.03m^3 raceway.

Treatments	Growth parameters (Mean ± SE)							
	Stocking weight (g)	Stocking number	Average stocking weight (g)	Average stocking length (cm)	Harvest weight (g)	Average harvest weight (g)	Average harvest length (cm)	Survival rate (%)
T1	166.5±0.8	15	11.1±0.0	10.3±0.1	227.9±31.4^c	37.7±1.6	15.4±0.4	48.9±3.8^b
T2	164.2±1.6	15	10.9±0.1	10.0±0.2	319.2±34.0^{bc}	38.6±5.1	15.8±0.2	55.6±2.2^b
T3	164.4±0.6	15	11.0±0.0	10.1±0.1	349.4±0.1^{ab}	44.4±5.5	16.2±0.2	53.3±3.8^b
T4	164.6±0.8	15	11.0±0.1	10.2±0.1	432.8±50.4^a	44.5±2.0	16.1±0.1	64.4±4.4^a
T5	164.3±0.8	15	11.0±0.1	10.0±0.1	435.9±43.0^a	54.9±4.0	15.9±0.4	53.3±3.8^b
LSD (=0.5)	3.10Ns	-Ns	0.21Ns	0.39Ns	108.99**	12.56Ns	0.99Ns	11.72*
CV (%)	1.04	-	1.04	2.16	16.97	15.68	3.43	12.08

T1= 0% citric acid and 0 FYT/kg phytase, T2= 1% citric acid and 500 FYT/kg phytase, T3= 1% citric acid and 1000 FYT/kg phytase, T4= 3% citric acid and 500 FYT/kg phytase and T5= 3% citric acid and 1000 FYT/kg phytase. Mean value with different superscript letter within same column are significantly different at p<0.05.

Table 4: Mean value of growth coefficients of rainbow trout during experimental period of 90 days. All data presented are for $0.03m^3$ raceway.

Treatments	Growth Coefficients (Mean ± SE)						
	Daily Growth Rate (g/day)	Specific Growth Rate (%/day)	Feed Conversion Ratio	Protein Efficiency Ratio	Thermal unit Growth Coefficient	Daily Growth Coefficient	Condition Factor
T1	0.29±0.02	1.01±0.06^{b}	2.29±0.06^{a}	0.26±0.12^{c}	0.07±0.00	1.24±0.06	1.03±0.04^{b}
T2	0.31±0.06	1.04±0.06^{b}	2.09±0.08^{b}	0.57±0.04^{b}	0.07±0.01	1.27±0.16	0.96±0.08^{b}
T3	0.37±0.06	1.15±0.06^{ab}	2.01±0.01^{b}	0.65±0.04^{ab}	0.08±0.01	1.45±0.17	1.03±0.08^{b}
T4	0.37±0.02	1.16±0.06^{ab}	1.93±0.08^{b}	0.78±0.02^{a}	0.08±0.00	1.47±0.06	1.06±0.03^{b}
T5	0.49±0.04	1.34±0.06^{a}	1.96±0.05^{b}	0.79±0.01^{a}	0.1±0.01	1.75±0.1	1.35±0.04^{a}
LSD (=0.5)	0.14Ns	0.15*	0.20*	0.2***	0.02Ns	0.38Ns	0.19**
CV (%)	21.09	11.96	5.38	17.79	14.61	14.61	9.63

T1= 0% citric acid and 0 FYT/kg phytase, T2= 1% citric acid and 500 FYT/kg phytase, T3= 1% citric acid and 1000 FYT/kg phytase, T4= 3% citric acid and 500 FYT/kg phytase and T5= 3% citric acid and 1000 FYT/kg phytase. Mean value with different superscript letter within same row are significantly different at p<0.05.

Water quality:

No significant effect was obtained on temperature, dissolved oxygen and pH of water with different concentrations of citric acid and microbial phytase (Table 5), and in line with water quality parameters results by the results of Singh et al. (2020) in rainbow trout.

Table 5: Means and Ranges of water quality parameters during experimental period of 90 days. All data presented are for $0.03m^3$ raceway.

Treatments	Water Quality Parameters (Mean ± SE)		
	Temperature (°C)	Dissolve oxygen (mg/L)	pH
T1	17.7±0.04 (17.1-18.4)	8.5±0.1 (8.4-8.7)	8.3 (8.1-8.4)
T2	17.7±0.04 (17.1-18.4)	8.4±0.1 (8.2-8.5)	8.2 (8.1-8.5)
T3	17.7±0.04 (17.1-18.4)	8.5±0.2 (8.2-8.5)	8.2 (8.1-8.4)
T4	17.7±0.04 (17.1-18.4)	8.5±0.1 (8.4-8.7)	8.3 (8.1-8.4)
T5	17.7±0.04 (17.1-18.4)	8.4±0.1 (8.2-8.5)	8.2 (8.2-8.5)

T1= 0% citric acid and 0 FYT/kg phytase, T2= 1% citric acid and 500 FYT/kg phytase, T3= 1% citric acid and 1000 FYT/kg phytase, T4= 3% citric acid and 500 FYT/kg phytase and T5= 3% citric acid and 1000 FYT/kg phytase. Mean value with different superscript letter within same row are significantly different at p<0.05.

CONCLUSION

From the experiment, it can be concluded that treatments treated with citric acid and microbial phytase showed better performance than the treatment not treated with citric acid and microbial phytase. In our experiment T5 (i.e. 3% citric acid and 1000 FYT/kg Phytase) showed the improved performance based on the highest daily growth rate, survival rate, and harvest weight and condition factor. The indicate that citric acid along with exogenous phytase enzyme supplementation has promising potential to improve growth and feed performance in rainbow trout fingerlings thereby helps to formulate cost effective feed. It will be necessary to conduct further more research for optimization of the graded amount of citric acid and phytase in rainbow trout feed at different development stages with varying phosphorus feed components in relation to nutrient absorption and utilization.

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COMBINED EFFECT OF TAMOXIFEN AND 17α -METHYL TESTOSTERONE ON SEX REVERSAL OF NILE TILAPIA (*Oreochromis niloticus*) FRY

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ABSTRACT
Mono-sex culture is most effective methods for addressing issue of prolific breeding behavior in Nile tilapia. There is continued effort to find an alternative to 17α -methyl testosterone (MT) due to its limited availability, cost and health concerns, and Tamoxifen (TAM), a non-steroidal aromatase inhibitor, can be a good option. An experiment was conducted to study the combined effect of MT and TAM in various proportions. Altogether six different combinations of hormones were prepared that includes Control (no hormone), 100%MT, 75%MT+25%TAM, 50%MT+50%TAM, 25%MT+75%TAM and 100%TAM in feed. Each treatment was conducted in triplicate in 1m x1m x 1m hapas for 28 days and then reared for additional 95 days in hapas of 1.5m x1m x 1m, all suspended in green ponds. The male proportion achieved in treatment with 100% MT and 50%MT+50%TAM was 100±0% and lowest in control. The daily weight gain during treatment phase was significantly higher in control and 100% MT group and there is no difference (P>0.05) in survival among treatments compared to other treatments. MT can be efficiently replaced up to 50% by the TAM without significant effect on the sex reversal in Nile tilapia. However, detailed study on its combined working mechanism is to be studied.

Keywords: Sex reversal, Aromatase inhibitor, MT, Tamoxifen

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is indigenous to Africa and the Middle East. Introduction of tilapia by different tropical and sub-tropical country of the world has been begun due to its culture potential (FAO 2009). Tilapia is termed as "poor man's fish" (Bhujel 2013) and "aquatic chicken" because of its wide range of environmental tolerance, acceptance of a wide variety of foods, and inexpensive cost of production (Fitzsimmons 2000; Canonico et al. 2005). Different strains of Nile tilapia were introduced in Nepal during 1985 to 2009 that includes the introduction of Chitralada strain from Thailand in 1985 (Shrestha 1994) and GIFT (Genetically improved Farmed Tilapia) strain from Thailand in 2001 and from Bangladesh in 2009 (Shrestha and Pandit 2017). The major drawback of tilapia culture is its prolific breeding behavior resulting in large number of recruitments leading to stunted growth of fish as well as posing threat to local biodiversity (Popma and Lovshin 1996; Tower 2005; El-sayad 2006). Thus, it took a long time to get legal status for farming in Nepal which has provided for the monosex culture in 2020 the Government of Nepal (CFPCC 2022).

Fish farming strategies to prevent the condition of overcrowding and stunting of tilapia include cage culture (Rakocy and McGinty 2005), polyculture with predator fish (Yi et al. 2004), culture of mono sex tilapia (Megbowon and Mojekwu 2014), and culture of sterile individuals (Mair and Little 1991). Mono sex tilapia can be obtained by different methods including hand sexing (Nwangwu and Abebayo 2012), feeding with hormonal diets like 17-α methyl testosterone (Megbowon and Mojekwu 2014; Prabhadevi 2018), aromatase inhibitor (Afonso et al. 2001, Wassermann and Afonso 2003; Ruksana et al. 2010), egg immersion (Cagauan et al. 2004) feeding with different plant extracts (Khalil et al. 2014), feeding with animal testes (Khanal et al. 2014; Ranjan et al. 2015; Yustiati et al. 2018) etc. Sex-specific physiological growth capacity and aggressive feeding behavior of male help them attain almost double growth rate than female, so males are generally preferred for culture (Fuentes-silva et al. 2013; Prabhadevi 2018). Sex of newly hatched tilapia is generally determined by their genotype but the morphology and phenotype can be altered leading to development of monosex population (Oluseun 2015). From hatching up to 2 weeks newly hatched tilapia have equal concentration of androgen and estrogen in their body (Fuentes-silva et al. 2013), so increasing the extent of male hormone by the application of Methyl testosterone induce masculinization. In Nepalese conditions, both tamoxifen (an aromatase inhibitor) and MT have been studied and proven to be successful, with a success rate of up to 98 percent. Because both hormones have different sex-reversal mechanisms, their combined action could result in an entirely male Nile tilapia fry population. The present study was carried out to study the combined effect of tamoxifen and methyl testosterone on sex reversal of Nile tilapia fry**.** If this study can guarantee 100% male tilapia fry production, it might be a game-changer for hatcheries that produce only male tilapia fry. However, because tamoxifen is easily available in the market and non-steroid in nature, it will help to minimize the use of synthetic steroid hormones.

MATERIAL AND METHODS

The study was carried out at the Hatchery Complex of the Fisheries Program, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University from May 2021 to September 2021. The hatchling of Nile tilapia with yolk sac was procured from Center for Aquaculture Agriculture Research and production Kathar, Chitwan. The hatchlings were reared in aquaria till the absorption of yolk sac. Water quality monitoring, proper aeration and water exchange were done in aquaria to provide a proper environment to the fry.

Treatment design

Complete randomized design (CRD) was used for the experiment with six treatments each in triplicate. The experiment was conducted in nylon hapas measuring 1 m x 1 m x 1 m suspended in 5 m x 5 m x 1.5 m cemented tanks. In treatment phase nine-day old hatch stocked at 200 individuals/m^2 whereas after the treatment phase was over, the fries were raised for an additional 95 days in hapas with stocking density of 15 fry/m^2 or gonadal development.

Diet combinations with different ratios of Tamoxifen (TAM) and 17α- Methyl testosterone (MT) was prepared and fed for 28 days to the yolk-sac absorbed free swimming hatchling. Feeding rate gradually reduced to 10% at last week of treatment phase from 25% at the beginning whereas after treatment fries were fed with commercial pellet of 28% CP at 5% of body weight. The fries were raised for additional 95 days after completion of treatment period.

Fish meal was purchased from the market which was the base material for each type of experimental diet. The verified and recommended doses of androgen MT and aromatase inhibitor (Tamoxifen) were used for the present experiment. The verified and recommended dose of 17α- methyl testosterone in 60 mg/kg of feed (Bhujel 2012) while the verified dose for tamoxifen is 1000 mg/kg of feed (Pandit et al. 2018). Based on these doses the treatment design was developed to proportionate the volume of aromatase and steroid. As 17α-MT is readily soluble in 95% ethanol, no processing was done for its dissolution, while the tamoxifen available as tablet was crushed using a mortar and pestle before dissolving in ethanol. The required amounts of both hormones were weighed using an electronic balance and transferred to a brown bottle using a funnel. 100 mL ethanol was used first to wash the funnel as well as dissolve the hormone and additional 100 mL were added to the solution to make it standard. Then, 200 mL of stocks of 17α-methyl testosterone and tamoxifen were made, with concentrations of 300 mg/L and 5000 mg/L, respectively. The stock solutions were kept in refrigerator for further use. Experimental diet of 100 g was made for each treatment in each lot using different combination of hormones shown in Table 1.

Table 1: Treatment design with ratio of MT and TAM in the experimental feed.

Treatment No.	Treatment Name	Ratio of MT of TAM in diet	
		MT (mg/kg)	TAM (mg/kg)
T_1	Control	0	0
T_2	100%MT	60	0
T_3	75%MT+25%TAM	45	250
T_4	50%MT+50%TAM	30	500
T_5	25%MT+75%TAM	15	750
T_6	100%TAM	0	1000

Analytical parameters

Growth analysis was done during the treatment phase weekly. Daily weight gain (DWG), specific growth rate (SGR), survival rate and apparent feed conversion ratio (AFCR) of fish fed with different diets for different were calculated using following formulas;

$$\text{Daily weight gain (g/fish/day)} = \frac{W_t - W_0}{\text{Culture period (days)}}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln W_t - \ln W_0}{\text{Culture period (days)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of harvested fish}}{\text{Number of stocked fish}} \times 100$$

Sex determination was carried out by sacrificing all fish after 95 days of post treatment phase for gonadal examination. Visual examination method of matured gonad was used for determine the sex however for small or ambiguous gonad a simple microscope was used. Gonado-somatic index (GSI) was calculated using the Berhaut equation (1973):

$$\text{Gonadosomatic index (\%)} = \frac{\text{Weight of gonad (g)}}{\text{Body weigt of fish}} \times 100$$

Water quality analysis

The most critical aspect for growth and disease restriction is good water quality. The water quality parameters like Dissolved oxygen (DO), pH and Temperature were measured daily using instruments Lutran pH 222, Lutran DO 5519 and Lutran pH 222, respectively.

Production cost for sex reversed fry

Production cost for sex reversed fry calculated based on the ingredient used for feeding, hatchling use per replication and miscellaneous cost on present retail price on market.

Statistical Analysis

Data analysis was done using MS-Excel and SPSS (V 21.0). Using one-way ANOVA, the effects of various feed types on masculinization, survival, daily weight gain (DWG), and specific growth rate (SGR) following treatment were examined independently. Mean comparisons were carried out by DMRT for significant differences. Means were expressed as mean±SE for all parameters except percentage data (masculinization and survival rate) which are expressed as mean±SD.

RESULTS

Water quality
Incubation phase

Mean and range of temperature and pH during egg incubation is shown in Table 2. There was no much change in pH between morning and afternoon, however, temperature increased by 2.5 to 3°C during afternoon.

Table 2: Average water quality parameter in Aquarium during incubation period (measured at 5-6 am and 4-5 pm)

Parameters	Time	1	2	3
pH	Morning	8.2 (8.1-8.2)	8.1 (8-8.2)	8.0 (7.9-8.2)
	Afternoon	8.2 (8.1-8.4)	8.2 (8-8.2)	8.1 (8-8.2)
Temperature (°C)	Morning	25.2±1.5 (23.7-27.5	25.3±1.4 (23.5-27)	25.1±1.7 (23.4-27.3)
	Afternoon	28.2±1.0 (27.2-29.7)	28.0±0.9 (26.7-29.2)	27.6±1.5 (25.8-29.4)

Treatment phase

The mean and range of dissolved oxygen (DO), pH, temperature, soluble reactive phosphorus (SRP), total ammonium nitrogen (TAN) and alkalinity during treatment phase were presented in Table 3. The average DO and temperature were not different between the ponds. However, there was increased DO and temperature between morning and afternoon.

Table 3: Water quality parameters (mean±SE) of different ponds during treatment phase (measured at 5-6 am and 4-5 pm)

Parameters	Time	1	2	3
Dissolved Oxygen (mg L^{-1})	Morning	8.1±1.4 (5.3-10.5)	8.1±1.4 (4.9-10.5)	8.2±1.5 (5-10.4)
	Afternoon	9.8±1.5 (5.1-12.1)	9.5±1.5 (5.2-11.9)	9.6±1.5 (5.4-11.7)
pH	Morning	8.8 (8.3-9.6)	8.8 (8.3-9.4)	8.8 (8.2-9.7)
	Afternoon	9.0 (8.1-9.7)	9.0 (8.5-9.6)	9.0 (8.5-9.6)
Temperature (°C)	Morning	29.0±1.5 (26.4-31.6)	29.0±1.5 (26.1-31.3)	28.9±1.5 (26.1-31.3)
	Afternoon	31.2±1.9 (27.9-34.4)	31.0±1.8 (27.8-33.9)	31.1±1.9 (27.5-34.1)
SRP (mg/L)		0.07±0.03 (0.021-0.232)	0.05±0.02 (0.016-0.119)	0.04±0.02 (0.004-0.148)
TAN (mg/L)		0.07±0.03 (0.000-0.180)	0.07±0.02 (0.013-0.148)	0.07±0.02 (0.005-0.145)
Alkalinity (mg/L as $CaCO_3$)		70.3±4.0 (59.3-86.2)	70.1±2.9 (61.7-83.1)	73.2±4.3 (64.1-92.3)

Post-treatment phase

The mean and range of dissolved oxygen (DO), pH and temperature of different ponds during post treatment phase were presented in Table 4. The mean dissolved oxygen (DO) and temperature of different ponds during the post-treatment phase were not different. However, there increased DO within the pond between morning and evening.

Table 4: Daily water quality parameter (mean±SE) during post treatment phase (measured at 5-6 am and 4-5 pm)

Parameters	Time	5	8	11
Dissolved Oxygen (mg/L)	Morning	3.5±1.6 (1.3-6.7)	3.3±1.8 (0.8-6.9)	3.2±1.8 (1-7)
	Afternoon	7.6±2.5 (3.2-12.8)	8.1±2.9 (2.7-13.8)	7.4±2.4 (2.8-12.5)
pH	Morning	7.7 7.2-8.5)	7.5 (7.1-8.4)	7.3 (6.9-8.6)
	Afternoon	7.9 (7.3-9.2)	7.9 (7.2-9.7)	7.6 (6.8-10.9)
Temperature (°C)	M orning	29.1±1.0 (26.5-31)	29.2±1.0 (27.1-31.2)	29.2±1.0 (27.2-31.1)
	Afternoon	30.5±1.5 (27.6-32.7)	30.5±1.6 (27.4-33.3)	30.6±1.5 (27.8-33)

Growth parameters during treatment phase

Growth parameter of fish fed with different feed types for 28 days during treatment phase is shown in Table 5. There is no difference ($p>0.05$) in the mean initial weight (mg/fish) between treatments. Mean final weight (g/fish) and DWG (mg/fish/day) of control and T_2 (100% MT) were significantly higher ($p<0.05$) than all other groups fed with different proportions of MT and TAM. However, SGR and survival of Nile tilapia (*Oreochromis niloticus*) during the treatment phase were not significantly different ($p>0.05$).

Table 5: Average growth parameter of fish during treatment phase of 28 days

Treatmen t	Mean initial weight (mg/fish)	Mean final weight (g/fish)	DWG (mg/fish/day)	SGR (%/day)	Survival (%)
T_1	129±1.3^{a}	2.06±0.01^{a}	73.0±0.19^{a}	18.15±0.35^{a}	92.1±4.2^{a}
T_2	138±1.0$^{a.}$	2.18±0.10^{a}	77.3±3.57^{a}	18.09±0.29^{a}	92.9±3.5^{a}
T_3	141±0.7^{a}	1.76±0.08^{b}	62.2±2.89^{b}	17.24±0.16^{a}	90.3±4.0^{a}
T_4	123±0.5^{a}	1.68±0.03^{bc}	59.7±1.04^{bc}	17.58±0.14^{a}	97.9±1.1^{a}
T_5	136±0.9^{a}	1.65±0.07^{bc}	58.4±2.39^{bc}	17.14±0.18^{a}	87.4±4.8^{a}
T_6	121±2.2^{a}	1.55±0.03^{c}	54.8±0.98^{c}	17.46±0.72^{a}	95.2±1.1^{a}

(Mean value with similar superscripts in column is not significantly different $p<0.05$)

Average body weight of fish during treatment phase in groups of fish fed with different proportions of MT and TAM containing feed for 28 days shown in Figure 1. Average weight of fish fed with feed containing 100% TAM remained lower than other groups of fish.

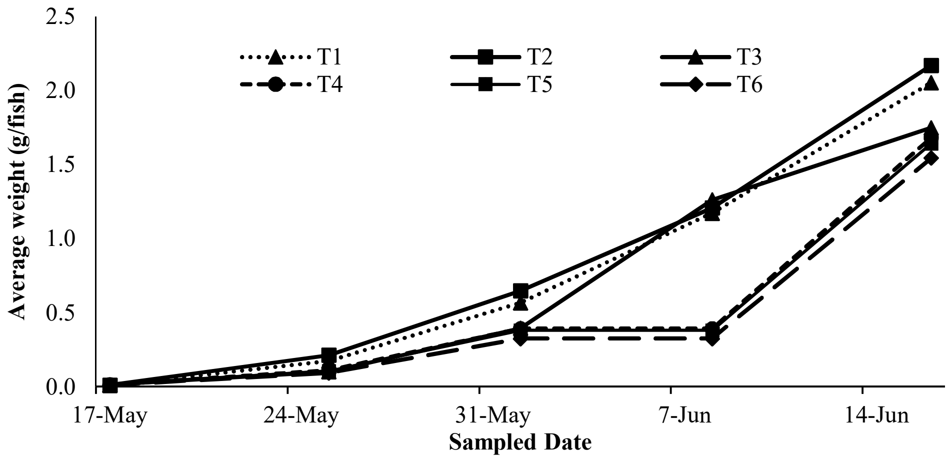

Figure 1: Average body weight of fish during treatment phase of 28 days.

Growth parameters during post-treatment phase

The average growth parameter of Nile tilapia (*Oreochromis niloticus*) during 95 days (16-June to 17-September) of post treatment phase is shown in Table 6. Mean initial weight (g/fish) and control and T_1 (100% MT) were significantly higher ($p<0.05$) than all other groups fed with different proportions of MT and TAM. There is no significant difference ($p>0.05$) in mean final weight (g/fish) between different treatments.

The DWG (mg/fish/day) of T_2 (100% MT) was significantly higher ($p<0.05$) than fish in T4 (50% MT + 50% TAM), T_6 (100% TAM and control where statistically at par with T_3 (75% MT + 25% TAM) and T_5 (25% MT + 75% TAM) respectively. There was no significant difference ($p>0.05$) in survival of fish in different treatment groups. The gonado-somatic index (GSI) of male under treatment control is significantly higher than the rest of all other treatments. However, it was lower in T_3 (75% MT +25% TAM) and T_4 (50% MT + 50% TAM).

Table 6. Average growth parameter of during 95 days of post-treatment phase

Treatment	Mean initial weight (g/fish)	Mean Final weight (g/fish)	DWG (mg/fish/day)	Survival (%)	GSI-Male (%)
T_1	2.06 ± 0.01^{a}	30.93 ± 1.13^{a}	303.98 ± 11.84^{b}	96.3 ± 0.6^{a}	0.31 ± 0.04^{a}
T_2	2.18 ± 0.10^{a}	33.63 ± 3.79^{a}	413.09 ± 55.72^{a}	91.7 ± 4.8^{a}	0.22 ± 0.01^{b}
T_3	1.76 ± 0.08^{b}	33.41 ± 0.91^{a}	333.15 ± 8.85^{ab}	95.0 ± 1.9^{a}	0.14 ± 0.04^{c}
T_4	1.68 ± 0.03^{bc}	28.81 ± 1.54^{a}	285.55 ± 16.38^{b}	91.5 ± 3.4^{a}	0.15 ± 0.02^{c}
T_5	1.65 ± 0.07^{bc}	33.39 ± 1.44^{a}	334.13 ± 14.57^{ab}	94.2 ± 1.1^{a}	0.15 ± 0.01^{bc}
T_6	1.55 ± 0.03^{c}	29.60 ± 0.51^{a}	295.30 ± 5.25^{b}	96.7 ± 0.3^{a}	0.21 ± 0.03^{b}

(Mean value with similar superscripts in columns are not significantly different $p<0.05$)

Average body weight of fish during post treatment phase fed with normal pellet feed for 95 days is shown in Figure 2.

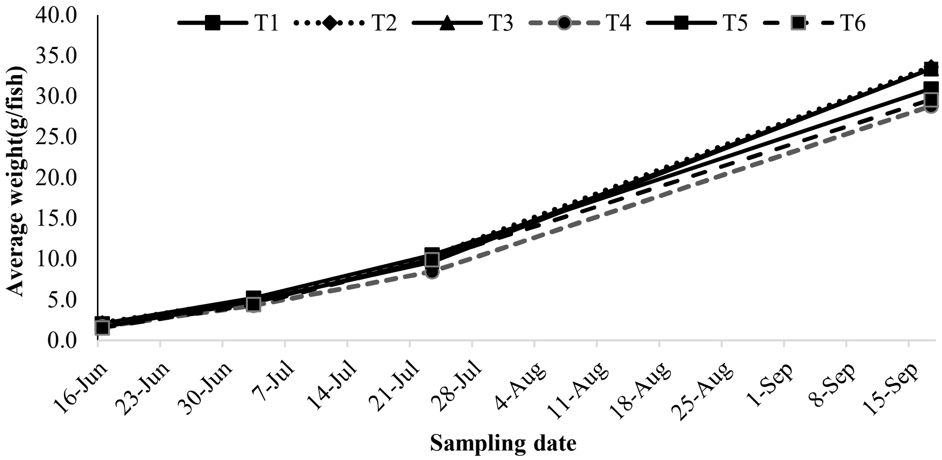

Figure 2: Average body weight of fish during post-treatment phase of 95 days

Sex reversal

Present study was carried out to study the combined effect of different proportions of MT and TAM on sex reversal efficiency in Nile tilapia. Figure 3 shows the combined effect of different proportions of MT and TAM on male proportion of Nile tilapia. Proportion of male on all treatments with combined action of both hormones and T_2 (100%) MT was significantly higher ($p<0.05$) than the rest of all other treatments. Average male population in control was found to be relatively lower than other treatments fed with different proportions of MT and TAM.

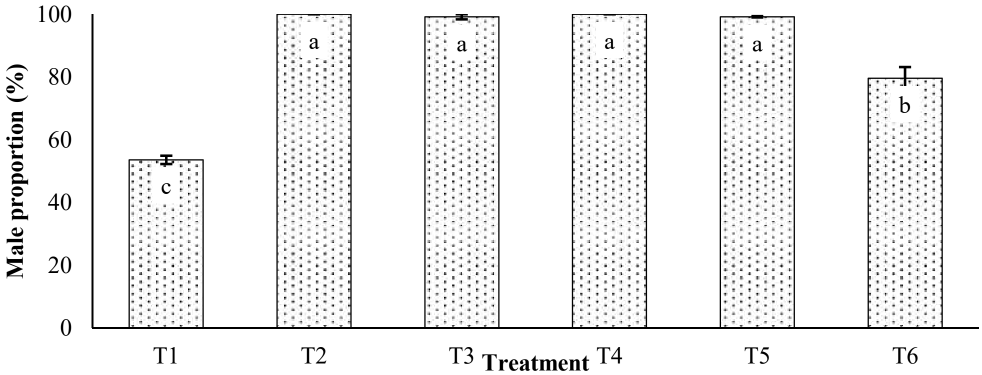

Figure 3: Proportion of male in different treatments

(Mean values with similar superscripts are not significantly different at $p<0.05$)

Cost analysis for monosex fry production

The cost of the hormonal diet, hatchling, and other incidentals were taken into account while calculating the production cost for each sex-reversed fry. Tamoxifen, alcohol, and 17α-methyl testosterone were used to make fish meal for a hormonal diet. Average labor, electricity, and other operational costs during the treatment phase are included under miscellaneous costs. Using one replication of each treatment, the production cost was calculated based on each individual fry production. The average cost of producing one sex-reversed fry for six different treatments is shown in Table 8. Average cost of monosex fry production was seems increasing when increasing the amount of Tamoxifen in diet.

Table 8: Production costs for Nile tilapia fry (NRs/fry) in different treatments

Particulars	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Hatchling cost	0.10	0.10	0.10	0.10	0.10	0.10
Cost of MT	0.00	0.27	0.20	0.13	0.07	0.00
Cost of TAM	0.00	0.00	0.16	0.32	0.49	0.65
Ethanol	0.00	0.44	0.44	0.44	0.44	0.44
Fish meal	0.60	2.22	2.22	2.22	2.22	2.22
Total feed cost	0.60	2.93	3.03	3.12	3.22	3.31
Miscellaneous	0.25	0.25	0.25	0.25	0.25	0.25
Total cost	0.10	0.10	0.10	0.10	0.10	0.10
Production cost / fry	0.95±0.12	3.28±0.13	3.38±0.19	3.47±0.32	3.57±0.14	3.66±0.27

DISCUSSION

Sex reversal

A huge number of mono-sex tilapia fry had to be produced with increased industrialization and increased demand for tilapia fillets. Having a high rate of reproduction when Nile tilapia is raised in a mixed-sex environment, they produce unmarketable-sized fish due to uncontrolled reproduction and stunted growth. For sex reversal in Nile tilapia, 17α-methyl testosterone is the most widely utilized and recognized steroid hormone. When this androgen is employed for sex reversal, Goudie et al. (1986) came to the conclusion that persons who eat fish that were fed MT as juveniles pose no health risk. However, in developing countries, there is greater concern regarding cost and availability. Both tamoxifen (an aromatase inhibitor) and MT have been researched and found to be effective mechanisms in Nepalese conditions; their combined action could result in low-cost production of totally male Nile tilapia fry.

In the present study, the combined effect of both MT and TAM in different proportions resulted in 99.2±0.8 to 100.0±0.0 % male population in contrast to about 50:50 male and female ratio in the natural population. The sex reversal potentiality of MT and TAM in different doses, duration has been already studied in different fish species. However, male proportion results from feed containing MT only in present study was higher than the result obtained from other 96.8±6% (Green and Teichert-Coddington 1993), in glass aquaria 97±4% (Okoko 1996), 21 days treatment 80±3.9% (Celik et al. 2011). The present study contradict the result obtained by Orose et al. (2016) which revealed 100% male Nile tilapia feeding for 4-month experimentation phase with 60 mg/kg MT in experimental diet, in *Betta splendens* 100% at 3 and 4 mg/kg for about eight weeks (Kipouros et al. 2011) and 100% male after 60 day's treatment (Jalabert et al. 1974). The result obtained by present research supported by Romerio et al. (2000) 98% male, Smith and Philps (2001) reported 99-100% male population of Nile tilapia when given MT at 60 mg/kg of feed. However, present study contradiction with Bhandari et al. (2006) who achieved 100% masculinization at the dose rate of 50 mg/kg MT in feed. Higher percentage of male population with higher amount of 17α-MT consumed was also reported by several authors (Mainardes-Pinto et al. 2000; Mengumphan et al. 2006; Marjani et al. 2009 and Ferdous and Ali 2011).

The present study examined possibility of using tamoxifen for monosex male production of Nile tilapia where TAM alone at the rate of 1000 mg/kg in the experimental diet produced 79.6±3.6% male population in treatment duration of 28 days which is less compared to result obtained by Pandit et al. (2018) for 30 days in Nile tilapia with male population 96.7±1.2% whereas quite similar with dose rate of 250 mg/kg feed in the same treatment. In Bagrid catfish fed tamoxifen-treated diets, a dose-dependent rise in the percentage of males was reported, with the highest dose of 200 ppm producing 90% males (Park et al., 2003). On the other hand, feeding tamoxifen at a level of 2 mg/g food to Nile tilapia fry at 8 days' post hatch for 150 days resulted in gonads having both testicular and ovarian tissue (Nakamura et al., 2003). Tamoxifen treatment of Nile tilapia juveniles with 200 g/L for 60 days resulted in 90% males in an immersion experiment (Singh et al., 2012). Tamoxifen has also been observed to impede the normal induction of vitellogenin in female medaka (*Oryzias latipes*) upon oral administration at high concentrations (Chikae et al. 2004) and immersion experiment (Sun et al., 2007). Tamoxifen's masculinizing impact has been linked to a blockage of estrogen function because it competes with endogenous estradiol for estrogen receptor binding and suppression of cyp19a expression (Kitano et al. 2007). In contrast to the current finding, Guiguen et al. (1999) found that tamoxifen did not cause masculinization in the gonadal sex differentiation in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). These findings reveal that the susceptibility of numerous fish species to tamoxifen in gonadal sex differentiation differs.

The current study revealed that raising the amount of TAM in the experimental diet and decreasing the proportion of MT in the diet results in the entire male population. This result indicates lower dose of MT can also result in all male Nile tilapia when it is incorporated in in high dose of tamoxifen. When MT was used in the meal at a modest level of 20 mg/kg, 57.1% of male Nile tilapia was generated (Celik et al. 2011). However, in present study 75 percent of MT is substituted by tamoxifen, the current study finds 99.2±0.2% male population. As a result, it appears that MT may have a stimulatory effect when combined with tamoxifen.

Growth and survival

When compared to females, tilapia males gain more weight and convert more feed in large-scale commercial settings and grow 18 to 25% faster than females (Macintosh & Little, 1995). The present study aimed to control reproduction in Nile tilapia by masculinization at gonadal differentiation phase with use of synthetic hormone. In the present study, DWG of control & 100% MT treated fish was higher than other treatment. Okoko (1996) reported no significant effect on tilapia DWG when he increased the oral administration of 17α-methyl testosterone from 0 to 1200 mg/kg of diet. The final weight gains of 100% TAM treated fish was lowest among the treatments but statistically higher than the research done by Pandit et al. (2018). The final weight gain of all treatments in the post treatment phase was similar. Guerrero III & Guerrero (1997) and Maine-Pinto et al. (2000), found that 17α-methyl testosterone has little or no effect on the growth and survival of Nile tilapia during hormonal treatment. The DWG of post treatment fry of Nile tilapia was lower than the result obtained by (Ibrahim and Naggar, 2010) that might be due to lower size of fry during post treatment phase. According to Bombardelli and Hayashi (2005), the lack of significant difference in weight and survival of Nile tilapia among treatment may be due to rapidly metabolizing and excretion of hormone causing no anabolic effect. Specific growth rates of all the treatments were similar in all treatments in the treatment phase. Similar results were obtained by (Jimenez and Arredondo, 2000). However, SGR values observed in the present study were higher than values of 2.97- 3.09 and 3.10%/day respectively demonstrated by Ahmed et al. (2013). The higher SGR value and growth of Nile tilapia could be due to a high protein diet combined with a favorable habitat, such as a temperature of around 30°C.

The survival of Nile tilapia throughout the treatment phase using different proportion of MT and TAM have not significantly differed. This means that neither hormone can alter the other's functions in the body. This value contradicts the values of 64.0±5.0% and 76.6±4.2% in the trial I and trial II of the experiment done by Khanal et al. (2014) using MT in the diet. The present study showed that tamoxifen feeding does not significantly affect the growth and survival of fry. However, high mortality was observed in Nile tilapia after dietary treatment with tamoxifen (Nakamura et al. 2003; Chikae et al. 2004; Pandit et al. 2018).

In this experiment, the gonad weight was recorded for both control and hormone treated groups. The gonadosomatic index of different treatments varied significantly among all the treatments. The result showed that the mean gonad weight of (T_1) control (0.31±0.04%) was significantly higher than all other treatments. This is followed by T_2 (100% MT) which has an average gonadosomatic index of 0.22±0.01% which is significantly higher than other groups of fish with hormonal feed treatment at different proportions of MT and TAM. Higher gonadosomatic index in T_1 (control) might be due to no hindrance in the gonadal development process. However, there is no significant difference in the mean final weight among different treatments.

Production cost

Production cost of monosex fry using different ratio of hormones was higher compared to control. This was mainly due to the additional cost of hormone used in feed preparation. However, there was an upward trend in the production expense per fry (NRs. 3.38 to 3.66) as tamoxifen dosage increased from T_3 to T_6 (Table 8). Tamoxifen costs eight times less than 17α-methyl testosterone at the same dosage (Pandit et al. 2017). However, the dose of tamoxifen was roughly 17 times greater than the amount of 17α –methyl testosterone used as the maximum dose for masculinization (Pandit et al. 2019). The use of non-streoidal hormone for the monosex production could be an advantage in terms of hormonal use but the gross margin must be taken into consideration when up scaling the method at commercial level.

CONCLUSION

The current investigation revealed that a group of fish fed 25% MT and 75% TAM generated a male population of 99.24±0.22%. Likewise, treatment with 100% MT and 50% MT and 50 % TAM resulted in 100% male production. During the treatment phase, there was no difference in the survival rate of the fry, indicating that these hormones have no deleterious impact on health or other metabolic functions. However, growth of fish was lower with the increase in TAM in experimental feed. Hence, combined effect of MT and TAM can generate masculinization with non-steroidal MT substitution. The cost analysis showed that the tamoxifen could be an expensive option for sex reversal but owing to its non-steroidal nature and easy availability in Nepalese market, it could be a good option if used in combination with the 17α- methyl testosterone.

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REPLACEMENT OF FISH MEAL WITH SOYBEAN MEAL IN HORMONAL FEED FOR SEX REVERSAL OF NILE TILAPIA (*Oreochromis niloticus*) FRY

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ABSTRACT
The study evaluated the effectiveness and feed cost of soybean meal (SM) as a replacement for fish meal in hormonal feed for sex reversal in Nile tilapia. Experiment was conducted at Aquaculture farm of Agriculture and Forestry University from October 2021 to February 2022. The experiment used five treatments in a completely randomized design (CRD) with three replicates. Treatments were: control T_1 (100%FM-based hormone feed), T_2 (75% FM+25%SM-based hormone feed), T_3 (50%FM+50%SM-based hormone feed), T_4 (25%FM+75%SM-based hormone feed), and T_5 (100%SM-based hormone feed). The experiment was carried out in two phases, viz. Treatment phase carried out for 28 days in 1m x1m x1m hapas and Rearing phase carried out for next 120 days in 2m x 1m x 1m hapas. There was no significant difference ($p>0.05$) in the male population among all treatments, with a range of 99.8±0.2% to 100.0±0.0% however, the survival in T_5 was significantly lower ($p<0.05$) compared to all other treatments. The study found that replacing fish meal with soybean meal decreases the production cost of all male monosex Nile tilapia fry, suggesting that 75% of fish meal replacement is more effective in terms of sex reversal, growth, survival, and in fry production cost.

Keywords: Steroid, Sex reversal, MT, Soybean meal, Fishmeal

INTRODUCTION

Aquaculture is constantly growing to meet the lack of animal nutrition in the modern world (Rahman 2019). Correct environmental and growth conditions must be made available for fish to grow properly and multiply. For the culture, a reliable source of fry is essential for the blooming of fish farming. Since the cost of fry plays a crucial role in determining the overall expenses of aquaculture, it is important to strive for a new understanding of seed production to enhance the financial viability of the aquaculture farm. The primary species of tilapia used in aquaculture include *Oreochromis niloticus, O. aureus, O. mossambicus,* and several species of *Sarotherodon* (Fitzsimmons 2000). Nile tilapia (*Oreochromis niloticus*), similar to other finfish and land-based animals, must have a well-balanced daily intake of protein fats, carbohydrates, minerals, and vitamins to match its nutrient and energy needs. Factors such as age, size, physiological state, and water temperature impact the nutritional and energy needs of fish. For instance, young tilapia need a diet that contains an elevated amount of protein, lipids, vitamins, and minerals, but is low in carbohydrates. Sub-adult and adult tilapia need a diet high in lipids and carbohydrates for energy and low in protein for rapid growth (Lovell 1989). The protein desires of tilapia vary, ranging from 40-45% Crude Protein (CP) for brood stock, 40-50% CP for fry/fingerlings, and 28-32% CP for grow-out fish (Stickney 1997). As tilapia matures, the required protein decreases. Tilapia, most farmed species in the 21st Century (Fitzsimmons 2000) and regarded as omnivorous fish, can use mutually animal and plant materials for its nutrient (Madalla 2008).

Agricultural waste products are seen as an affordable alternative to fishmeal as protein sources in aqua-feed (Daniel 2018). The most frequently utilized agricultural by-products as protein sources in fish like soybean meal, rapeseed meal, coconut seed cake, and cotton seed cake (Cho and Slinger

1979). Fishmeal can be substituted by soybean meal due to the growing demand, unpredictable availability, and rising charge of fish meal, leading fish nutritionists to seek substitute protein sources. Plant-based feed alternatives have demonstrated lower performance compared to fishmeal, especially for carnivorous and omnivorous species. Soybean-based products are a prime focus as alternative feed due to their accessibility, local production, low cost, and nutritional content. However, the use of processed soybean has had mixed results (Wang et al. 2015). The main challenge in raising tilapia is managing their uncontrolled breeding in grow-out ponds, which results in overcrowding, food competition, and stunting growth in aquaculture systems. Males exhibit faster growth and are bigger in size compared to females (El-Griesy and El-Gamal 2012). Monosex culture is a common technique for managing tilapia populations in many countries for fish farming. The Monosex culture method involves manually separating the sexes, altering the environment, hybridizing, using hormones for sex changes (sex reversal), and genetic manipulation techniques such as androgenesis, gynogenesis, polyploidy, YY male technology and transgenesis. A mono-sex population has benefits, including faster growth and avoiding unwanted population growth.

The most widely used method for producing mono-sex male tilapia involves feeding them synthetic steroid 17-Methyl testosterone (MT) in feed for 21-28 days after the yolk sac has been absorbed. This process is known as hormonal sex reversal. This method was employed as an overcome to the issue of early sexual maturity and uncontrolled spawning (Popma and Green, 1990). Feeding tilapia larvae with a fish meal containing the male hormone 17α-methyltestosterone (MT) leads to the physical development of a male phenotype with male function, and a male phenotype with female genotype (XX) (Beardmore et al. 2001). To grow and function reproductively as males, the male steroid hormone is given to newly hatched fry so that in genetically female individuals, testicular tissue develops from undifferentiated gonadal tissue. (Watanabe et al. 2002). As a gift from the King of Thailand to the King of Nepal in 1985, the Chitralada strain of Nile tilapia was introduced in Nepal from Thailand. The GIFT strain of Nile tilapia was introduced by NARC in 2001 from AIT, Thailand, and by IAAS in 2009 from Bangladesh (Shrestha and Pandit 2017). Numerous studies on culture, reproduction control, etc. are still being done in order to establish it on a commercial scale. Even after three decades, its cultivation has not flourished yet. There are two major reasons for this. The first is the lack of government's appropriate policy to introduce such an exotic species with a general fear that they can displace indigenous species, since they are prolific breeders, creating a negative impact on local biodiversity maintenance. Second, it has been yet regarded as a low-value-trash fish (Popma and Lovshin 1995). In Nepal, it was formally legalized to farm tilapia commercially in 2020 by the Government of Nepal (CFPCC 2022). Today, the demand, price, and future outlook for tilapia have greatly improved. The objectives of the present study is to produce monosex male Nile tilapia by using soybean meal based hormone feed as an alternative source of fish meal based hormone feed.

MATERIALS AND METHODS

Experimental site

The research was conducted in the facilities of Fisheries Program of Agriculture and Forestry University (AFU), Bharatpur-15, Rampur, Chitwan from October 2021 to Febuary 2022. The fish seed with yolk sac was procured from the CAARP Hatchery, Kathar, Chitwan, and put into the glass aquarium. Before the experiment began, the fish was accustomed to the test environment.

Experimental setup

Following a completely randomized design (CRD), the uniformly sized, healthy fry were randomly assigned to five experimental treatments each with three replicates. Each nylon hapa (1 m x 1 m x 1 m) suspended in 5 m x 5 m x 1.5 m cemented ponds with each treatment was stocked with 200 first-feeding (9 dah) fry of Nile tilapia. Following the treatment phase (which lasted 28 days), the fish from each replication of each treatment were transferred to another nylon hapa (2 m x 1m x 1m) for rearing for 120 days. During the treatment phase, fry from each replication of each treatment were fed four times a day. During the first week, fry were fed 25% of their total body weight, and this was regularly reduced to 5% each week in the treatment phase. The fish were fed a commercial pellet of 28% CP two times a day at 5% body weight during the growth phae experiment.

Experimental diet preparation

The verified and recommended dose of 60 mg/kg of 17α-methyltestosterone (MT) (Bhujel 2012) were used in the experiment. MT of 60 mg was thoroughly mixed with 200 mL of 95 percent ethanol to make the stock solution. The stock solution was put into a dark brown bottle. The stock solution was refrigerated for future use. Both fish meal and soybean meal were bought from the market and ground into a fine powder with the help of an electric mill. Initially, the basal (control) diet is made entirely of fish meal (FM) and MT. The first experimental feed, T_1 was prepared using 100 g of FM mixed with 20 mL of hormone stock solution. For the complete dissolution of hormones in fish meal, an additional 20 mL of 95% ethanol was also used. To ensure complete mixing, the required volume of hormone was mixed in the fish meal in two steps. The prepared feed was dried in the shade overnight to enable ethanol evaporation. The dried feed was stored in an airtight container for further use. The container was stored in a cool and dry place. For the preparation of other experimental diets, the feed preparation process was similar, but two ingredients, such as fish meal (FM) and soybean meal (SM) were taken, and the fish meal was replaced by soybean meal (25, 50, 75, and 100%). Table 1 shows the specifics of the experimental diets.

Table 1: Showing treatment, ingredients composition, and the ratio of mixing

Treatment	Ingredients
T_1	Control (100 % FM with MT)
T_2	75 % FM + 25 % SM with MT
T_3	50 % FM + 50 % SM with MT
T_4	25 % FM + 75 % SM with MT
T_5	0 % FM + 100 % SM with MT

FM= Fish meal; SM= Soybean meal

Water quality analysis

Temperature, dissolved oxygen (DO), and pH of water inside the hapas were recorded twice a day (5-6 A.M. and 4-5 P.M.) by Lutran pH 222, Lutran DO 5519, and Lutran pH 222, respectively.

Growth Parameters

During the treatment period, weekly fish growth samples were determined by sampling. During the sampling, at least 20 % (40) of the fry was taken from each hapa and the respective average weight was determined. The following standard formula was used to analyze the fish growth parameters, including daily weight gain, specific growth rate, survival rate:

$$\text{Survival rate (\%)} = \frac{\text{Number of harvested fish}}{\text{Number of stocked fish}} \times 100$$

$$\text{Daily weight gain (g/day)} = \frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{\text{Culture period (day)}}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln W_t - \ln W_0}{\text{Culture period}} \times 100$$

Where,

W_t= Final weight

W_0= Initial weight

Sex Confirmation

Fish from each hapa were dissected individually once the experiment was over, and sex was confirmed via morphological examination of the testes and ovary.

Statistical analysis

The gathered data were entered into an Excel spreadsheet and analyzed with the SPSS (V 21.0) software program. The effect of different feed types on male population proportion, survival rate, daily weight gain (DWG), and specific growth rate (SGR) was investigated using one-way ANOVA. The DMRT performed a mean comparison in cases where there was a significant difference in the outcome. Means were presented as the mean±SE for all parameters except mean±SD for percent data.

Production cost of monosex fry

The fry cost was calculated on the basis of the current retail price at the time of the experiment. The price of hatchlings, fish meal, soyabean meal, methyl testosterone and ethanol were 10 paisa (100 paisa =1 NRs) per hatchling, 1200 NRs per kg, 150 NRs per kg, 12000 NRs per 5 mg and 600 NRs per litre respectively, and other miscellaneous items were calculated. The hormonal feed consumed by each hapa fry was calculated. The total cost of hormonal feed consumed during the treatment phase of each treatment was calculated, and the cost of each fry was determined.

RESULTS AND DISCUSSION

Sex reversal

The male proportion of Nile tilapia was not significantly different with different feed compositions of FM and SM. The present study conducted that the replace the hormonal fish meal with hormonal soybean meal in the Nile tilapia sex reversal process. The male proportion (%) of Nile tilapia with respect to the different composition of feed is shown in Table 2. The male proportion (%) of all treatments found no significant difference ($p>0.05$) with the male proportion 100 % with different feed compositions of FM and SM.

Table 2: Effect of different treatements on the male population with a treatment duration of 28 days (Mean±SD).

Treatment	Male Proportion (%)
T_1	100.0±0.0
T_2	100.0±0.0
T_3	100.0±0.0
T_4	100.0±0.0
T_5	99.8±0.2

In the present study, feeding 17α-Methyltestosterone treated feed with various compositions of fish meal and soybean meal to sexually undifferentiated Nile tilapia for 28 days resulted in 99.8±0.2 to100.0±0.0% male population. There are anumber of studies carried out using fish meals for sex reversal. However, the result of MT treated soybean meal feeding measured during the current study was higher than 97.3% reported by Celik et al. (2011), and higher than result obtained with MT treated with a fish meal (95-97%) (El-Greisy and El-Gamal 2012). The results of the present study was similar to the results obtained by Boussou et al. (2017) using heat treatment (36°C) for sex reversal and feeding with soybean meal (97.3±0.2 %) while it was higher than as reported by Wahbi and Shalaby (2010) which reported 86.7-90.0% using 30% crude protein diet with 60 mg/kg MT in which fish meal and soybean meal was used. The current study's findings were aided by the findings of Popma and Green (1990) which produced 97-100% male fry using fish meal with MT. Ajiboye et al. (2015) obtained 90% males using farm made feed (25.2% CP) treated with MT which is quite lower than present study. For many years, costly fish meal protein diets for *Oreochromis niloticus* have been largely replaced with soybean meal (Ma et al. 1996; Bamba 2007). It is reported that eliminating fish meals from the diets of first-feeding larvae of Nile tilapia did not affect their development or survival (Gonzales et al. 2007). Consequently, the finding of the current study suggests that soybean meal can successfully replace fish meal in the diet of larvae during the hormone treatment for masculinization of Nile tilapia fry.

Jensi et al. (2016) and Vinarukwong et al. (2018) supported the current study using soybean meal is used in place of fish meal treated with the most researched and recommended dose of MT, i.e., 60 mg/kg in feed, on the development of male monosex tilapia. They obtained that higher doses of MT, such as 70 mg/kg and 80 mg/kg, did not significantly improve growth and survival rates (for the same species, lower length and weight values were found at an 80 mg/kg MT dose as opposed to a 60 mg/kg MT dose (El-Greisy and El-Gamal 2012). The highest reported survival rates for sex-reversed O. *niloticus* fed a 60 mg MT/kg diet were 84.1% (Jensi et al. 2016), 88% (Alcantar-Vazquez et al. 2015), and 80% (Kefi et al. 2013); an account of 90-95% of males were obtained (Ajiboye et al. 2015); whereas Zaki et al. (2021) at a dose of 60 mg MT reported 90% of male populations. Therefore, MT at 60 mg/kg in feed was used in the current study. Finally, the current study again proved that the dose of MT 60 mg/kg in feed was sufficient for the male monosex production process because it reported a 99.8–100% male proportion.

Photo 1: Showing the testis of male Nile tilapia after dissecting

Growth and survival

The Table 3 showed that the average growth parameter during 28 days of the treatment phase. The mean final weight (mg/fish) and DWG (mg/fish/day) of control (100% FM) was significantly higher ($p<0.05$) than T_4 (25% FM+75% SM) and T_5 (100% SM) while statistically at par with T_2 (75% FM+25% SM) and T_3 (50% FM+50% SM). Similarly, SGR (%/day) of T_2 (75% FM+25% SM) compared to all other treatments was significantly higher ($p < 0.05$). Similarly, SGR (%/day) of control (100% FM) was significantly ($p<0.05$) than T_5 (100% SM) while statistically at par with T_3 (50% FM+50% SM) and T_4 (25% FM+75% SM) after a 28-day treatment period. No significant differences ($p>0.05$) were found in survival (%) of control (100% FM), T_2 (75% FM+25% SM), T_3 (50% FM+50% SM), and T_4 (25% FM+75% SM) but significantly higher ($p<0.05$) than T_5 (100% SM).

Table 3: Growth parameters of fry during 28 days of the treatment phase (Mean±SE)

Treatment	Mean initial weight (mg/fish)	Mean final weight (mg/fish)	DWG (mg/fish/ day)	SGR (%/day)	Survival (%)
T_1	$14.0{\pm}0.8^{a}$	$652.4{\pm}0.01^{a}$	$22.8{\pm}0.5^{a}$	$13.9{\pm}0.3^{b}$	$87.4{\pm}1.7^{a}$
T_2	$10.0{\pm}0.7^{b}$	$644.3{\pm}0.03^{ab}$	$22.5{\pm}1.1^{ab}$	$14.8{\pm}0.4^{a}$	$86.2{\pm}1.6^{a}$
T_3	$14.0{\pm}0.1^{a}$	$543.7{\pm}0.08^{abc}$	$18.7{\pm}3.1^{abc}$	$13.1{\pm}0.3^{bc}$	$85.6{\pm}1.5^{a}$
T_4	$12.0{\pm}0.5^{ab}$	$507.1{\pm}0.02^{bc}$	$17.6{\pm}0.6^{bc}$	$13.5{\pm}0.1^{bc}$	$84.1{\pm}0.8^{a}$
T_5	$13.0{\pm}0.04^{ab}$	$447.3{\pm}0.018^{c}$	$15.4{\pm}0.6^{c}$	$12.7{\pm}0.18^{c}$	$78.5{\pm}1.8^{b}$

(Mean value with similar superscripts in column are not significantly different $p<0.05$)

As compared to controls, Nile tilapia fry-fed T_4 (25% FM+75% SM) and T_5 (100% SM) feed had significantly lower daily weight gain (DWG) and specific growth rate (SGR). The main reason for this lower growth was a lower proportion of crude protein availability in these feeds as compared to the control (100% FM) and other treatment feeds. Fry growth is improved with increased dietary protein composition. Larvae of tilapia are found to be carnivores while after maturing, they become more herbivorous (Kaiser 2005). The first feeding larva stage of tilapia needs to contain higher concentrations of crude protein in their diet than raise diets (Gonzales et al. 2007). The findings revealed that as the SM level in the feed increased, the DWG and SGR decreased due to lower dietary protein levels in the diet. Sarker et al. (2022) described that the DWG and SGR ranged from 31.6±2.9-42.3±4.6 mg/fish/day and 15.8±0.3-16.8±0.4 %/day when 60 mg/kg of MT treated with commercial feed fed 5 times a day during 28 days, which is higher than the present study. DWG and SGR obtained in the present study were 15.4±0.6-22.8±0.5 mg/fish/day) and 12.7±0.2-14.8±0.4 %/day). The lower DWG and SGR reported in the present study were due to the lower size of fry used in the present study. The final body weight (g/fish) at the treatment phase of the current study was ranked as control, T_2, T_3, T_4 , and T_5 and ranged between 0.44 ± 0.01-0.7 ± 0.01 g/fish, as supported by Omar et al. (2021). They obtained the final body weight of 0.7 ± 0.01-0.8 ± 0.01 g/fish. It has been reported that the final body weight was 0.76 ± 0.02 (g/fish) when MT was treated with 30% CP (farm-made feed), which was almost parallel to the present study Sarbajna et al. (2010).

The survival (%) in the T_5 (100% SM) was significantly lower as compared to the other group of treatments. The survival (%) of the treatment phase of the present study ranged between 78.5 ± 1.8 and 87.5 ± 1.2 %. The result showed that there was lower survival as the soybean meal level increased the feed. (Drummond et al. 2009) reported that the average survival of Nile tilapia fed 60 mg/kg of MT treated with fish meal result obtained (65.6-84.3%). They were almost similar to the present study. Although the lower survival rate in treatments fed on soybean meal during the treatment phase cannot be justified, it is possible that some anti-nutritional factors in soybean meal is to blame for the lower survival rate. To make this clearer, additional research on the chemical makeup of soybean meals must be done. With increasing dietary protein intake, tilapia's protein requirement declines with age and size, and protein needed for fry is 30–56% (Winfree and Stickney 1981; Jauncey 1982) whereas protein availability in raw soybean meal contained 30.53 % (Zainab et al. 2021), 37.69% (Etiosa et al. 2018) and soybean flour contained 37-49 % crude protein (Franck et al. 2002). It has been reported that when defatted and ground into grits or flour, they contained nearly 50–54% protein; the created soy protein concentrates with 60

70% protein after flavoring compounds and flatulence sugars were extracted with ethanol or acidic waters. They could be also processed into soy protein isolates with 90–100% protein by alkali extraction of the protein, fiber dismissal by centrifugation and re-precipitation, and drying of the protein (Lusas and Riaz 1995).

In the present study, we used milled soybean flour for the sex reversal of Nile tilapia fry and obtained no significant difference in male proportion (%) in all treatments. Thus, it is assumed that protein availability for *O. niloticus* fry in sex reversal is sufficiently provided by the soybean meal used in the present study.

Production cost of monosex fry

The total cost of monosex fry production was estimated on a general basis and shown in Table 4. The total diet given to each hapa during the treatment period was estimated, and the total cost of the diet was calculated. From the feed supplied to each hapa, the total production cost of each fry was estimated.

Table 4: Production cost of monosex fry production

Particulars	Treatments				
	T_1	T_2	T_3	T_4	T_5
Hatchling cost (NRs./replication)	20.0	20.0	20.0	20.0	20.0
Cost of MT (NRs./370 mg)	53.3	53.3	53.3	53.3	53.3
Ethanol (NRs./ 370 mL)	88.8	88.8	88.8	88.8	88.8
Fish meal (NRs./370 gm)	444	333	222	111	0
Soybean meal (NRs./370 gm)	0	13.9	27.8	41.6	55.5
Total feed cost	606.1	509	411.8	314.7	217.6
Miscellaneous	50	50	50	50	50
Total cost	656.1	559.0	461.8	364.7	267.6
Production cost (NRs./ fry)	3.28	2.79	2.31	1.82	1.34

The production cost of monosex fry was highest in T_1 and lowest in T_5 during the treatment phase. The current study shows that there was a decrease in the production cost of monosex fry with increasing the SM contained in their diet which could be clearly due to lower cost of soybean meal compared to fish meal. However, lower growth and survival rate also must be taken into account which could have been due to the lower crude protein level in soybean meal as compared to fish meal (Winfree and Stickney 1981; Jauncey and Ross 1982; Siddiqui et al. 1988; El-Sayed and Teshima 1992).

CONCLUSION

The results of the current study found that the proportion of males in all treatments was significantly at par to each other. It was also evident from the result of present study that the growth and survival of monosex male Nile tilapia fry was also at par with the use of fish meal with the replacement of fish meal by soybean meal upto 50 %. However, the survival as well as growth of fry were significantly lower in case of only soybean meal hormonal feed. The production cost of all male monosex Nile tilapia fry decreased with the increasing replacement of fish meal by soybean meal upto 75%. Overall, replacing 75% of fish meal by soybean meal was found to have better sex reversal coinsidering the sex ratio, growth and survival as well as fry production cost. The use of soybean meal has the potential to be used in place of fish meal to produce a population of all-male Nile tilapia.

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SOME COMMON DRUGS AND CHEMICALS USED IN AQUACULTURE IN NEPAL

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ABSTRACT
Common drugs and chemicals used in aquaculture practices of Nepal are rarely documented. Present work was carried out to list out some common drugs and chemicals used in aquaculture farms. This compilation of drugs and chemical is based on discussion with fish managers, fish farmers and my personal experiences as fish farm manager for more than two decades. It is not necessary that all farmers might be using the enlisted drugs and chemicals in their farms. There might be farms which may not use any chemicals. In general, the use of drugs and chemicals in aquaculture is very limited to certain hatcheries working for research and academic purposes. A detailed survey is advised to obtain more specific details on drugs and chemicals in aquaculture practices used in Nepal.

Key words: Prophylactic, Antibiotics, Injuries, Transport

INTRODUCTION
Aquaculture in Nepal is one of the rapidly growing food industry (Shrestha and Pant 2012; Gurung 2014; Kunwar and Adhikari 2017). With increasing aquaculture activities, processes such as handling for breeding, selling, growth checkup, size segregation and transportation of fish become more inevitable. Often inappropriate handlings may cause physical injuries to fish ranging from stages of embryo, brood including all intermediate stages of fry, fingerling, juvenile and adult fish. The injuries, if not treated might cause devastating losses. So, to maintain healthy fish stock, one might require some prophylactic measures to prevent and control infectious and other diseases that could be caused by virus, bacteria, fungus, pest and parasites. Similarly, stocking of fish in new cage, tank, pond, raceway require cleaning of nets, floor and walls of holding tanks to avoid harmful germs. In many occasions, especially in carp, tilapia, trout, and pangas aquaculture and others, the pond/raceway/tanks require some management practices to make the water suitable for fish stocking, maintaining reasonable range of pH, dissolved oxygen and other water quality parameters etc. The management measures are known to break down the life cycles of certain pathogen and parasites that might require two or more hosts to complete their life cycle. The pond water quality management is also mandatory to control undesirable copepods and other organisms (Kumar et al. 2012) as these might injure and harm delicate hatchlings, fry and fingerlings.

In aquacultural practices, to facilitate spawning of fish in hatchery some drugs are indispensable (Kumar and Roy 2017), because certain cultivable fishes cannot spawn spontaneously (Rottman et al. 1991; Jha and Neupane 2023). Such fish require certain stimulating hormone, which facilitates spawning with hatchery personal support to take out mature ova and milt for fertilization. Luteinizing Releasing Hormone-analogue (LRH-*a*) and Ovaprim are used to propagate many fish species, especially carp, catfishes and others.

Tilapia has shown relatively higher growth in male compared to female of same age (Bhujel and Suresh 2000). So, for commercial production of tilapia, all male fry is pre-requisite to be more economical. To promote all male fry population certain drugs are used such as 17 α-Methyl testosterone (MT) hormone (Guerrero 1975; 1982; Sarker et al. 2022).

Some common chemical used traditionally to maintain hygiene and cleanness of fish hatcheries are lime, formalin and common salt (Ali et al. 2014; FAO 2023). There could be a long list of such drugs and chemical use in fish farming. However, there are rarely such listing of common drugs used in aquaculture. Therefore, to find out list of common drugs used in fish farms has always been difficult. Hence, this paper presents compilation and listing of chemicals and drugs used in aquaculture for fish health and environmental safety in Nepal.

MATERIALS AND METHODS

Information on drugs and chemicals used in fish farms were collected through discussion with farm managers and hatchery owners of private sector besides other secondary sources and my own experiences on the use of drugs while working in fish farm for more than two decades in Nepal and abroad such as Japan and Sri Lanka.

RESULTS AND DISCUSSION

A list of drugs and chemical used in aquaculture practices has been given in Table 1. Most aquaculture practitioners use these chemicals as prophylactic agents such as lime, common salt and $KMnO_4$ etc. Since lime is recommended as a disinfectant and pond water cleaning agent its use could be most common in fish farms. The other disinfectant commonly used was the common salt to control external parasites and treat some of fungal infection in fish. The use of $KMnO_4$ was recommended earlier to control the outspread of ulcerative disease syndrome (UDS). Other more common drug is acriflavin used as prophylactic agents in wounds for healing open injuries to keep away fungus and bacterial infections.

Fish breeding hormone

Use of Luteinizing Releasing Hormone-analogue (LRH-a), and Ovaprim were most common among carp hatcheries. Both of these common breeding hormones are not produced in Nepal, but are marketed from abroad. LRH-a, is more common to use to breed *Ctenopharyngodon idella* (grass carp), *Hypophthalmichthys molitrix* (silver carp), *Aristichthys nobilis* (bighead carp). Ovaprim is most effective to breed *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Naini) and *Catla catla* (Bhakur). This drug is popular in carp and catfish hatcheries with restricted distribution to certain limitation in Nepal. The ovaprim have also been effectively used for breeding *Labeo dero* (Gardi), *Pangasianodon hypophthalmus* (Pangas), *Clarias batrachus* (Asian catfish) and *Clarias gariepinus* (African catfish). Before, Ovaprim and LRHa hormone in fish breeding, use of pituitary gland extract of mature fish was common and reliable practice in Nepal. Now the use of mature fish pituitary glands for fish breeding is rare, but may require to improvised and reintroduce.

17 Methyl testosterone hormone

17-α Methyl testosterone hormone (MT) is a hormone use to promote all male population of tilapia. Tilapia is one of most popular fish all over the world. In nature, male has faster body growth comparing to female. So to be more proficient in tilapia production, male tilapia are preferred. For all male fry production, MT is used to promote all male population.

Water and soil treatment products

For fish farming, pond preparation is an initial step towards enhancing of the fish production. Such preparation before stocking fish in pond are required for mineralization of organic matter, adjusting pH, disinfection, removing predators and competitors. The chemicals used in this regard are lime in the form of lime stone ($CaCO_3$), slaked lime Ca (OH_2) or un-slaked lime (CaO). Some literatures (Subhashinge et al. 1996; Sharker et al. 2014) have shown that aqua medicines like Geotox, Zeolite, Zeocare, Lime, Bio Aqua, Aquanone, Zeo prime are used for the pond preparation and water quality management. Disinfection and anaesthetized of dried ponds are also done using active iodine or potassium permanganate ($KMnO_4$). However, there are inadequate information on the use of these chemicals including the use of iodine as broad-spectrum microbicide against bacteria, fungi, viruses, and protozoans in Nepal, as Zhang et al. (2023) has described elsewhere.

Table 1: Some aquaculture drugs usually used in aquaculture farms in Nepal

S.N.	Drug	Commercial Name	Usage and doses	Target species
1	LRH-a	Luteinizing Releasing Hormone-analogue	Fish spawning hormone. Improves the spawning function in male and female brood finfish. The dose of LRH-a use is 5–10µg/kg; for male dose is reduced to half (FAO 1985).	Grass carp, Bighead carp, Silver carp
2	Ovaprim	Liquid peptide preparation, an analog of salmon gonadotropin releasing hormone (sGnRHa) and brain neurotransmitter (dopamine) inhibitor.	Promote and improve the spawning function in male and female mature brood fish. Usually a dose of 0.5 mL/kg of bodyweight Ovaprim is used. For male the dose is reduced down to half. Usually, two doses are applied.	Rohu, Naini, Catla, Gardi (*Labeo dero*), Silver carp, Bighead carp, Grass carp,
3	17α-Methyl testosterone hormone	17α-Methylandrost-4-en-17β-ol-3-one	60 mg 17α-MT per kg feed dose for 28 days (Sarker et al. 2022). It promotes to produce all male population.	Tilapia
4	Formalin	Formaldehyde solution	Control protozoan, monogenetic trematodes, Saprolegnia infection. A dose of 15 to 25 mg/L is used for prolonged bath to remove for controlling fish parasites (Francis-Floyd 1996)	Fin fish, Rainbow trout
5	Folic acid	N-[p-[[(2-amino-4-hydroxy-6-pteridinyl) methyl]-amino] benzoyl]-L-glutamic acid	A dose of 0.3 and 0.6 mg/kg for survival and growth (Cowey and Woodward 1993)	Rainbow trout
6	Mineral mixture	Mineral mixture contains essential minerals that required in trace amounts	Dose requirement is 1% of the feed for improving the feed quality of fish.	All carp, trout and Tilapia feed.

		(iron, zinc, manganese, copper, iodine, cobalt, selenium, sodium, calcium, Sulphur, phosphorus, magnesium, potassium and chlorine.		
7	Tricaine Methanosulphonate	Triacaine-S MS-222	It used during the transportation fish as an anesthesia. The dose vary according to species. In general a dose at 30 mg/L for may recommended (Liu et al. 2022; Zhang et al. 2023)	All cultivable brood fish, such as carp
8	Acetic acid	Acetic acid	Use 1000-2000 ppm dip for 1-10 minutes (ASEAN Secretariat 2013)	Juvenile and broods of carp
9	Acriflavin	Acriflavinium chloride 3, 6-Diamino-10-methylacridinium chloride mixt. With 3, 6-acridinediamine	Long Bath (3 – 5 days) – Apply 200 mg of Acriflavine per 10 gallons (5ppm) of either aquarium water or stock holding tank (Aqua world 2023).	All carp
10	Sodium Chloride	NaCl or common salt	Use to control external parasites. Does may depend on size and species and other factors. In general 5 to 10 g/L are well tolerated (Burgdorf-Moisuk 2011) or 3% dip for 10-30 min (ASEAN Secretariat 2013)	Carp, Trout
11	Calcium Oxide	Quick lime or burnt lime	Control external parasite and break the chains of parasite life cycle stages. Improves water soil and water quality. During pond preparation, lime is applied to the pond bottom at doses of 100-8,000 kg/ha or to the water during the rearing period at 10-500 kg/ha (ASEAN Secretariat 2013)	Carp, Tilapia, catfish ponds for disinfection.
12	Calcium Carbonate	$CaCO_3$. Most common agricultural lime		

13	Trichlorofon (TCF)	Acetylcholinesterase (AChE)	Organophosphate compound used extensively as an anti-parasitic in aquaculture. The dose requirement 0.1 to 1.0 mg/L could be recommended (Lopes et al. 2006)	In carp ponds
14	Quinaldine	2-methylquinoline is an organic compound with the formula $CH_3C_9H_6N$	Used for fish anesthesia. 175 to 400 µL/L in the case of Rohu transportation (Hasan et al. 2013)	Mostly in carp
15	Benzocaine	Benzocaine is chemically very similar to MS222	Used for fish anesthesia (25–30 mg/L for most fish in less than 3.5 min, and fish may recovered in less than 10 min after 15 min of exposure. Safety margins were narrow; 30 mg/L for about 20 min, 25 min of exposure causes deaths (Gilderhus 1990).	Carp
16	Malachite Green	Malachite green is prepared from benzaldehyde and dimethylaniline	Its use is controversial, has been banned in USA and UK. Use 1 teaspoon (approximately 5 mL) per 10 gallons of water (Sharpe 2023)	Use to disinfect the trout eggs and use in carp pond as disinfectant in some research hatcheries.
17	Some antibiotics use 1. Amoxicillin The dosage of antibiotic has been suggested to be 4.5 grams of antibiotic per 100 pounds of fish per day. 2. Erythromycin	Amoxicillin is a penicillin antibiotic, used in fish tanks. It effectively combats gram-positive and certain gram-negative bacteria in fish. Erythromycin helps broad spectrum treatment and control of bacterial disease, such as body slime, furunculosis, gill and fin rots.	One tablespoon for each 5 gallons (19 L) of aquarium.	Aquarium fish in general. May be cultivable fishes can also be treated.

Disinfectants used in aquaculture

Disinfectants are antimicrobial pesticides which are used almost in regular intervals to kill bacteria, viruses and other pests in fish hatcheries and production locations for throughout the production cycle for achieving better survival and productivity (Newman 2006). Some of the products use to disinfect the rearing and hatchery facilities are Bleach, Aquakleen, Benzalkonium chloride (BKC), Ethylenediaminetetraacetic acid (EDTA), Efinol, Formalin as disease disinfectants. Among them most common in Nepal may be Formalin especially in research facilities in general, but rarely in private sector hatcheries and farms. The formalin are used commonly to control protozoan disease and effective against many external pathogens, mostly to treat fry.

Piscicides

A piscicide is a chemical substance which is used to kill undesirable fish from water bodies. Most common examples of piscides are Rotenone, Saponins, Niclosamide etc. These all chemicals are toxic, poisonous that kills fish (Das et al. 2017). In general, no detail account of the use of piscicides are available from Nepal.

Anesthetics used for fish

Anesthetics are chemical or physical agents used to calm animals and cause them to progressively lose their mobility, equilibrium, consciousness, and finally their reflex action (Husen and Sharma 2014; Mustafa and Aileru 2021). The anesthetics are helpful for reducing the stress caused during handling and transport of juvenile, adult and brood fish. The most common anesthetic drugs used in fish are MS-222 (Tricaine), Benzocaine, Isoeugenol, Metomidate, 2-phenoxyethanol, and Quinaldine (Sneddon 2012).

Feed additives

Fish feed additives are those pigments, vitamins, chemo attractants and preservatives such as mold inhibitors and antioxidants which are used in small quantity in the fish feed during feed preparation to increase feed efficiency and to prevent certain diseases. These all additives actually add the nutritive values of the feed to make supplementary feeds as complete and absolute type of feed for a cultivating specific species. Other additives, such as probiotics, prebiotics aim to target improve intestinal health, reduce stress and increase the resistance of diseases.

Antibiotics use and antimicrobial resistance in fish farms

Antibiotics are drugs which kill, prevent, control or treat bacteria to spread further. Often it is not necessary to use the antibiotic drugs because some mild bacterial infections may recover without the use of antibiotics. Several types of bacterial infections are known to appear in fish farms situated in warmer belt as well as from colder regions. One of the most serious diseases where use of antibiotics might be desirable is Epizootic Ulcerative Syndrome (EUS), a bacterial-fungal disease in Nepal. Beside these other bacterial diseases causing serious problems reported are furunculosis, columnaris, dropsy, vibriosis, bacterial gill disease and fin rot/tail rot (Shrestha et al. 2019; Timilsina et al. 2022). Bacterial diseases are known to be best treated by antibiotics such as penicillin, amoxicillin, or erythromycin and others. However, there is very limited studies on specific bacterial infections and treatment in aquaculture in Nepal. Therefore, it is difficult to find fish specific antibiotics in markets in Nepal. In such cases, if highly desirable than it may be the case that common antibiotics which are used for human or in poultry or in animal husbandry may also be used in aquaculture. Some antibiotics used for research and academic purposes in Nepal could be amoxicillin and erythromycin. It is likely that aquarium shops might be using antibiotics to control bacterial diseases. However, further verification might require to confirm the use of antibacterial drugs. Earlier there has been no guidelines on the usage and doses prescribed on antibiotics specific to aquaculture, but recently government has been cautious about the cases of antimicrobial resistance, in general. Recently, Timilsina et al. (2021) reported that in an antibiotic susceptibility test in many rainbow trout farms, the fish were resistant to Amoxicillin, Azithromycin, Neomycin, Oxytetracycline Cephalexin, Doxycycline and Streptomycin antibiotics. These implying that there is dire need of further studies and investigation on bacterial diseases and the use of antibiotics in fish farms in Nepal.

CONCLUSION

The use of certain drugs and chemicals in aquaculture practices might be controversial. Some chemicals might have environmental or impact on human health, if not used appropriately. Calculation of dose requirement is one of the essential parts with the drugs and chemical use. Miscalculation of such drugs and chemicals might impact negatively on the environment, targeted fish species as well as on human health. So, care should be taken to use drugs and chemicals in proper doses. In some countries certain chemicals have been banned for the use considering the impact. Many farmers and other users might not be aware of the impact of such chemicals and drugs. For example, malachite green has been banned in many countries but the same may not be applied in other countries. In such conditions the drugs and chemicals chartered should be applied from concerned governing agencies from time to time to avoid the negative impact of drugs and chemicals.

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