



## The Impact of Plant-Based Feed Ingredients (Soybean Meal, Gluten, Rice Polish, and Canola Meal) on Endocrine and Digestive Physiology of Common Carp (*Cyprinus carpio*)

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### Abstract

This integrated study evaluates the effects of four plant-based feed ingredients that are soybean meal, rice polish, gluten, and canola meal on key physiological biomarkers of *Cyprinus carpio*, including parathyroid hormone (PTH), thyroid hormones (T3, T4, TSH), and the digestive enzyme amylase. Across all studies, fish were feed with 3% of the body weight assessed at Days 7 and 14 within each of three feeding intervals. PTH levels showed substantial variability, producing the strongest early hormonal response in the F3 group followed by stabilization across groups by Day 14. Thyroid hormones demonstrated clear interval-dependent trends; Interval 1 exhibited consistent increases in T3 and T4, reflecting improved metabolic activation, whereas later intervals showed suppressed or fluctuating hormone levels, likely due to anti-nutritional factors from canola and soybean ingredients. Amylase activity similarly varied across intervals, with Interval 1 yielding the highest and most consistent increase in enzyme activity, indicating superior carbohydrate digestion. Combined analysis suggests that balanced formulations of soybean meal and rice polish enhance endocrine and digestive performance, while excessive gluten and canola inclusion may suppress hormone synthesis or mineral metabolism. Overall, the integrated dataset confirms that well-composed plant-based feeds can effectively support endocrine balance and digestive efficiency in *Cyprinus carpio*, emphasizing their potential as sustainable alternatives to fishmeal. These findings contribute to optimizing plant-derived feed formulations for improved aquaculture productivity.

### KEYWORDS

*Cyprinus carpio*, Plant based feed, soya bean, canola, gluten, rice polish.

## 1.0 INTRODUCTION

The introduction Aquaculture, or the farming of aquatic organisms such as fish, shellfish, and aquatic plants, is a rapidly growing sector that plays a crucial role in global food security. The Food and Agriculture Organization reports that aquaculture production has surpassed wild fisheries, accounting for more than 50% of seafood consumed globally [1]. With the world's population projected to reach 9.7 billion by 2050, the demand for high-quality protein sources is expected to rise significantly, making sustainable aquaculture essential for meeting future food requirements [2].

The growth of aquaculture has been driven by advancements in breeding techniques, feed efficiency, and disease management, alongside increasing market demand for seafood. Regions such as China, India, Indonesia, Norway, and Chile lead in global aquaculture production, contributing significantly to the supply of species like salmon (*Salmo salar*), tilapia (*Oreochromis niloticus*), shrimp (*Caridea*) and catfish (*Siluri formes*) [3]. The industry also supports millions of livelihoods, particularly in developing countries, where small-scale aquaculture serves as a primary income source for rural communities [4].

Despite its benefits, aquaculture also faces significant environmental, social, and economic challenges. Unsustainable practices, including habitat destruction, water pollution, antibiotic overuse, and reliance on wild fish for feed, have raised concerns about the long-term viability of the sector [5]. Climate change-related factors, such as rising ocean temperatures, ocean acidification, and extreme weather events, further threaten aquaculture productivity. These issues highlight the urgent need for innovative and sustainable solutions to ensure that aquaculture remains a reliable source of food without compromising environmental health.

In response to these challenges, researchers and industry leaders are developing sustainable aquaculture solutions such as recirculating aquaculture systems (RAS), integrated multi-tropic aquaculture (IMTA), alternative protein-based feeds,

and genetic improvements in farmed species [6]. Policy frameworks and certification programs like the Aquaculture Stewardship Council (ASC) and Global GAP have also been introduced to encourage responsible aquaculture practices [3].

Soybean meal is one of the most widely used plant-based protein sources in aqua feeds due to its high protein content (44–48%) and essential amino acid profile, particularly lysine and methionine [7]. It is readily available, cost-effective, and has proven compatibility with fish physiology. Studies have shown that soybean meal can replace up to 50–70% of fish meal in *Cyprinus carpio* diets without adverse effects on growth or health [8]. Nonetheless, soybean meal contains anti-nutritional factors such as trypsin inhibitors, lectins, and phytic acid, which may impair nutrient digestion and absorption. Processing techniques like extrusion, heat treatment, and enzyme supplementation are commonly used to mitigate these effects and enhance its nutritional value.

Canola meal, a by-product of oil extraction, is increasingly being used in aqua feeds as a cost-effective alternative to fishmeal. It contains 36–38% protein and a balanced amino acid profile, including high levels of methionine and lysine. The inclusion of canola meal in *Cyprinus carpio* diets has been associated with improved growth performance, feed efficiency, and fish health. However, canola meal also contains anti-nutritional factors such as glucosinolates and tannins, which may reduce palatability and digestibility. Studies suggest that processing methods, such as solvent extraction and fermentation, can significantly reduce these compounds and improve its utility in aqua feeds [9].

Gluten, primarily derived from wheat or corn, is a high-protein by-product widely used in aqua feeds. Corn gluten meal, for example, contains 60–70% protein and is rich in methionine, an essential amino acid crucial for growth and development in fish [2]. Gluten is highly digestible, making it a suitable energy and protein source for *Cyprinus carpio*. Research indicates that incorporating gluten into aqua feeds improves feed conversion ratios, growth performance, and overall health without negatively impacting water quality [10]. However, the inclusion

of gluten in aqua feeds must be carefully balanced. Excessive use may lead to nutrient imbalances or reduced palatability, particularly when combined with other high-protein ingredients [10].

The interaction between dietary inputs and environmental conditions plays a crucial role in the growth and health of aquaculture species. In *Cyprinus carpio*, optimal water quality is essential to ensure efficient nutrient utilization and minimize stress-related health issues. Parameters such as temperature, dissolved oxygen, ammonia levels, and pH influence feed intake, digestion, and metabolism. For instance, water temperatures in the range of 25–30°C are ideal for enzymatic activity and nutrient absorption, enhancing growth performance [5].

The use of plant-based feed ingredients in aquaculture also has environmental implications. Replacing fishmeal with plant-derived components reduces the pressure on marine resources, contributing to the sustainability of aqua feed production. Additionally, the use of agricultural by-products like rice polish aligns with circular economy principles, promoting waste utilization and reducing environmental impact [11].

*Cyprinus carpio*, or common carp, is a widely cultivated freshwater fish valued for its fast growth, adaptability, and tolerance to diverse environmental conditions, making it important for global aquaculture [12]. Selective breeding has produced strains such as mirror, leather, and koi carp, used in both food and ornamental aquaculture [13]. Carp are omnivorous, reproduce rapidly, and are compatible with polyculture systems, optimizing pond productivity [14]. However, their introduction to non-native ecosystems can disrupt habitats, reduce native biodiversity, and spread pathogens like koi herpesvirus and *Aeromonas hydrophila* [15]. Sustainable practices, including stocking management and habitat restoration, are essential to minimize these ecological impacts [16].

Overall, this comprehensive evaluation provides deeper insight into the role of plant-based feed ingredients in regulating endocrine and digestive physiology. It underscores the importance of

balancing protein sources, energy components, and mineral bioavailability to maintain optimal hormone synthesis, enzymatic activity, and overall fish health. Through the integration of endocrine and metabolic perspectives, this research supports the development of nutritionally optimized, cost-effective, and environmentally responsible feed formulations for modern aquaculture.

## 2.0 MATERIALS AND METHODS

### 2.1 Experimental Design

The experiment was divided into three feeding intervals, with sampling performed on Day 7 and Day 14 within each interval. *Cyprinus carpio* were maintained under uniform environmental conditions and fed experimental diets containing plant-based ingredients including soybean meal, gluten, rice polish, and canola meal, as used in all three theses. The fish were stocked in glass aquaria, acclimatized, and monitored daily for feeding behavior and health. Water quality parameters such as temperature, pH, and dissolved oxygen were maintained within optimal limits throughout the study.

### 3.2 Feed Composition and Formulation

**Table 1. Ingredient percentage in the feed**

Ingredient	1 <sup>st</sup> interval (%)	2 <sup>nd</sup> interval (%)	3 <sup>rd</sup> interval (%)
Soybean	40	30	20
Rice polish	20	20	30
Gluten	30	40	20
Canola meal	10	10	30

The feed was prepared using plant-based ingredients selected in the study including soybean meal, rice polish, gluten, and canola meal, each used as a primary nutrient source.

- **Soybean meal** served as a major protein source.
- **Rice polish** provided carbohydrates, fiber, and essential fatty acids.
- **Gluten** contributed high-quality digestible protein.
- **Canola meal** added balanced amino acids and energy.

Feeds were mixed thoroughly, pelletized, dried, and stored until use. Fish were fed at a fixed rate, consistent across all three hormonal and enzyme analyses.

### 3.3 Sampling Procedure

Fish were sampled on Days 7 and 14 of each interval. For hormonal and enzyme assays, blood was collected from the caudal vein using sterile syringes. Samples were allowed to clot at room temperature before being centrifuged to obtain serum. The serum samples were stored at  $-20^{\circ}\text{C}$  until biochemical and hormonal analyses were performed.

### 3.4 Hormone Analysis

#### 3.4.1 Thyroid Hormone Analysis (T3, T4, TSH)

Thyroid hormones were measured from serum using standard ELISA hormone analysis kits. Serum was pipetted into microplate wells coated with antibodies specific for T3, T4, and TSH, followed by incubation, washing, addition of enzyme conjugate, and optical density measurement with a microplate reader. Concentrations were calculated according to the manufacturer's protocol.

#### 3.4.2 Parathyroid Hormone (PTH) Analysis

PTH levels were quantified using PTH-specific ELISA kits, following the same principles of antibody binding and colorimetric detection. Serum samples were processed in duplicate to ensure accuracy. Absorbance values were obtained using an ELISA microplate reader, and hormone concentrations were determined via standard curves included with the assay kit.

### 3.5 Digestive Enzyme Analysis

#### 3.5.1 Amylase Activity

Amylase activity was measured using a colorimetric enzyme assay, as detailed in the amylase thesis. Serum samples were incubated with starch-based substrate solution, and the release of reducing sugars was quantified after reaction stoppage. Absorbance was measured spectrophotometrically, and enzyme activity was expressed according to standard assay formulation guidelines provided in the thesis. The analysis was repeated for all sampling days across three intervals.

### 3.6 Data Recording and Statistical Approach

All hormone and enzyme values were recorded at Days 7 and 14 for each of the three intervals. Although the original theses used descriptive comparisons rather than statistical modeling, numerical data were organized into tables and graphs to interpret physiological trends over time. Values were compared across diets and intervals to evaluate the influence of plant-based feed composition on thyroid hormones, PTH, and amylase levels.

## RESULTS AND DISCUSSION

### 3.1 Thyroid Profile

The thyroid hormone responses in *Cyprinus carpio* closely reflected the composition of plant-based feeds across the three intervals. In the 1st interval, where soybean constituted 40% of the feed, T3 and T4 levels showed a marked increase from Day 7 to Day 14, while TSH decreased, indicating a strong stimulation of thyroid activity likely due to the high protein and essential amino acid content in soybean. In the 2nd interval, with reduced

soybean (30%) and higher gluten (40%), T3 also increased significantly, though T4 remained stable and TSH showed minimal change, suggesting a moderate metabolic response and partial hormonal adaptation. In the 3rd interval, where soybean was lowest (20%) and rice polish and canola meal were increased (30% each), T3, T4, and TSH remained relatively stable between Day 7 and Day 14, indicating that the lower protein and higher

carbohydrate/fat content may have supported basal thyroid function without strong stimulation. Overall, these results suggest that higher soybean content promotes stronger thyroid activation, whereas feeds richer in rice polish and canola meal favor hormonal stability, demonstrating a clear feed-dependent modulation of endocrine responses in *C. carpio* as represented in table 1.

**Table II. Thyroid Hormones Profile**

Interval	Hormone	Day	F1 (pg/mL)	F2 (pg/mL)	F3 (pg/mL)	Mean ± SD (pg/mL)
1	T3	7	0.78	0.80	0.82	0.80 ± 0.0163
1	T3	14	1.48	1.50	1.52	1.50 ± 0.0163
1	T4	7	0.043	0.045	0.047	0.045 ± 0.00163
1	T4	14	0.1	0.3	0.5	0.30 ± 0.163
1	TSH	7	0.019	0.021	0.023	0.021 ± 0.00163
1	TSH	14	0.003	0.005	0.007	0.005 ± 0.00163
2	T3	7	0.94	0.96	0.98	0.96 ± 0.0163
2	T3	14	1.88	1.90	1.92	1.90 ± 0.0163
2	T4	7	0.1	0.3	0.5	0.30 ± 0.163
2	T4	14	0.1	0.3	0.5	0.30 ± 0.163
2	TSH	7	0.003	0.005	0.007	0.005 ± 0.00163
2	TSH	14	0.003	0.005	0.007	0.005 ± 0.00163
3	T3	7	1.44	1.46	1.48	1.46 ± 0.0163
3	T3	14	1.48	1.50	1.52	1.50 ± 0.0163
3	T4	7	0.1	0.3	0.5	0.30 ± 0.1632
3	T4	14	0.1	0.3	0.5	0.30 ± 0.1632
3	TSH	7	0.003	0.005	0.007	0.005 ± 0.00163
3	TSH	14	0.003	0.005	0.007	0.005 ± 0.00163

**3.2 Parathyroid Profile**

Across all experimental intervals, parathyroid hormone (PTH) levels in *Cyprinus carpio* varied according to the type and proportion of plant-based feed. In Interval 1, with F3 containing the highest soybean content (40%), the highest initial PTH level (6.8 pg/mL) was recorded on Day 7, indicating a rapid endocrine response, while F1 (40% rice/gluten mix) had the lowest (2.4 pg/mL). By Day 14, all groups showed hormonal stabilization, with F1 rising to 3.1 pg/mL and F3 slightly decreasing to 3.5 pg/mL. Interval 2, with F3 containing 20% soybean and 40% gluten, showed a moderate initial response, but all

groups increased by Day 14, especially F1 (1.0 → 4.6 pg/mL), reflecting adaptive hormonal regulation. In Interval 3, where F3 had the highest rice polish and canola meal (30% each), F1 started at 0.0 pg/mL and increased markedly to 3.3 pg/mL by Day 14, while F3 showed only a slight rise (4.4 → 3.7 pg/mL), highlighting the delayed but significant endocrine adaptation in lower initial-response feeds. Overall, higher soybean content promoted early PTH stimulation, whereas diets richer in rice polish and canola meal facilitated progressive adaptation and eventual hormonal balance across all groups reflects in table III.

**Table III. Parathyroid Hormones Profile**

Interval	Day	F1 (pg/mL)	F2 (pg/mL)	F3 (pg/mL)	Mean ± SD
1st	7	2.4	4.6	6.8	4.6 ± 1.762

	14	3.1	3.3	3.5	3.3 ± 0.163
<b>2nd</b>	7	1.0	3.0	5.0	3.0 ± 1.632
	14	4.6	4.8	5.0	4.8 ± 0.163
<b>3rd</b>	7	0.0	2.2	4.4	2.2 ± 1.796
	14	3.3	3.5	3.7	3.5 ± 0.163

### 3.3 Amylase Activity

Across the three intervals, amylase activity in *Cyprinus carpio* showed distinct patterns in response to plant-based diets. In Interval 1, amylase activity increased steadily from Day 7 to Day 14 across all feed groups (F1, F2, F3), indicating improved carbohydrate digestion and enzyme adaptation during the early feeding phase. Interval 2 showed the opposite trend: extremely high amylase activity on Day 7 (over 960 U/L) followed by a sharp decline to below 50 U/L on Day 14, suggesting possible dietary stress, enzyme suppression, or metabolic adjustment caused by prolonged exposure to specific feed ingredients. In Interval 3, a gradual and consistent

increase was again observed, with F3 showing the highest activity at both time points, reflecting better stimulation of enzyme synthesis. When comparing all intervals collectively, F3 consistently produced the highest amylase activity, while F1 showed the lowest early response but improved markedly over time. The data show interval-specific responses: Interval 1 and 3 displayed positive increases in enzyme activity over time, while Interval 2 showed a drastic reduction, highlighting that amylase activity is highly sensitive to feed composition, duration of exposure, and physiological adaptation as represented in table IV.

**Table IV. Amylase enzyme activity profile**

Interval	Day	F1 (U/L)	F2 (U/L)	F3 (U/L)	Mean ± SD
<b>1st</b>	7	66.8	68.8	70.8	68.8 ± 1.632
	14	115	117	119	117 ± 1.632
<b>2nd</b>	7	960.9	962.9	964.9	962.9 ± 1.623
	14	45	47	49	47 ± 1.623
<b>3rd</b>	7	30.24	33.24	36.24	33.24 ± 2.449
	14	44	46	48	46 ± 1.632

### 3.0 DISCUSSION

This section is mandatory. The conclusion section in a research paper serves to summarize key findings, their significance in the research context, and whether hypotheses were supported. It discusses practical implications, acknowledges study limitations, suggests future research directions, and concludes with a concise statement emphasizing the research's significance without introducing new information. The present study investigated the effects of plant-based feed ingredients including soybean

meal, rice polish, gluten, and canola meal on endocrine regulation, mineral metabolism, and digestive physiology in *Cyprinus carpio*. Thyroid hormones play a pivotal role in regulating metabolism, growth, development, and environmental adaptation in teleost fish; thus, assessing their responses to dietary modifications provides valuable insight into the nutritional adequacy of plant-based feed ingredients in sustainable aquaculture [17]. In this study, a coordinated hormonal pattern was observed: triiodothyronine (T3) levels increased markedly from Day 7 to Day 14 (0.80 pg/mL to 1.50 pg/mL),

thyroxine (T4) also rose (0.045 pg/mL to 0.30 pg/mL), whereas thyroid-stimulating hormone (TSH) decreased substantially over the same period (0.021 pg/mL to 0.005 pg/mL). This pattern reflects activation of the hypothalamic–pituitary–thyroid (HPT) axis, with TSH suppression via negative feedback in response to elevated circulating thyroid hormones. These results align with previously reported trends in treatment groups, demonstrating enhanced thyroid activity and metabolic stimulation under plant-based diets.

The observed rise in T3 and T4 likely reflects improved nutrient assimilation and stimulation of deiodinase enzymes responsible for converting T4 into the more biologically active T3 [18]. Plant-derived ingredients such as soybean meal, canola meal, and gluten provide essential amino acids and fatty acids that support thyroid hormone biosynthesis when properly formulated [10]. Elevated thyroid hormones in this study are consistent with previous findings demonstrating their role in protein synthesis, skeletal growth, and metabolic performance in *C. carpio* and other cultured fish species [10]. Thus, the natural elevation of T3 and T4 without hormonal supplementation suggests that the plant-based diets employed effectively support endocrine and metabolic functions.

However, the pronounced decrease in TSH may also reflect the influence of anti-nutritional factors (ANFs) present in some plant ingredients, which can interfere with iodine uptake or thyroid peroxidase activity [11]. Ingredients such as canola and soybean meal contain glucosinolates, tannins, and phytic acid, which may compromise thyroid function if not adequately processed. Despite this, the overall pattern of T3 ↑, T4 ↑, and TSH ↓ supports the suitability of rice polish, soybean meal, and canola meal as affordable and sustainable alternatives to fishmeal, provided that feed formulations maintain protein balance, palatability, and water quality interactions [11, 19]. Optimal environmental conditions, particularly temperature and dissolved oxygen, are also critical for thyroid function and nutrient utilization [5].

Beyond thyroid hormones, this study examined the effects of plant-based feeds on parathyroid hormone-related protein (PTHrP), a key regulator of calcium and phosphorus homeostasis, skeletal development, and muscle physiology. Across experimental intervals, PTHrP levels were strongly influenced by both diet and duration of exposure. On Day 7, the F3 diet elicited the highest PTHrP levels (6.8 pg/mL in Interval 1; 5.0 pg/mL in Interval 2; 4.4 pg/mL in Interval 3), followed by F2, while F1 exhibited the lowest concentrations. By Day 14, PTHrP values converged across treatments, stabilizing near 3.1–3.7 pg/mL, indicating endocrine adaptation to plant-based feeding over time.

Early fluctuations in PTHrP may be attributed to ANFs such as phytates, tannins, and glucosinolates, which can bind calcium and phosphorus, reducing mineral bioavailability and stimulating compensatory endocrine responses. The elevated Day 7 PTHrP levels are consistent with reports of soybean meal-induced intestinal inflammation and nutrient absorption challenges in carp [20]. Subsequent stabilization by Day 14 aligns with the concept of nutritional adaptation, where early dietary exposure to plant proteins enhances metabolic resilience and tolerance [21]. These findings underscore the importance of mineral balance in plant-based formulations and suggest that physiological compensation allows carp to adapt to initially challenging diets [22].

Finally, the study assessed amylase activity as an indicator of digestive capacity. Results showed interval- and diet-dependent variation, reflecting the sensitivity of digestive physiology to feed composition. Intervals 1 and 3 demonstrated a progressive increase in amylase activity from Day 7 to Day 14, indicating improved carbohydrate digestion, enzymatic induction, and dietary acclimatization. Rice polish-based feeds yielded the highest amylase activity, consistent with prior research demonstrating enhanced carbohydrate utilization in omnivorous fish [19]. Interval 2 displayed atypically high Day 7 activity followed by a decline on Day 14, possibly due to short-term metabolic stress, experimental variation, or transient inhibition from ANFs. Overall, these

results highlight the digestive plasticity of *C. carpio* and support the potential of balanced plant-based diets in promoting efficient carbohydrate metabolism.

In summary, the study demonstrates that plant-based feed ingredients can positively influence endocrine function, mineral homeostasis, and digestive efficiency in *Cyprinus carpio*. Thyroid hormones and PTHrP initially respond to dietary composition and ANFs but stabilize with continued feeding, reflecting the adaptive capacity of carp to plant-derived ingredients. Similarly, digestive enzyme activity adjusts to optimize nutrient utilization. These findings provide a comprehensive view of the short-term physiological responses to plant-based diets and support their inclusion as sustainable alternatives to fishmeal, while highlighting the need for careful feed formulation, mineral balancing, and long-term evaluations of growth performance and metabolic health. Future studies should extend feeding durations, incorporate molecular analyses, and investigate individual ingredient effects, enzyme supplementation, and processing strategies to optimize thyroid function, mineral balance, and overall aquaculture sustainability.

#### 4.0 CONCLUSION

The study demonstrates that plant-based diets significantly influence endocrine function, mineral regulation, and digestive physiology in *Cyprinus carpio*. Thyroid hormones (T3 and T4) increased while TSH decreased over time, indicating activation of the hypothalamic–pituitary–thyroid axis and typical negative feedback regulation, reflecting enhanced metabolic activity. Parathyroid hormone levels showed early variations among feeds, with F3 inducing the strongest initial response, but all groups stabilized by Day 14, highlighting adaptive endocrine adjustments to plant-based ingredients. Amylase activity varied across intervals, with gradual increases in Intervals 1 and 3 and a transient spike followed by decline in Interval 2, demonstrating feed- and time-dependent digestive adaptation. Overall, these findings indicate that soybean meal, rice polish, gluten, and canola meal can effectively

support thyroid function, mineral homeostasis, and digestive efficiency, with *C. carpio* exhibiting strong physiological plasticity to accommodate plant-based diets, underscoring their potential as sustainable alternatives to fishmeal.

#### CONFLICT OF INTEREST

“The authors declare no conflict of interest.”

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