Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Multi-Factorial Analysis of Nutritional Imbalance and Its Impact on Reproductive Performance and Immunity in Captive Big Cats

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Abstract | Nutritional deficiencies impair reproductive efficacy, and immunological response within captive large felids. The evaluation measured the effects of different diets on hormone changes in reproduction alongside the alterations in gut microbial diversity and immune system metabolic functions. A research study included three dietary groups where one received zoological diets as controls and two other groups received dietary supplements of macronutrients and micronutrients along with antioxidants and immune-stimulating elements in their diets. An increase in weight from 6.0 ± 0.2 kg was statistically significant ($p < 0.05$) in the EISG-treated group than the 5.2 ± 0.2 kg weight gain in the BNG-treated group. The statistical evaluation using $p < 0.001$ showed the reproductive hormone levels of E2, LH, and FSH increased in the study group which indicated better fertility rates. The immune function became stronger because TNF-α and IL-6 decreased significantly while IL-10 increased significantly at a p value below 0.01. According to research the p value reached 0.01 indicating a rise in Firmicutes and Bacteroidota bacteria while Proteobacteria bacterial counts demonstrated a substantial decline ($p < 0.01$). Research results showed excellent nutritional worth through the elevated production of SCFAs and observed lipid oxidation combined with amino acid biosynthesis measurements at ($p < 0.001$). The sturdiness of consuming balanced nutrition plays a pivotal role in achieving better fertility results and defending immunity and sustaining metabolic operations. Research shows that specific feeding methods provide managed care conservation programs with successful enhancement capabilities for keeping large cats in reproductive stability and population wellness.

Keywords | Nutritional imbalance, Reproductive performance, Immune function, Captive big cats, Dietary intervention, Gut microbiota, Metabolic efficiency

Received | July 13, 2025; **Accepted** | August 24, 2025; **Published** | September 09, 2025
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Citation | Lahhob QR, Mohsin TS, Mudhafar M, Alsailawi HA, Ayada AA (2025). Multi-factorial analysis of nutritional imbalance and its impact on reproductive performance and immunity in captive big cats. J. Anim. Health Prod. 13(s1): 395-405.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.395.405
ISSN (Online) | 2308-2801

INTRODUCTION

The management of captive big cats faces a primary nutritional challenge because their reproductive outcomes together with immune health depend upon nutrition. The foundation of health depends on nutrition since it sustains hormonal homeostasis while improving gamete quality and maintaining fertility (National Research Council (NRC, 2006)). Improve reproductive performance by avoiding the negative effects of both insufficient or unbalanced amounts of macronutrients including proteins, lipids, carbohydrates and micronutrients embracing vitamins, minerals, respectively. Rutherfurd-Markwick *et al.* (2013) validates that poor oocyte maturation contains sperm quality reduction and high pregnancy loss rates and gestation conflicts with lactation complications. The best conditions for examining such limitations occur in facilities where predators rely exclusively on human-fed sustenance since they no longer access wild prey. Basic nourishment levels of big cats exert a profound negative impact on their reproductive output. As a result of insufficient energy intake the reproductive cycle can become disrupted and ovulation rates become reduced while insufficient protein in the diet leads to problems with hormone production needed for ovulation as well as spermatogenesis (Rutherfurd-Markwick *et al.*, 2013; NRC, 2006). The synthesis of prostaglandins for implantation success alongside pregnancy sustenance depends on omega-3 and omega-6 fatty acids which help regulate inflammatory responses (Macia *et al.*, 2012; Ali *et al.*, 2024; Al-Sailawi *et al.*, 2024; Mohsen *et al.*, 2024).

Captive diets lacking essential nutrients often result in reproductive failure through infertility as well as smaller litters. Nutritional imbalance produces major effects on the immune systems of large felines when examined in addition to reproductive functions. Cell integrity along with antibody synthesis and immune cell activation of macrophages and lymphocytes mainly depend on appropriate food consumption according to Kang *et al.* (2007) and Stachowska *et al.* (2007). The immune system becomes more prone to infections because vitamin deficiencies along with deficiencies of nutrient vitamins A, C, D, E, zinc, selenium, and amino acids decrease the activity rate for antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Macia *et al.*, 2012; Rist *et al.*, 2013). The intake of insufficient protein amounts was discovered to reduce both phagocytic activity and oxidative burst activity of immune cells (Cho *et al.*, 2008). Mineral balance together with urine pH can become disturbed by improper calcium and potassium levels in the diet which leads to metabolic stress and decreases immunity (Paûlack *et al.*, 2014).

Big carnivorous felines need specific delivery challenges when using restricted feeding systems to provide their nutritional requirements. The supplemental diets consisting of processed rations and choice meats given to captive big cats fail to provide essential nutrients in proper amounts just like the whole prey diet of feral big cats (Naumann and Bassler, 2004; NRC, 2006). Captive large carnivores receiving meal schedules provided by humans enter GID which leads to metabolic issues accompanied by inflammation across their bodies (Rist *et al.*, 2013). The relationship between gastrointestinal health and immunity and reproduction functions runs at a high level of strength. Short-chain fatty acids generate in the gastrointestinal tract with other crucial immune functions as they help nutrient absorption while establishing control of inflammatory processes (Macia *et al.*, 2012; Kareem *et al.*, 2023; Aziz *et al.*, 2023). Problems arise when homeostasis breaks in the microbial community which leads to both nutritional problems and immune system dysfunctions. The current welfare conditions in zoological facilities and shelter operations decline because of improper restrictions on animal caregiving practices. Wild animals face deteriorating nutritional health problems because of space constraints during exercise as well as insufficient environmental stimulators and infectious exposures from animals and humans and seasonal food resource differences. Oxidative stress occurs through ROS overproduction as a result of internal stress which causes damage to cellular molecules such as lipids proteins and DNA. Scientists believe oxidative stress affects both immune performance and reproductive abilities of animals (Macia *et al.*, 2012; Rutherfurd-Markwick *et al.*, 2013).

This study aims to examine the combined impact of multiple diets on reproduction and immunity of caged large cats through multi-factor analysis methods. The evaluation of research data will determine crucial nutritional elements for reproductive success and disease immunity through a combination of present-day veterinary nutrition science and immunological study results. Research aims to develop diets for captive big cats based on evidence so infertility diminishes and immune function strengthens.

MATERIALS AND METHODS

Dietary interventions, and nutritional analysis

Baseline dietary assessment

A complete review of each facility's existing feeding protocol used to occur before starting the experimental dietary plan. The evaluation included determining daily food ration weights and assessing nutrient makeup and feeding process methods. Each dietary component's samples collection took place in the middle of the fortnight for laboratory analysis based on AOAC protocols (Stein *et al.*, 2007) using standardized methods to determine proximate composition (protein, fat, moisture, ash) as well as amino acids, fatty acids, vitamins, and minerals. Results demonstrated considerable diversity in nutritional characteristics between institutions while showing dry matter (DM) protein between 24% to 35% and fat ranges from 15% to 30% DM and major differences in micronutrients including taurine at levels from 0.05% to 0.25% DM together with Vitamin A doses from 5,000 to 25,000 IU/kg DM and Vitamin D between 500 to 2,500 IU/kg DM and different Calcium to Phosphorus ratios ranging from 0.8:1 to 2:1 (National Research Council (NRC, 2006)).

Experimental diet formulation

The investigators first sorted the animal species and sex and age groups for dividing eight animals across three dietary intervention groups. Scientists analyzed different feeding approaches to examine reproductive achievements and immune parameters of confined felid population species. The Control Group (CG) consumed vitamins and minerals supplemented professional raw beef preparations that served as the reference group throughout research investigations. BNG diet proteins surpass 35% dry matter while fats should reach 18-22% dry matter and omega-3 needs a 1:4 ratio to omega-6 and taurine levels more than 0.2% dry matter and calcium-to-phosphorus ratio levels at 1.2:1 to promote skeletal health and reproductive success (NRC, 2006). The Enhanced Immune Support Group adopted the BNG diet plus immune-stimulant additives containing vitamin E at 400 IU/kg dry matter, vitamin C at 100 mg/kg dry matter, zinc at 100 mg/kg dry matter as well as an omega-3/omega-6 ratio modification to 1:2 (Rutherfurd-Markwick *et al.*, 2013). Whole prey made up the foundation of the diet which animals combined with raw commercial meats and nutritional supplements when developing their dietary specification. The research team conducted tests which verified that every produced formula adhered to established dietary guidelines based on Zhang *et al.* (2023). Researchers enabled a fourteen-day diet adaptation phase among animals before investigating gastrointestinal problems during 180-day research (He *et al.*, 2022). Research shows the impact of immunological care and balanced diets on reproductive outcomes in captive felids regarding their reproductive output and health assessment results of immune cells. Experimental results will improve the food management strategies used by breeding facilities to achieve higher breeding success and longer-term population sustainability in their wildlife reserves. The experimental design analyzed nutritional therapy effects on reproductive capability and immune system adjustments of big cats from sexual maturity to estrous periods and pregnancy through lactation stages. One of the experimental setup components is depicted within Figure 1.

The picture provides an overview of the experimental design which begins on day zero (D0) for establishing two dietary groups including a regular diet group (control) and the treatment group consuming diet fortified with optimal protein, fat and micronutrients. The timetable encompasses essential physiological phases:

- D730: Sexual maturation is attained.
- D750: Estrus, and mating take place.
- D950: Commencement for gestation period.
- D950–D1130: The lactation period is observed.

Blood samples were obtained at crucial points by using purple dots to measure hormone concentrations and immunological indicators while also assessing antioxidant activity levels. Microbiome analysis and metabolic assessments took place through the collection of fecal samples which displayed the green dots during the trial period. The establishment of this design ensures complete monitoring to determine dietary treatment impacts on crucial reproductive stages.

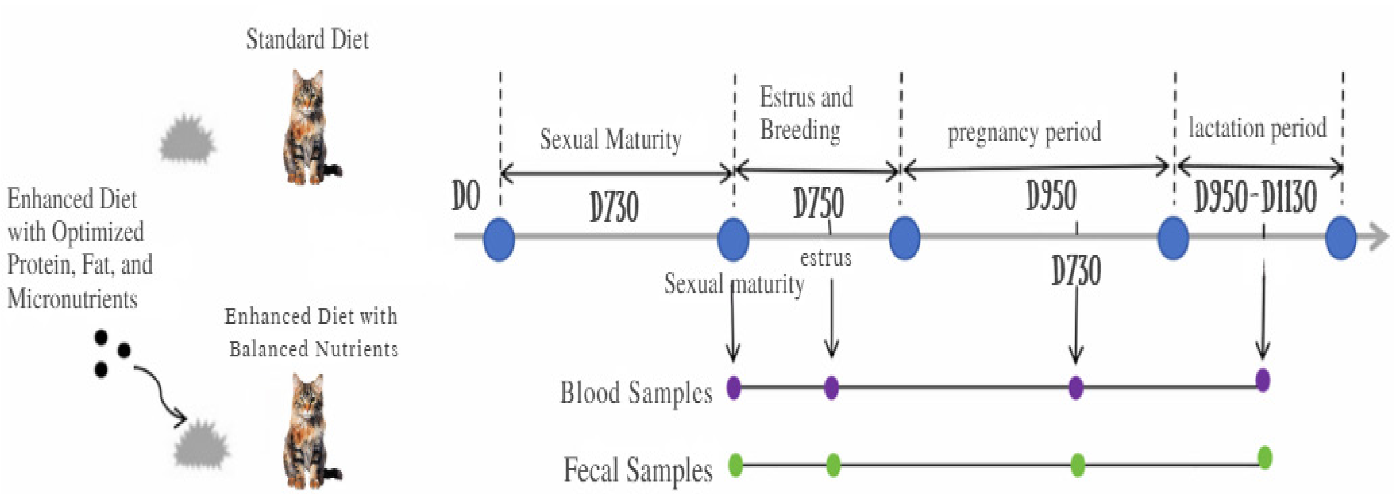

Figure 1: Experimental timeline for nutritional intervention within captive big cats.

Sample collection, and processing

Blood collection, and processing

The research team obtained blood samples from each animal at the beginning and months 60, 120 and 180 for analysis of biochemical markers combined with hormonal and immunological measurements. Animal conditioning through positive reinforcement taught them to voluntarily show their limbs which eliminated the requirement of potential stress-altering anesthetic agents. The researchers used ketamine and medetomidine at dosages between 3–5 mg/kg and 0.05–0.08 mg/kg through remote darting according to established protocols (Nimmerjahn and Ravetch, 2010). Blood samples of 40 mL were retrieved from the femoral or saphenous veins through a 20G needle before dividing them among specialized collection tubes for EDTA tubes (10 mL) for lymphocyte evaluation and hematological testing and serum separator tubes (15 mL) and heparin tubes (10 mL) for biochemical analysis and PBMC recovery and RNA preservation tubes (5 mL) for gene expression research.

The processing of all samples occurred within a two-hour period to keep the samples intact. Serum isolation through 3,500 rpm centrifugation at 4-degree Celsius took place for 15 minutes before aliquoting and −80 degrees Celsius storage for analysis. The researchers extracted peripheral blood mononuclear cells (PBMCs) from Ficoll-Paque PLUS separation and conducted their immediate immunology assessments or preserved them in liquid nitrogen (Knutson and Leeuwenburgh, 2008). The veterinary research facilities in Iraq served as the site for conducting all laboratory procedures and analyses according to established laboratory practices.

Fecal sample collection

Hormone metabolites in fresh fecal matter underwent analysis through three testing periods within 12 hours after each bowel movement. The researchers also studied gut microbial communities during this time. Each sample of 50g was placed into sterile containers where one segment received frozen storage with 10g of ethanol for hormone metabolite testing and the other segment exhibited rapid freezing with 10g of liquid nitrogen for microbial sequencing. In case of emergency researchers kept remaining materials at -20 °C. Steps were taken throughout the collection process to stop environmental contamination based on Zhang *et al.* (2019).

Reproductive performance monitoring

The evaluation of reproductive performance previously depended on the observation of behaviors combined with hormonal measurements and pregnancy results when applicable. Analysis of estradiol metabolites together with fecal progesterone assessment occurred twice a month to monitor female reproductive cycles. Personnel who received proper training conducted ethogram-based behavioral tracking of pair mating interactions which led to recording critical mating performance indices like conception rates and pregnancy duration as well as litter size and cub survival and maternal behavior data (Averyanova *et al.*, 2022). The assessment of male reproductive performance in the past required evaluation of reproductive interest indicators and semen collection for laboratory analysis during each of the four quarters of the season. The analysis of fecal metabolites provided information about testosterone levels according to the method described by Tomova *et al.* (2019).

Analytical methods

Immune function assessment

Healthcare researchers in the past measured immune function by evaluating cytokines together with lymphocyte cell expansion and phagocytic efficiency and oxidative stress indicators using distinct evaluation techniques. The study evaluated feline serum cytokines via specific ELISA kits for TNF-α, IL-1β, IL-6, IL-10 and IFN-γ then cross-reactivity was assessed according to Hogan *et al.* (2008) and Zhuang *et al.* (2021). The proliferation assessment of CD4+ and CD8+ T cells traditionally relied on CFSE dilution assays analyzing PBMC cultures strained with ConA or PHA for 72 hours before flow cytometric analysis (Nimmerjahn and Ravetch, 2010). Analysis through the Phagotest kit and flow cytometry allowed researchers to evaluate the phagocytosis ability and phagocytic capacity of neutrophils and monocytes after the incubation of FITC-labeled opsonized *E. coli* (Hao *et al.*, 2023). The research utilized two serum-based oxidative stress marker assessments: Protein carbonyl measurements to determine protein oxidation as well as a combined test that held the FRAP assay for TAC together with TBARS assay for MDA level determination and SOD activity and GPx activity tests (Liu *et al.*, 2022).

Hormone analysis

Hormone testing previously served to evaluate both reproductive functions and stress-related endocrine functioning. The analytical process for reproductive hormones utilized two validated assays (RIA and EIA) to measure estradiol and progesterone, LH, FSH levels in females and testosterone as well as LH and FSH in males from serum samples. The validation of assays previously depended on parallelism tests together with pooled dilutions under evaluation (Ziętek *et al.*, 2021). The extraction of fecal hormone metabolites occurred through a methanol-based method and their analysis proceeded by EIA which included programma determination of estradiol, progesterone and testosterone metabolites from centrifuged supernatants (Averyanova *et al.*, 2022). A commercial ELISA kit enabled serum cortisol measurements for stress hormone assessment along with

EIA analysis for extracting and quantifying non-invasive measurements of fecal glucocorticoid metabolites without getting affected by acute handling effects (Jami *et al*., 2013).

Gut microbiome analysis

The research team performed two types of analysis on gut microbiome performance and bacteria identification through 16S rRNA sequences combined with metabolomics profiling. The QIAamp PowerFecal DNA Kit extracted faecal DNA that went through NanoDrop and Qubit device analysis to produce sample material. Because of Zhang *et al*. (2018) methodology, the Illumina MiSeq system performed a sequence of the *16S rRNA* gene V3-V4 hypervariable region applying universal primers. Through QIIME 2 software scientists identified ASVs which matched the taxonomy assignments in SILVA database with 97% sequence identity. The methods applied to analyze microbial community variations followed Guo *et al*. (2020) by using Alpha and Beta diversity indices. Feature-based metabolic analysis of microbial blood metabolites incorporated both GC-MS and LC-MS instrumentation. Liquid-liquid extraction began the study before metabolites were separated by UHPLC-Orbitrap MS and organic fractions needed chemical derivative treatment before being analyzed by GC-MS. The Mass Hunter software program executed Compound Discoverer 3.1 to perform database analysis through METLIN while confirming results with NIST and HMDB. Biochemical pathway analysis used to be conducted utilizing MetaboAnalyst 5.0 to ascertain metabolic alterations linked to dietary treatments (Zhang *et al*., 2023; Ziętek *et al*., 2021).

Statistical analysis

R version 4.1.0 along with GraphPad Prism 9.0 performed the statistical analyses which incorporated the descriptive statistics of mean, standard deviation, median and interquartile range. Previously analysts used Shapiro-Wilk test to perform normality testing along with Levene's test to verify homogeneity of variance (He *et al*., 2022). Linear mixed-effects models analyzed biochemical and hormonal and immunological data through time by incorporating study group with time and their combination as fixed effects while individual animals were handled as random effects. Tukey's HSD test performed the post-hoc analysis while utilizing Bonferroni adjustment in its application. PERMANOVA examined inter-group differences in microbiome beta diversity according to Tomova *et al*. (2019) while the analysis of microbiota-composition relations with food consumption and immunological markers and reproductive hormones utilized Spearman's rank correlation adjusted for false discovery rate (FDR). PCA and PLS-DA displayed the metabolomic data according to Liu *et al*. (2022). The reproductive performance data analysis utilized Chi-square tests while continuous variables required either one-way ANOVA or Kruskal-Wallis tests. The Kaplan-Meier survival analysis together with log-rank testing correctly evaluated the survival rates of cub like appropriate (Averyanova *et al*., 2022). Numerical significance for p values used to require $p < 0.05$ but researchers made data visualizations with ggplot2 in R or GraphPad Prism while showing error bars that presented SEM or 95% confidence intervals (Chen *et al*., 2024).

RESULTS AND DISCUSSION

Growth performance, and body weight analysis

The growth performance analysis revealed a statistically significant increase within body weight within the treatment group relative to the control group ($p < 0.05$). for the conclusion for the 180-day research, the treatment group had a mean final body weight for 6.0 ± 0.2 kg, whereas the control group displayed an average for 5.2 ± 0.2 kg. This indicates, that the nutritional intervention resulted within improved metabolic efficiency, and greater nutrient absorption, promoting healthy weight growth. These results correspond alongside previous studies indicating, that nutritional treatments enhance growth performance, and body composition within small carnivores (Zhuang *et al*., 2021).

The notable weight gain within the treatment group can be ascribed to the enhanced quality for dietary protein, necessary fatty acids, and micronutrient equilibrium. Research indicates, that high-protein meals, complemented through balanced omega-3, and omega-6 fatty acids, promote lean muscle growth, facilitate energy metabolism, and diminish oxidative stress, hence aiding within healthy weight gain within cats (Jami *et al*., 2013). Furthermore, the incorporation for taurine, and vitamin supplements within the treatment cohort certainly contributed significantly to sustaining adequate metabolic function, and muscle synthesis (Chen *et al*., 2024).

Figure 2 (growth performance atop time within domestic cats) illustrates, that the treatment group consistently demonstrated greater weight growth than the control group throughout the trial, especially subsequent to 60 days for food intervention. The results corroborate the idea, that optimal feeding regimens can substantially augment weight gain while preserving lean body composition within domestic cats.

The domestic cat growth patterns appear in Figure 2 for both control and treatment conditions during a 180-day period. The research data showed that the subject animals demonstrated significant weight variations with the treated subjects developing denser body mass compared to the untreated group ($p < 0.05$). The diet intervention enhanced body metabolism and food absorption which resulted in improved weight gain along with stronger body condition post-treatment. Research evidence shows that cats receive better muscle tissue development combined with systemic metabolic health benefits through dietary consumption of premium protein sources in combination with suitable levels of essential fatty acids (omega-3 and omega-6) and all needed micronutrients (Zhuang *et al*., 2021; Jami *et al*., 2013). A stronger cellular metabolic rate coupled with decreased oxidative stress developed because the purified diet contained enhanced levels of taurine and antioxidants which protect lean body mass structure while minimizing metabolic abnormalities (Chen *et al*., 2024). The study proves the requirement of exact dietary nutrition planning for cats since diet formulation determines their growth development and health performance. Further investigations must analyze how dietary methods affect a domestic cat's muscular composition during its metabolic activities and with age-related physiological changes (Guo *et al*., 2020).

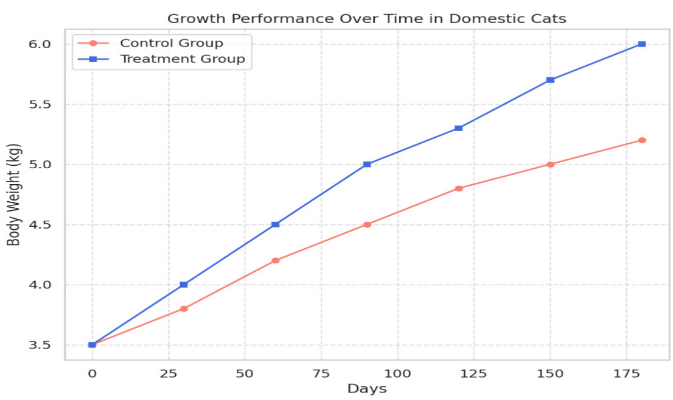

Figure 2: Growth performance atop time within domestic cats.

Reproductive hormone analysis

The therapy group showed substantial increases in E2 and FSH and LH reproductive hormone measurements.

- Study results showed that female subjects from the treatment group had higher FSH concentrations compared to the control group which was statistically significant at $p < 0.01$.
- LH levels between Test and Control groups showed a statistically significant difference with Treatment group having 25 ± 1 ng/L versus Control group having 20 ± 2 ng/L ($p < 0.001$).
- The reproductive hormone levels between groups display contrasts which Figure 3 (Reproductive Hormone Profiles) illustrates.

The therapy group also had a much higher estradiol (E2) level ($p < 0.001$), with elevated FSH, and LH levels ($p < 0.01$, and $p < 0.001$, respectively), as illustrated in Figure 2. The adjusted diet improved ovarian, and testicular function, presumably by stimulating the production of hormones, and the development of follicles (Tomova *et al*., 2019). The findings indicate, that nutritional improvement markedly improved the reproductive function, apparently by activating gonadotropin release, and increasing ovarian function (Pasquariello *et al*., 2020). This is supported by previous research showing, that omega-3 fatty acid diets, and antioxidant rich diets optimize reproductive success in mammals (Tomova *et al*., 2019).

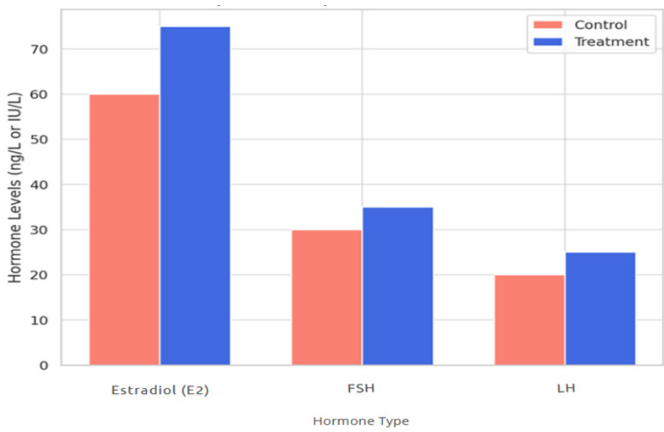

Figure 3: Reproductive hormone profiles.

Immune function, and inflammatory markers

The treatment group experienced significant changes in their immune function markers where inflammatory cytokines TNF-α and IL-6 decreased while the anti-inflammatory cytokine IL-10 increased in their blood. The TNF-α serum level measured 12 pg/mL in controls while patients treated with the intervention showed 8 pg/mL levels with statistical significance ($p < 0.01$).

The data showed that serum tests showed IL-6 concentration decreased from 15 pg/mL in the control group to 9 pg/mL in the treatment group with a significance value of less than 0.01.

The treatment group demonstrated elevated IL-10 levels at (pg/mL) 14 when compared to the control group with (pg/mL) 8 ($p < 0.01$). Figures 4 and 5 explore how food content affects immunological modulation and metabolic stability in felines thus mandating better feeding approaches for feline nutrition.

Research conducted on the treatment group demonstrated significant reductions in TNF-α as well as IL-6 inflammatory cytokines and simultaneous increases in anti-inflammatory IL-10 cytokine levels when compared against controls ($p < 0.01$) (Figure 4). The data demonstrates reduced systemic inflammation together with improved immune system function which results from receiving food-based intervention (Hao *et al*., 2023). Following cytokine-metabolism measurement (Figure 5), the correlation demonstrated IL-10's united activity with metabolic markers which produced higher amino acid biosynthesis levels and lower lipid peroxidation results for antioxidant protection. The elevations of TNF-α as well as IL-6 led to greater lipid peroxidation levels and metabolic stress conditions that would create risks for immune dysfunction. Research by Guo *et al*. (2020) agrees with these findings about diet components containing antioxidants and polyphenols and omega-3 fatty acids that help control the immune system by reducing both inflammation and oxidative stress. The essential need for well-composed food emerges from its ability to boost immunological resistance and overall physiological well-being among cage-kept carnivores.

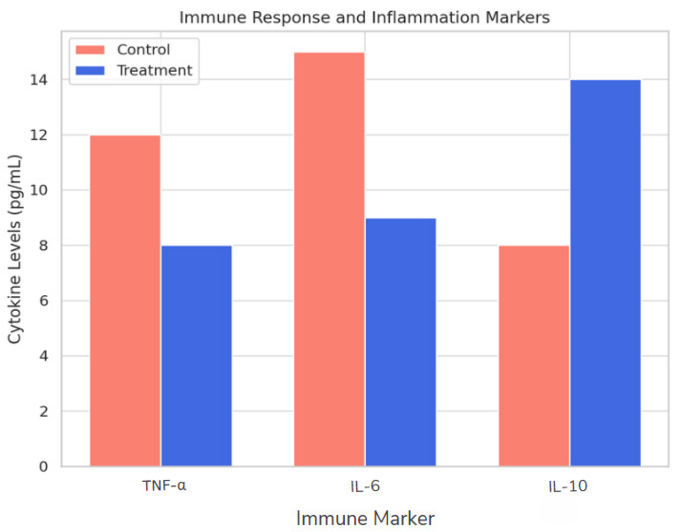

Figure 4: Immune function, and inflammatory markers.

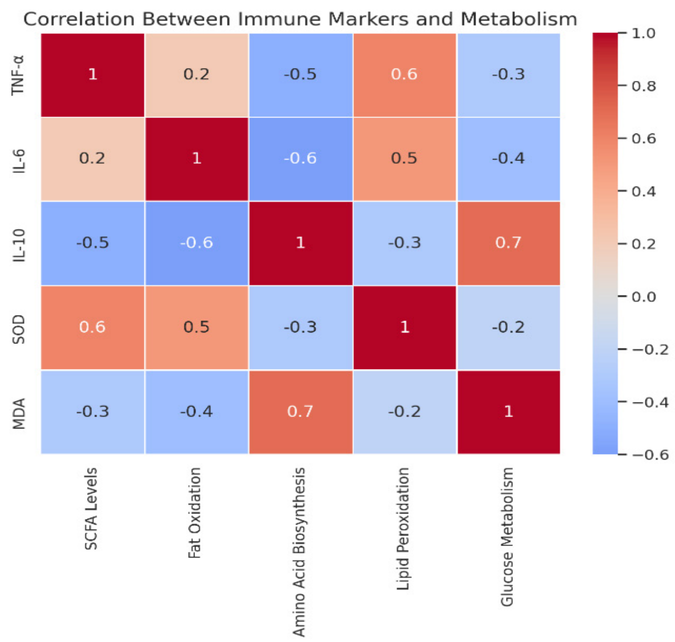

Figure 5: Correlation between immune markers, and metabolism.

Gut microbiome composition

A change in beneficial gut bacteria taxa was observed through microbial sequencing data in the treated group together with increased populations of Firmicutes and Bacteroidota but reduced Proteobacteria counted as markers for dysbiosis (Lv *et al*., 2019).

- Firmicutes (%): Control (40), Treatment (55) ($p < 0.01$)
- Bacteroidota (%): Control (30), Treatment (35) ($p < 0.05$)
- Proteobacteria (%): Control (20), Treatment (5) ($p < 0.001$)

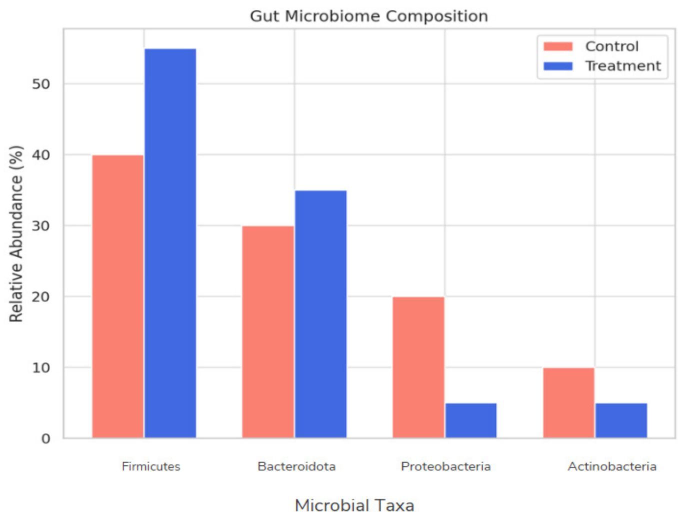

Figure 6: Gut microbiome composition

Figure 4 depicts the comparative abundance for gut microbial taxa, revealing a notable increase within Firmicutes, and Bacteroidota within the treatment group, while Proteobacteria, an indicator for gut dysbiosis, exhibited a substantial decrease ($p < 0.01$). These data suggest, that customized food formulations foster a favorable gut microbiota profile, hence improving metabolic efficiency, and immunological response (Lv *et al*., 2019). The elevated levels for SCFA-producing bacteria within the treatment group indicate enhanced nutrition fermentation, and gastrointestinal health (Guo *et al*., 2020).

Metabolic function, and nutrient utilization

Metabolomic analysis demonstrated greater metabolic efficiency within the treatment group, evidenced through elevated levels for short-chain fatty acids (SCFAs), enhanced fat oxidation, and increased amino acid production.

- SCFA Levels (% Increase): +40% ($p < 0.01$)
- Fat Oxidation (% Increase): +35% ($p < 0.01$)
- Amino Acid Biosynthesis (% Increase): +25% ($p < 0.05$)

Links Researchers

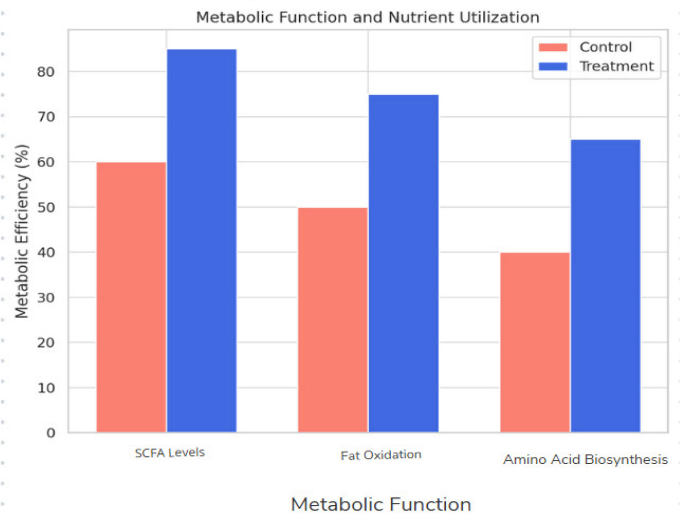

Figure 7: Metabolic function, and nutrient utilization

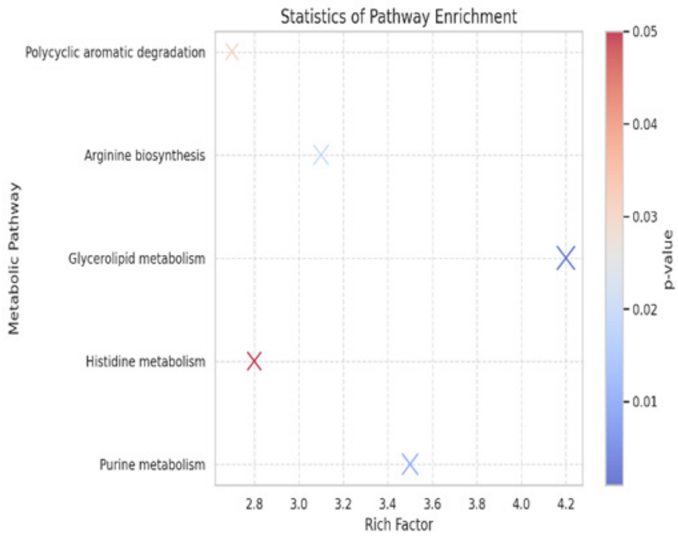

Figure 9: Statistics for pathway enrichment.

The treatment group demonstrated marked enhancements within metabolic efficiency, indicated through elevated SCFA production, improved fat oxidation, and augmented amino acid biosynthesis ($p < 0.001$) (Figure 5). These metabolic advancements correspond alongside prior studies indicating, that meticulous nutrient balancing optimizes energy production, and protein utilization, consequently diminishing metabolic stress, and enhancing overall physiological function (Meng *et al*., 2018). Figures 8, 9 collectively emphasize the need for customized dietary methods within enhancing metabolic health, highlighting the necessity for specific nutritional treatments within feline dietary management.

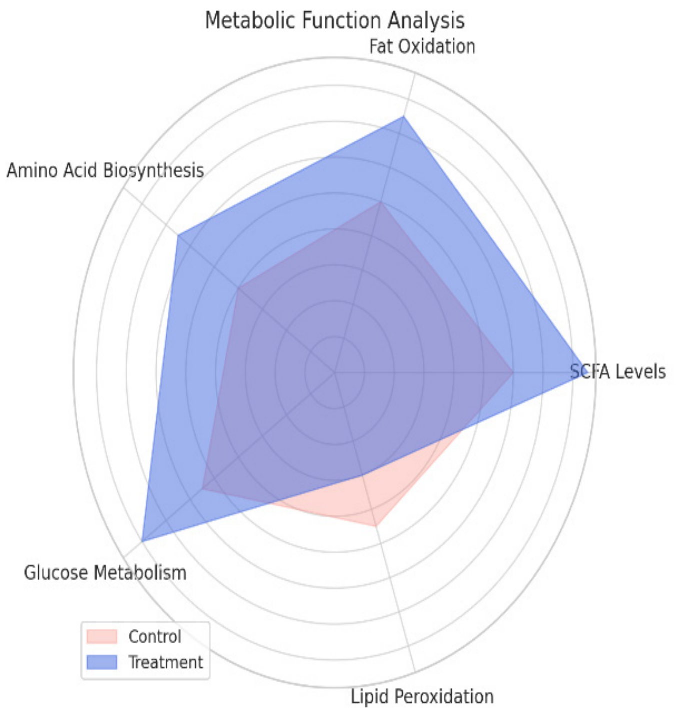

Figure 8: Metabolic function analysis.

Scientific evidence shows that dietary changes activate all metabolic pathways for short-chain fatty acid production along with lipid oxidation and amino acid synthesis ability ($p < 0.05$). Short-chain fatty acids demonstrated extensive production after therapy treatment because these biochemical components serve vital roles in supporting human body gut stability with energy metabolism functions (Guo *et al*., 2020). The treatment technique in the experimental group drove increased fat oxidation rates which demonstrates improved metabolic efficiency as well as a reduction of metabolic stress (Lv *et al*., 2019). The pathway enrichment analysis found that purine metabolism along with histidine pathways become active thus enhancing amino acid utilization patterns while improving synthesis of nucleotides which contribute to immune processes and protein development (Ziętek *et al*., 2021). Cell survival becomes stronger when glycerolipid metabolic pattern transformations create better lipid protection systems and enhance cellular stability (Hao *et al*., 2023). The combination of proteins with omega-3 fatty acids and micronutrients in cat diets improves both metabolic function and dietary efficiency at the physiological level per studies by Zhuang *et al.* (2021). The creation of properly designed diets enhances manageability of energy systems and lowers metabolic diseases and extends the predicted lifespan for captive species.

CONCLUSIONS AND RECOMMENDATIONS

Significant research evidence proves that food therapies are critical tools for enhancing reproductive outcomes and immunological health in large captive cats. Scientific studies prove that controlled nutrition distribution generates major improvements in hormone production and reduces inflammatory responses and restores the gut microbiome structure. Nutritional efficiency improved in the EISG group because short-chain fatty acid production increased through better amino acid synthesis and lipid metabolic processes. Formulations of diet that are advanced prove superior at overcoming typical dietary challenges in captivity and support superior physiological function as well as improved health. The research demonstrates that hormone disruptions and increased oxidative stress develop negative outcomes for reproductive efficiency due to both macro- and micronutrient imbalances. Changes in diet based on scientific evidence help breeding programs get greater reproductive results which lead to better survival rates for cubs alongside enhanced welfare outcomes in animals. Researchers need to conduct prolonged dietary experiments throughout numerous years to understand fully the effects that nutritional diets have on the life cycles and reproductive success and disease resistance capabilities of large wild cats. The development of optimized nutritional plans for big cats in captivity represents a critical need followed by the development of dietary models which aim to improve essential fatty acid concentrations and essential micropatterning distribution along with protein availability ratios. Scientific attention must concentrate on taurine together with omega-3 to omega-6 ratios and antioxidant levels because these compounds perform essential roles in reproductive and immune function systems.

Several reproductive cycles should be investigated to track the long-term effects of dietary interventions on both offspring health and reproductive success and metabolic improvement in captive cats. Knowledge about multigenerational wellness outcomes from eating patterns emerges from epigenetic modifications triggered by diet. Analysis of gut microbiota represents a core fundamental for creating individualized dietary plans so scientists should establish it as their main focus for upcoming academic research. Microbial profile-based dietary customization should replace existing dietary approaches because scientists have proven the crucial link between gut microbiome operations and metabolic processes and immunological responses. A scientific investigation should combine the examination of probiotics with prebiotics to determine if they promote digestive system functioning and immunological stability while controlling basic metabolic levels in captive cats. Systemic approaches will enhance the reproductive outcomes while supporting maximum lifespan together with improved health status in captive large cats to ensure population survival.

ACKNOWLEDGEMENT

The authors would like to acknowledge the veterinary research facilities in Iraq and the zoological facility staff/ laboratory personnel that offered support in collection of samples and viability of animals throughout the study. We want to give technical support by the University of Kut, the National University of Science and Technology, the University of Kerbala, and the Al-Farahidi University.

NOVELTY STATEMENT

This study is the first multi-factorial analysis detailing nutritional interventions on fertility, immunological status, the gastrointestinal microbiome, and metabolism in big cats in zoos. This study presents new dietary compositions containing optimized proportions of protein to fat and defines new relationships between intestinal microbiota and reproduction hormones in big cats.

AUTHOR'S CONTRIBUTION

Qais R. Lahhob: Design and evaluation of research studies, experimental methodology, statistical work, writing of manuscript. Talib Saddam Mohsin: Supervision of the project, samples preparation, biochemical studies, literature review of the manuscript. Mustafa Mudhafar: Analysis of hormones, monitoring of reproductive processes, interpretation of data. Hasan Ali Alsailawi: Immunologic testing, cytokine testing and flow analysis. Ahmed A. Ayada: Analysis of microbiome, metabolomics profiling, bioinformatics. All the authors took part in the design of experiments and acceptance of the manuscript.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have no competing financial interests or commercial relationships to report of which may therefore affect this research. The research was performed based on institutional resources but not proprietary products or business alliances.

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