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Research Article

Influence of Sleep Loss and Diet on Growth Parameters, and Biological Stress Markers

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Abstract

Background: In today's demanding world, poor nutrition, caffeine reliance, and inadequate rest contribute to chronic fatigue and health issues. This study examines the interplay between sleep, an amino acid-deficient diet, and caffeine, focusing on their impacts on metabolism, growth, oxidative stress, and cognitive health. It highlights the critical role of quality sleep in supporting cognitive function.

Methods: The study used adult male Wistar rats divided into 10 groups (A–J) based on cage conditions and diet. Groups A–E were housed in normal cages and fed diets with varying levels of tryptophan and other essential amino acids, while Groups F–J were subjected to sleep deprivation via the disk-over-water method and given diets with varying tryptophan levels and caffeine. Over two weeks, blood samples were collected on days 1, 4, 7, and 13 for biochemical analysis. On day 14, the rats were sacrificed for blood collection, biochemical assays, and brain histology.

Results: The 2-week study found that caffeine and sleep deprivation reduced melatonin, serotonin, testosterone, and growth hormone levels, highlighting potential biomarkers and effects on growth and sex characteristics. Increased TNF-α, interleukin-1β, oxidative stress, and histopathological abnormalities suggest heightened health risks and cellular damage from combined caffeine use and sleep deprivation.

Conclusion: This study highlights the links between sleep, diet, hormonal, and inflammatory responses, emphasizing caffeine's potential neurotoxic risks when combined with sleep deprivation. It calls for further research into dietary strategies to mitigate these effects and improve health.

Keywords: Sleep Deprivation; Caffeine; Balanced Diet; Cognitive Dysfunction

Summary Statement

This study reveals how sleep deprivation, diets, and caffeine impair metabolism, growth, and cognition, highlighting oxidative stress, inflammation, and neurotoxicity as key risks, while emphasizing the importance of dietary strategies for improved health outcomes.

Introduction

In today's fast-paced society, many individuals rely on caffeine to maintain wakefulness and alertness, often accompanied by a nutritionally deficient diet consumed while on the move. This routine, characterized by insufficient rest and poor nutrition, perpetuates a cycle of chronic fatigue and suboptimal health. Amino acids, essential for protein synthesis and the production of neurotransmitters and hormones, play a critical role in maintaining physiological functions. Protein quality is measured by its ability to meet the nutritional requirements of essential amino acids (EAAs). Deficiencies in EAAs, adversely affect growth, bone development, and protein metabolism, as demonstrated in animal models [1]. The adult human brain requires approximately 20% of the body's energy, primarily derived from glucose, to sustain neurotransmission. The brain's high metabolic activity is maintained even during sleep, with local neuronal activity varying significantly depending on sensory or motor stimulation and wakefulness transitions, thereby maintaining proper brain function [2]. Diet and mental health are closely linked, with evidence suggesting that amino acids such as tryptophan play a critical role in mood regulation and cognitive function. Severe dietary restrictions, as seen in anorexia nervosa, are associated with depressive symptoms and impaired cognitive functions, highlighting the importance of adequate nutrition for mental health [3].

Although caffeine has been shown to have short-term benefits in improving memory and cognition, its long-term effects are less clear, with studies presenting conflicting results regarding its association with cognitive decline and dementia risk [4]. Sleep is essential for overall health, with non-rapid eye movement (NREM) and rapid eye movement (REM) stages serving distinct functions, including memory consolidation and growth hormone release, with memory deficits associated with sleep deprivation [5].

In this study, we investigated the hypothesis that a combination of sleep deprivation and essential amino acid-deficient diet exacerbate the learning and memory impairment induced by each factor alone.

Methods

Experimental feed preparation and analysis

The experimental feeds utilized in this study included standard animal chow (Topfeed), milled maize, and pelletized red and white sorghum. These feeds were sourced from the Malete farm market, Kwara State Nigeria, while the maize and sorghum were manually cleaned to remove any visible farm dirt. Subsequently, the cleaned maize and sorghum were milled and pelletized at an Animal Feed Mill, located in Ilorin, Kwara State Nigeria. Each feed type underwent proximate and amino acid composition analysis to evaluate and compare their nutritional profiles. The analyses were carried out in triplicates (n = 3).

Determination of amino acid profile

The amino acid profile of the experimental feeds was determined following the method described by Otter [6] with slight modifications. The sample was dried at 70°C to constant weight, defatted, hydrolyzed, evaporated using a rotary evaporator, and then analyzed using Applied Biosystems PTH Amino Acid Analyzer, California U.S.A.

Proximate analysis of moisture, protein, fat, ash, and crude fibre

The proximate analysis of the experimental feeds was determined following the method described by Thiex [7,8].

Experimental design

The animal protocol used in this study was approved by the Faculty of Pure and Applied Sciences Animal Ethics Committee, following recommendations from the Research Committee at Kwara State University, Malete. Eighty adult male Wistar rats (weighing 145g-150g) were obtained from the animal holding unit of the Biochemistry Department at Summit University, Kwara State, Nigeria, and acclimatized for one week. The rats were then divided into two sets, labeled groups A-J, with eight rats in each group as outlined in Table 1. The experimental phase, during which the dietary and housing conditions were implemented according to the table, was conducted for two weeks. Caffeine powder was purchased from First Octopus Chemicals, Ogbomosho, Nigeria. All other reagents used were of analytical grade, and the water used in the experiments was glass distilled.

Group	Housing Condition	Diet	Caffeine Administration
A	Regular animal cage housing	Regular animal chow and water ad libitum	None
B	Regular animal cage housing	Milled maize and water ad libitum	None
C	Regular animal cage housing	Pelletized sorghum and water ad libitum	None
D	Regular animal cage housing	Milled maize and water ad libitum	Bi-daily administration of 20 mg/kg body weight of caffeine powder
E	Regular animal cage housing	Pelletized sorghum and water ad libitum	Bi-daily administration of 20 mg/kg body weight of caffeine powder
F	Disk-over-water cage housing	Regular animal chow and water ad libitum	None
G	Disk-over-water cage housing	Milled maize and water ad libitum	None
H	Disk-over-water cage housing	Pelletized sorghum and water ad libitum	None
I	Disk-over-water cage housing	Milled maize and water ad libitum	Bi-daily administration of 20 mg/kg body weight of caffeine powder
J	Disk-over-water cage housing	Pelletized sorghum and water ad libitum	Bi-daily administration of 20 mg/kg body weight of caffeine powder

Table 1: Summary of Experimental Group Treatments Based on Dietary and Housing Condition Variations.

Sleep deprivation procedure

The disk-over-water method was utilized to induce sleep deprivation while minimizing the effects of social isolation by housing two rats each in separate compartments sharing a common round disk that formed an elevated partial floor, with shallow water covering the cage floor beneath the disk. The cages were constructed from glass, with punctured holes at the top to allow for respiratory exchange. Care was taken not to puncture the bottom of the cages to prevent the rats from using the holes to climb up and escape the rotating platform. The cages were constructed from glass, with punctured holes at the top to allow for respiratory exchange. Care was taken to avoid puncturing the bottom of the cages, preventing the rats from using the holes to climb up and escape the rotating platform. The disk automatically rotated at a slow speed in response to the rats' movements. When a sleep-deprived rat fell asleep and lost muscle tone, it would fall into the water, triggering wakefulness and disturbing the balance of the rotating disk, which could cause the other rats to also fall into the water. The rats would then swim out of the water and return to the platform. A 2-hour daily break was provided, during which a small, non-rotating platform was placed beside the rotating disk, allowing the rats to sleep without falling into the water. The rats were pre-trained for one week before the experiment began to recognize the non-rotating platform as a steady platform. After the 2-hour break, the non-rotating platform was removed, and the sleep deprivation procedure resumed. This protocol was maintained for two weeks, resulting in the sleep-deprived rats achieving only approximately 16% of their normal sleep.

Blood sample preparation and animal sacrifice and blood sample preparation

Blood samples were collected from rats via tail vein in each group on days 1, 4, 7, 10, and 13 to carry out biochemical assays.

Elisa assay for testosterone, IGF-1, serotonin, melatonin, interleukin-1 B, and TNF- A

The concentrations of testosterone, IGF-1, serotonin, melatonin, interleukin-1 beta, and TNF-alpha were determined in the prepared plasma using enzyme-linked immunosorbent assay (ELISA) kits, employing the sandwich ELISA principle [9].

Evaluation of oxidative stress

The evaluation of oxidative stress in the serum of the Wistar rats in each experimental group was determined using the method described by Munteanu and Apetrei [10], with modifications.

Statistical analysis

All data were expressed as the mean of six replicates ± standard error of the mean (S.E.M) (except for the proximate and amino acid content analysis where three replicates were used). Statistical evaluation of data was performed using SPSS version 27.0 using one-way analysis of variance (ANOVA) followed by Duncan's posthoc test for multiple comparisons. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Results

The proximate analysis and amino acid composition of the experimental diets (Tables 2 and 3) revealed that maize had the highest tryptophan and protein, comparable to the standard rat chow, although significant differences exist. Sorghum, both white and red, has lower tryptophan composition and protein content, although it has higher lysine, histidine (g/100g protein), and carbohydrates (%).

Sample	Crude Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Regular Animal Chow	$10.19^{b} \pm 0.28$	$6.44^{b} \pm 0.23$	$4.21^{b} \pm 0.11$	$1.83^{b} \pm 0.03$	$74.61^{c} \pm 0.65$
Milled Maize	9.68^{b}+0.21	$7.81^{b} \pm 0.13$	$4.39^{b} \pm 0.13$	$1.68^{b} \pm 0.04$	$79.15^{c} \pm 0.58$
Red Sorghum	$5.39^{a} \pm 0.25$	$5.31^{a} \pm 0.18$	$3.31^{a} \pm 0.07$	$1.62^{a} \pm 0.02$	$84.37^{c} \pm 0.61$
White Sorghum	$5.54^{a} \pm 0.19$	$5.57^{a} \pm 0.17$	$3.9^{b} \pm 0.14$	$1.61^{a} \pm 0.03$	$83.38^{c} \pm 0.51$

Table 2: Proximate analyses result (%) of the experimental feeds used. Results are expressed as Mean ± S.D, (n = 3). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Amino Acid	Regular Animal Chow (g/100g protein)	Milled Maize (g/100g protein)	Red Sorghum (g/100g protein)	White Sorghum (g/100g protein)
Leucine	$13.51^{c} \pm 0.21$	$13.0^{b,c} \pm 0.28$	$12.78^{b} \pm 0.23$	$12.69^{b} \pm 0.18$
Lysine	$2.36^{c} \pm 0.12$	$2.46^{c,e} \pm 0.16$	$2.49^{e} \pm 0.10$	$3.1^{c} \pm 0.14$
Isoleucine	$3.99^{b} \pm 0.14$	$3.17^{b} \pm 0.12$	$3.08^{b} \pm 0.17$	$3.4^{b} \pm 0.12$
Phenylalanine	$4.87^{d} \pm 0.28$	$4.87^{b} \pm 0.22$	$4.78^{c} \pm 0.14$	$5.23^{a} \pm 0.31$
Trytophan	$1.23^{a,b} \pm 0.08$	$0.91^{a} \pm 0.03$	$0.12^{c} \pm 0.03$	$0.14^{c} \pm 0.04$
Valine	$5.41^{a} \pm 0.05$	$4.24^{a} \pm 0.02$	$4.21^{a} \pm 0.01$	$4.5^{a} \pm 0.01$
Methionine	$1.98^{c} \pm 0.06$	$0.72^{a} \pm 0.02$	$0.88^{a} \pm 0.01$	$1.12^{a} \pm 0.03$
Proline	$8.94^{d} \pm 0.14$	$4.06^{b} \pm 0.10$	$3.55^{b} \pm 0.16$	$4.26^{b} \pm 0.11$
Arginine	4.81 ± 0.21	4.64 ± 0.26	6.36 ± 0.25	4.56 ± 0.23
Tyrosine	$3.96^{e} \pm 0.24$	$3.78^{e} \pm 0.23$	$3.78^{e} \pm 0.24$	$3.78^{e} \pm 0.21$
Histidine	$2.17^{b} \pm 0.11$	$2.91^{c} \pm 0.18$	$3.67^{c} \pm 0.24$	$3.38^{c} \pm 0.27$
Cystine	$2.24^{b} \pm 0.21$	$1.45^{d} \pm 0.18$	$1.33^{e} \pm 0.12$	$1.27^{e} \pm 0.12$
Alanine	$9.29^{e} \pm 0.42$	$4.85^{c} \pm 0.26$	$5.23^{b} \pm 0.24$	$5.19^{c} \pm 0.28$
Glutamic acid	$20.52^{f} \pm 0.88$	$12.87^{b} \pm 0.68$	$14.08^{c} \pm 0.46$	$13.02^{d} \pm 0.47$
Glycine	$3.21^{e} \pm 0.37$	$3.42^{d,e} \pm 0.25$	$3.23^{d} \pm 0.32$	$3.68^{e} \pm 0.28$
Threonine	$3.16^{b} \pm 0.18$	$3.64^{e} \pm 0.26$	$3.05^{a} \pm 0.18$	$3.27^{b} \pm 0.24$
Serine	$4.4^{c} \pm 0.14$	$3.97^{b} \pm 0.12$	$3.86^{b} \pm 0.12$	$3.83^{c} \pm 0.18$
Aspartic acid	$7.6^{a} \pm 0.24$	$6.73^{d} \pm 0.38$	$6.6^{d} \pm 0.27$	$6.85^{c} \pm 0.28$

Table 3: Total Individual Amino Acids Content in Experimental Feeds. Results are expressed as Mean ±S.D, (n = 3). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

The analysis of serum testosterone levels across various experimental groups reveals significant insights into the interactions between diet, caffeine intake, sleep deprivation, and hormonal balance (Table 4). A pronounced reduction in testosterone was particularly evident in rats housed in disk-over-water cages, with the most significant declines observed in Groups I and J, which received a combination of daily caffeine administration (20 mg/kg body weight) and were housed in these cages. Statistical significance in the reduction was noted for Groups G and H, which were also housed in disk-over-water cages during the experimental period, compared to those in normal cages and those in normal cages receiving 20 mg/kg body weight caffeine (Groups D and E). Conversely, Groups D and E demonstrated a marked increase in testosterone levels when caffeine was administered in normal cages over 13 days.

Group	Testosterone level Day 1 (ng/dL)	Testosterone level Day 4 (ng/dL)	Testosterone level Day 7 (ng/dL)	Testosterone level Day 10 (ng/dL)	Testosterone level Day 13 (ng/dL)
A	$0.648^{c} \pm 0.025$	$0.509^{b} \pm 0.021$	$0.612^{b} \pm 0.037$	$0.536^{a} \pm 0.052$	$0.651^{a} \pm 0.028$
B	$0.625^{c} \pm 0.031$	$0.622^{b} \pm 0.047$	$0.599^{b} \pm 0.084$	$0.581^{a} \pm 0.027$	$0.692^{b} \pm 0.083$
C	$0.856^{b} \pm 0.029$	$0.822^{c} \pm 0.037$	$0.834^{c} \pm 0.022$	$0.818^{c} \pm 0.092$	$0.824^{c} \pm 0.038$
D	$0.884^{b} \pm 0.048$	$0.832^{c} \pm 0.042$	$1.325^{a} \pm 0.087$	$1.619^{b} \pm 0.095$	$1.556^{b} \pm 0.097$
E	$0.977^{a} \pm 0.029$	$1.112^{d} \pm 0.078$	$1.254^{c} \pm 0.193$	$1.992^{d} \pm 0.129$	$1.678^{d} \pm 0.074$
F	$0.509^{c} \pm 0.083$	$0.556^{a} \pm 0.028$	$0.486^{e} \pm 0.098$	$0.382^{e} \pm 0.049$	$0.324^{e} \pm 0.028$
G	$0.667^{c} \pm 0.027$	$0.627^{b} \pm 0.022$	$0.632^{d} \pm 0.037$	$0.564^{a} \pm 0.038$	$0.483^{g} \pm 0.033$
H	$0.934^{b} \pm 0.038$	$0.884^{e} \pm 0.029$	$0.888^{c} \pm 0.084$	$0.832^{i} \pm 0.035$	$0.796^{f} \pm 0.084$
I	$0.882^{b} \pm 0.094$	$0.434^{c} \pm 0.085$	$0.328^{a} \pm 0.028$	$0.253^{e} \pm 0.019$	$0.283^{e} \pm 0.023$
J	$0.912^{a} \pm 0.034$	$0.523^{b} \pm 0.038$	$0.487^{e} \pm 0.081$	$0.345^{d} \pm 0.094$	$0.319^{d} \pm 0.038$

Table 4: Serum testosterone concentration (ng/dL) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Groups A, B, and C, which were housed in regular cages without caffeine, exhibited relatively stable testosterone levels. Group D, which had a maize diet and caffeine but no sleep deprivation, initially exhibited a rise in testosterone levels that later escalated significantly, possibly due to caffeine's role in increasing testosterone in non-sleep-deprived animals. Notably, group E, which was subjected to a sorghum diet and caffeine, experienced a significant rise in testosterone levels, peaking on Day 10. This increase could be attributed to the combined effects of tryptophan-rich sorghum and the stimulatory influence of caffeine. In contrast, Groups F to J, which were housed in disk-over-water cages indicative of sleep deprivation, demonstrated sharp declines in testosterone levels. Particularly in Groups I and J, which also received caffeine, the marked hormonal decrease suggests a compounded negative effect of sleep deprivation and caffeine. Furthermore, the serum testosterone analysis indicated that caffeine intake and a tryptophan-deficient diet significantly impact testosterone levels, suggesting a reduced sex drive. This reduction may contribute to observed behaviors such as loss of fur, irritability, and loss of muscle function in experimental rats, as testosterone levels decreased throughout the experiment.

The analysis of serum IGF-1 levels further highlights the detrimental effects of sleep deprivation and caffeine administration. Correspondingly, IGF levels followed a similar trajectory, showing significant drops in sleep-deprived groups, particularly those with caffeine, indicating a stress-induced catabolic state (Table 5). A drastic decrease in IGF-1 levels was observed in groups subjected to sleep deprivation using the disk-over-water cage or a combination of both caffeine administration and disk-over-water housing, indicating potential growth hormone deficiency and malnutrition. Interestingly, the trend observed in rats from Groups D and E showed an increase in serum IGF-1 concentrations as the duration of caffeine administration lengthened, suggesting a change in physiological condition and increased growth hormone secretion.

Groups	IGF level Day 1 (ng/ml)	IGF level Day 4 (ng/ml)	IGF level Day 7 (ng/ml)	IGF level Day 10 (ng/ml)	IGF level Day 13 (ng/ml)
A	$304.968^{c} \pm 15.384$	$328.502^{e} \pm 23.845$	$305.282^{c} \pm 18.857$	$312.745^{b} \pm 25.819$	$304.746^{c} \pm 13.344$
B	$309.759^{d} \pm 21.637$	$321.656^{c} \pm 22.736$	$301.356^{b} \pm 12.832$	$302.247^{b} \pm 11.938$	$302.743^{b} \pm 12.864$
C	$321.784^{e} \pm 18.302$	$322.173^{b} \pm 11.908$	$304.463^{c} \pm 14.927$	$317.387^{d} \pm 13.935$	$318.636^{d} \pm 19.624$
D	$316.739^{c} \pm 15.295$	$322.656^{e} \pm 21.338$	$378.373^{d} \pm 19.226$	$345.864^{c} \pm 16.459$	$347.846^{f} \pm 11.426$
E	$369.657^{e} \pm 22.927$	$319.288^{c} \pm 17.835$	$382.443^{d} \pm 29.382$	$358.375^{c} \pm 23.638$	$352.749^{b} \pm 18.395$
F	$308.826^{a} \pm 14.327$	$206.571^{c} \pm 10.942$	$179.546^{a} \pm 11.538$	$172.745^{b} \pm 10.882$	$162.974^{d} \pm 14.026$
G	$344.927^{b} \pm 15.024$	$205.546^{d} \pm 11.924$	$176.684^{a} \pm 11.935$	$171.387^{b} \pm 13.375$	$161.739^{c} \pm 12.824$
H	$389.785^{e} \pm 27.827$	$203.468^{c} \pm 12.294$	$182.745^{b} \pm 15.352$	$172.352^{a} \pm 11.942$	$163.947^{a} \pm 10.284$
I	$399.645^{c} \pm 18.482$	$212.847^{f} \pm 10.204$	$178.469^{b} \pm 13.911$	$135.467^{c} \pm 10.294$	$121.937^{c} \pm 9.832$
J	$396.378^{d} \pm 14.924$	$219.378^{b} \pm 11.739$	$183.478^{e} \pm 13.284$	$138.372^{c} \pm 12.117$	$124.387^{c} \pm 11.934$

Table 5: Serum Insulin-like Growth Factor (IGF) concentration (ng/ml) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Analysis of serum melatonin levels (Table 6) revealed a steady decrease in the control groups A and B across the experimental days, except for Group C, which exhibited a gradual increase. This rise in Group C may be attributed to the intake of tryptophan-deficient sorghum. In contrast, a marked increase in serum melatonin levels was observed in all sleep-deprived groups (D-J), particularly in Groups I and J, where rats experienced both caffeine administration (20 mg/kg) and housing in disk-over-water cages. The significant elevation in melatonin levels suggests that the experimental rats may have experienced disrupted circadian rhythms, evidenced by their lack of vigor and pronounced behavioral changes, such as massive fur loss and delirium in Groups I and J.

Groups	Melatonin level Day 1 (pg/ml)	Melatonin level Day 4 (pg/ml)	Melatonin level Day 7 (pg/ml)	Melatonin level Day 10 (pg/ml)	Melatonin level Day 13 (pg/ml)
A	$45.029^{b} \pm 3.827$	$43.739^{b} \pm 3.849$	$42.092^{a} \pm 3.783$	$41.927^{b} \pm 3.919$	$41.385^{a} \pm 3.206$
B	$58.881^{d} \pm 5.782$	$56.028^{b} \pm 4.567$	$57.183^{d} \pm 6.091$	$54.922^{c} \pm 4.348$	$53.371^{c} \pm 4.294$
C	$39.985^{a} \pm 4.297$	$41.954^{a} \pm 4.892$	$40.395^{c} \pm 3.291$	$43.753^{b} \pm 4.294$	$42.951^{b} \pm 4.104$
D	$54.458^{b} \pm 4.265$	$56.168^{c} \pm 4.721$	$56.927^{c} \pm 4.183$	$58.293^{a} \pm 4.948$	$62.279^{d} \pm 5.826$
E	$53.371^{b} \pm 4.028$	$54.458^{c} \pm 5.927$	$57.294^{b} \pm 4.992$	$59.827^{c} \pm 5.119$	$61.938^{a} \pm 5.204$
F	$62.018^{b} \pm 3.927$	$63.145^{d} \pm 5.835$	$63.638^{d} \pm 5.174$	$68.295^{c} \pm 7.295$	$74.225^{a} \pm 5.891$
G	$58.276^{d} \pm 4.826$	$58.882^{a} \pm 5.218$	$60.295^{a} \pm 5.013$	$62.193^{c} \pm 4.205$	$62.822^{c} \pm 4.711$
H	$49.275^{b} \pm 3.674$	$52.914^{d} \pm 4.239$	$53.252^{d} \pm 4.725$	$65.023^{c} \pm 4.739$	$71.395^{e} \pm 6.386$
I	$68.205^{e} \pm 5.082$	$71.395^{b} \pm 6.129$	$101.948^{a} \pm 8.217$	$96.294^{a} \pm 8.204$	$98.029^{c} \pm 6.925$
J	$63.104^{e} \pm 4.953$	$74.039^{a} \pm 6.983$	$94.439^{a} \pm 6.814$	$106.709^{b} \pm 8.926$	$102.822^{b} \pm 9.205$

Table 6: Serum melatonin concentration (pg/ml) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Serum serotonin analysis (Table 7) showed stable levels across the control groups, with a peak observed in Groups D and E, which received caffeine while housed in regular cages. However, a decrease in serotonin levels was evident in all groups (F-J) subjected to sleep deprivation, particularly in Groups I and J.

Groups	Serotonin level Day 1 (pg/ml)	Serotonin level Day 4 (pg/ml)	Serotonin level Day 7 (pg/ml)	Serotonin level Day 10 (pg/ml)	Serotonin level Day 13 (pg/ml)
A	$56.294^{b} \pm 4.784$	$57.224^{b} \pm 5.022$	$58.294^{a} \pm 3.928$	$54.823^{a} \pm 3.284$	$56.925^{a} \pm 4.193$
B	$58.012^{c} \pm 3.126$	$58.921^{c} \pm 3.053$	$62.329^{e} \pm 4.109$	$62.937^{c} \pm 3.992$	$64.248^{b} \pm 4.285$
C	$54.221^{c} \pm 3.018$	$54.924^{c} \pm 3.847$	$51.835^{a} \pm 3.185$	$53.205^{b} \pm 4.209$	$56.392^{c} \pm 3.812$
D	$56.729^{c} \pm 4.627$	$59.295^{b} \pm 3.105$	$63.028^{d} \pm 6.295$	$63.925^{c} \pm 4.288$	$66.823^{d} \pm 5.284$
E	$54.839^{a} \pm 3.918$	$56.925^{a} \pm 4.290$	$58.205^{c} \pm 5.285$	$64.205^{b} \pm 3.837$	$66.935^{a} \pm 4.298$
F	$57.195^{c} \pm 5.021$	$48.202^{e} \pm 3.183$	$41.094^{a} \pm 3.927$	$33.924^{b} \pm 2.149$	$28.453^{b} \pm 2.358$
G	$56.973^{c} \pm 3.205$	$53.175^{c} \pm 3.129$	$49.238^{b} \pm 3.353$	$42.592^{d} \pm 3.936$	$31.924^{a} \pm 4.428$
H	$58.394^{c} \pm 5.593$	$55.928^{a} \pm 4.026$	$51.395^{b} \pm 4.734$	$43.294^{d} \pm 3.295$	$36.894^{f} \pm 2.472$
I	$52.295^{d} \pm 3.831$	$42.235^{b} \pm 3.905$	$38.284^{e} \pm 2.846$	$28.026^{c} \pm 2.148$	$18.937^{a} \pm 2.161$
J	$58.282^{e} \pm 4.852$	$52.763^{c} \pm 4.852$	$46.935^{d} \pm 3.304$	$32.734^{a} \pm 2.856$	$24.048^{b} \pm 2.478$

Table 7: Serum serotonin concentration (pg/ml) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

The analysis of serum pro-inflammatory markers TNF-α (Table 8) and IL-1β (Table 9) showed a steady decrease in control groups but an increase in all sleep-deprived groups, with the highest levels observed in rats receiving daily caffeine administration and housed in disk-over-water cages. This increase in inflammatory markers in Groups I and J underscores the systemic inflammation induced by chronic sleep loss, which is exacerbated by caffeine.

Groups	TNF- α level Day 1 (pg/ml)	TNF- α level Day 4 (pg/ml)	TNF- α level Day 7 (pg/ml)	TNF- α level Day 10 (pg/ml)	TNF- α level Day 13 (pg/ml)
A	$24.122^{c} \pm 3.892$	$23.029^{a} \pm 2.485$	$21.825^{a} \pm 2.593$	$21.296^{b} \pm 2.039$	$19.823^{a} \pm 2.114$
B	$20.986^{b} \pm 2.941$	$20.853^{b} \pm 2.103$	$20.048^{b} \pm 3.295$	$19.753^{c} \pm 2.104$	$19.395^{c} \pm 2.942$
C	$21.829^{c} \pm 2.324$	$21.293^{b} \pm 3.298$	$20.739^{c} \pm 2.840$	$20.216^{b} \pm 2.947$	$19.249^{b} \pm 2.592$
D	$21.193^{c} \pm 2.191$	$21.872^{b} \pm 2.842$	$23.739^{c} \pm 3.492$	$23.801^{c} \pm 3.927$	$24.144^{a} \pm 1.925$
E	$20.938^{a} \pm 2.112$	$21.439^{b} \pm 2.495$	$22.932^{b} \pm 2.238$	$23.626^{a} \pm 2.894$	$24.586^{c} \pm 2.042$
F	$22.839^{c} \pm 2.485$	$22.989^{a} \pm 3.383$	$23.222^{a} \pm 3.204$	$25.063^{d} \pm 3.812$	$27.029^{d} \pm 3.104$
G	$23.592^{d} \pm 3.294$	$23.826^{e} \pm 3.185$	$24.184^{a} \pm 2.592$	$24.896^{b} \pm 3.640$	$27.925^{b} \pm 3.425$
H	$20.876^{b} \pm 2.292$	22.852 ± 2.483	22.937 ± 2.384	23.538 ± 3.103	26.203 ± 3.683
I	$23.618^{e} \pm 2.352$	$24.291^{b} \pm 3.298$	$29.472^{c} \pm 2.506$	$31.409^{a} \pm 3.294$	$56.028^{e} \pm 4.205$
J	$23.457^{a} \pm 3.378$	$25.144^{e} \pm 3.916$	$30.975^{d} \pm 3.839$	$32.522^{c} \pm 3.101$	$59.962^{d} \pm 4.812$

Table 8: Serum Tumor Necrosis Factor-alpha (TNF- α) concentration (pg/ml) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Groups	IL-1β level Day 1 (pg/ml)	IL-1β level Day 4 (pg/ml)	IL-1β level Day 7 (pg/ml)	IL-1β level Day 10 (pg/ml)	IL-1β level Day 13 (pg/ml)
A	$31.597^{b} \pm 2.849$	$31.294^{c} \pm 3.210$	$30.295^{b} \pm 2.104$	$29.051^{a} \pm 2.014$	$27.525^{d} \pm 2.903$
B	$30.889^{a} \pm 2.130$	$29.128^{b} \pm 2.393$	$29.114^{b} \pm 2.845$	$27.346^{a} \pm 2.188$	$25.088^{a} \pm 2.148$
C	$31.842^{d} \pm 3.193$	$31.032^{d} \pm 3.027$	$29.782^{c} \pm 3.103$	$29.015^{e} \pm 2.307$	$28.952^{e} \pm 2.302$
D	$30.958^{c} \pm 2.732$	$32.395^{c} \pm 3.116$	$38.201^{e} \pm 3.292$	$54.781^{b} \pm 4.294$	$61.405^{c} \pm 5.587$
E	$29.859^{a} \pm 2.204$	$33.913^{d} \pm 3.203$	$37.126^{d} \pm 3.014$	$48.194^{c} \pm 2.286$	$64.035^{b} \pm 5.920$
F	$31.284^{c} \pm 3.832$	$38.946^{a} \pm 3.982$	$44.804^{d} \pm 3.847$	$56.721^{e} \pm 3.938$	$75.714^{g} \pm 6.258$
G	$32.728^{a} \pm 3.291$	$39.244^{d} \pm 4.193$	$51.992^{e} \pm 4.029$	$74.297^{d} \pm 4.205$	$88.238^{g} \pm 6.986$
H	$33.697^{e} \pm 2.621$	$46.295^{f} \pm 3.856$	$59.987^{a} \pm 4.194$	$76.679^{d} \pm 4.946$	$89.884^{f} \pm 7.374$
I	$30.465^{c} \pm 2.718$	$69.285^{b} \pm 4.394$	$88.735^{b} \pm 4.829$	$104.883^{e} \pm 8.386$	$158.651^{f} \pm 13.592$
J	$31.485^{d} \pm 3.018$	$66.268^{c} \pm 4.195$	$89.935^{g} \pm 5.048$	$107.649^{f} \pm 9.421$	$175.056^{a} \pm 16.435$

Table 9: Serum Interleukin-1 beta (IL-1β) concentration (pg/ml) over time in the experimental groups. Results are expressed as mean ±S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Correspondingly, the serum superoxide dismutase (SOD) activity (Table 10) revealed a steady increase in the control groups. At the same time, a sharp reduction was noted in sleep-deprived groups, particularly in Groups I and J. Additionally, catalase activity (Table 11) and glutathione levels (Table 12) demonstrated a statistically insignificant decrease in the control groups (A-E) but showed significant reductions in those housed in disk-over-water cages (F-J) alongside caffeine administration.

Groups	SOD (U/L) Day 1	SOD (U/L) Day 4	SOD (U/L) Day 7	SOD (U/L) Day 10	SOD (U/L) Day 13
A	$1.192^{e} \pm 0.028$	$1.349^{e} \pm 0.031$	$1.428^{b} \pm 0.028$	$1.849^{c} \pm 0.016$	$2.257^{c} \pm 0.069$
B	$1.139^{b} \pm 0.013$	$1.364^{d} \pm 0.023$	$1.578^{c} \pm 0.039$	$1.528^{c} \pm 0.023$	$1.984^{d} \pm 0.035$
C	$1.060^{e} \pm 0.008$	$1.118^{b} \pm 0.025$	$1.342^{c} \pm 0.018$	$1.697^{d} \pm 0.027$	$1.858^{d} \pm 0.052$
D	$1.243^{a} \pm 0.021$	$1.01^{d} \pm 0.016$	$0.816^{c} \pm 0.005$	$0.729^{b} \pm 0.012$	$0.732^{c} \pm 0.006$
E	$1.155^{b} \pm 0.019$	$0.981^{e} \pm 0.007$	$0.732^{d} \pm 0.007$	$0.619^{f} \pm 0.008$	$0.666^{f} \pm 0.004$
F	$1.281^{b} \pm 0.024$	$0.975^{d} \pm 0.003$	$0.894^{e} \pm 0.004$	$0.788^{c} \pm 0.006$	$0.872^{c} \pm 0.008$
G	$1.088^{d} \pm 0.009$	$1.021^{e} \pm 0.009$	$0.928^{a} \pm 0.007$	$0.854^{b} \pm 0.014$	$0.731^{c} \pm 0.009$
H	$1.142^{a} \pm 0.011$	$1.042^{b} \pm 0.006$	$0.946^{b} \pm 0.008$	$0.982^{e} \pm 0.008$	$0.799^{d} \pm 0.005$
I	$1.238^{c} \pm 0.029$	$1.038^{a} \pm 0.013$	$0.911^{b} \pm 0.010$	$0.513^{e} \pm 0.006$	$0.127^{d} \pm 0.003$
J	$1.034^{e} \pm 0.014$	$0.994^{d} \pm 0.008$	$0.863^{a} \pm 0.008$	$0.489^{c} \pm 0.004$	$0.212^{b} \pm 0.004$

Table 10: Serum Superoxide Dismutase (SOD) activity (U/L) over time in the experimental groups. Results are expressed as mean ±S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Groups	CAT (U/L) Day 1	CAT (U/L) Day 4	CAT (U/L) Day 7	CAT (U/L) Day 10	CAT (U/L) Day 13
A	$16.396^{b} \pm 1.053$	$16.294^{c} \pm 1.948$	$16.253^{c} \pm 1.836$	$15.867^{b} \pm 1.304$	$15.938^{b} \pm 1.305$
B	$15.358^{a} \pm 1.397$	$15.283^{a} \pm 1.437$	$15.038^{c} \pm 1.204$	$15.184^{b} \pm 1.743$	$15.395^{b} \pm 1.593$
C	$13.034^{d} \pm 1.830$	$13.402^{e} \pm 1.039$	$13.257^{b} \pm 1.958$	$13.028^{c} \pm 1.483$	$13.635^{b} \pm 1.942$
D	$15.532^{d} \pm 1.932$	$15.082^{d} \pm 1.842$	$13.997^{b} \pm 1.437$	$13.284^{b} \pm 1.290$	$13.018^{b} \pm 1.224$
E	$14.924^{a} \pm 1.527$	$14.284^{b} \pm 1.338$	$14.028^{c} \pm 1.753$	$13.392^{b} \pm 1.394$	$13.149^{b} \pm 1.685$
F	$13.883^{b} \pm 1.068$	$13.193^{c} \pm 1.749$	$12.485^{e} \pm 1.458$	$12.294^{d} \pm 1.479$	$11.692^{a} \pm 1.453$
G	$14.924^{c} \pm 1.944$	$13.989^{a} \pm 1.832$	$13.341^{b} \pm 1.579$	$12.038^{f} \pm 1.120$	$11.839^{e} \pm 1.892$
H	$13.098^{d} \pm 1.822$	$12.395^{f} \pm 1.136$	$10.395^{c} \pm 1.021$	$9.843^{a} \pm 1.092$	$7.659^{b} \pm 0.429$
I	$17.165^{b} \pm 1.643$	$12.114^{e} \pm 1.285$	$10.145^{a} \pm 1.038$	$9.246^{c} \pm 1.142$	$8.386^{c} \pm 0.822$
J	$16.827^{a} \pm 1.750$	$13.283^{e} \pm 1.489$	$11.294^{c} \pm 1.028$	$8.518^{b} \pm 1.038$	$7.576^{d} \pm 0.338$

Table 11: Serum Catalase (CAT) activity (U/L) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

Groups	GSH (mg/dL) Day 1	GSH (mg/dL) Day 4	GSH (mg/dL) Day 7	GSH (mg/dL) Day 10	GSH (mg/dL) Day 13
A	$0.673^{b} \pm 0.023$	$0.664^{b} \pm 0.037$	$0.682^{c} \pm 0.038$	$0.639^{a} \pm 0.071$	$0.648^{b} \pm 0.037$
B	$0.641^{c} \pm 0.041$	$0.629^{c} \pm 0.043$	$0.653^{d} \pm 0.025$	$0.673^{b} \pm 0.052$	$0.639^{c} \pm 0.024$
C	$0.584^{a} \pm 0.054$	$0.593^{a} \pm 0.034$	$0.582^{c} \pm 0.041$	$0.598^{b} \pm 0.028$	$0.602^{e} \pm 0.013$
D	$0.571^{b} \pm 0.069$	$0.562^{b} \pm 0.075$	$0.587^{c} \pm 0.038$	$0.534^{b} \pm 0.041$	$0.521^{a} \pm 0.026$
E	$0.608^{d} \pm 0.071$	$0.598^{d} \pm 0.069$	$0.593^{d} \pm 0.051$	$0.589^{d} \pm 0.062$	$0.584^{d} \pm 0.021$
F	$0.575^{a} \pm 0.042$	$0.543^{e} \pm 0.027$	$0.521^{b} \pm 0.025$	$0.506^{b} \pm 0.028$	$0.482^{c} \pm 0.037$
G	$0.627^{d} \pm 0.084$	$0.552^{a} \pm 0.048$	$0.589^{b} \pm 0.043$	$0.538^{c} \pm 0.034$	$0.546^{c} \pm 0.029$
H	$0.784^{e} \pm 0.063$	$0.631^{a} \pm 0.082$	$0.628^{b} \pm 0.031$	$0.579^{c} \pm 0.052$	$0.489^{c} \pm 0.038$
I	$0.792^{b} \pm 0.038$	$0.695^{c} \pm 0.021$	$0.626^{c} \pm 0.043$	$0.549^{a} \pm 0.038$	$0.459^{e} \pm 0.021$
J	$0.714^{c} \pm 0.043$	$0.672^{e} \pm 0.029$	$0.638^{a} \pm 0.027$	$0.583^{b} \pm 0.026$	$0.389^{f} \pm 0.013$

Table 12: Glutathione (GSH) concentration (mg/dL) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

An increase in malondialdehyde (MDA) levels was observed across all experimental groups (Table 13), with the highest increase in those housed in disk-over-water cages. This trend may indicate oxidative stress, tissue damage, and chronic inflammatory conditions, particularly given the erratic behavior, social anxiety, and muscle function loss observed in the experimental rats. The combination of these stressors, evidenced by elevated MDA levels, suggests significant oxidative damage that could explain the severe behavioral and physiological deterioration.

Discussion

This study explores how sleep deprivation, caffeine intake, and diet affect biochemical biomarkers involved in growth, sleep, inflammation, and oxidative stress. The results of the proximate analysis and amino acid composition corroborate the findings by Pan., *et al*. [11] who reported a lower tryptophan and protein composition in a sorghum-based diet compared to a corn-based diet, potentially influencing sleep-related biomarkers negatively.

Groups	MDA (µmol/L) Day 1	MDA (µmol/L) Day 4	MDA (µmol/L) Day 7	MDA (µmol/L) Day 10	MDA (µmol/L) Day 13
A	$3.944^{b} \pm 0.285$	$4.082^{b} \pm 0.383$	$4.285^{a} \pm 0.392$	$4.184^{c} \pm 0.284$	$4.358^{b} \pm 0.293$
B	$4.964^{d} \pm 0.434$	$5.294^{a} \pm 0.492$	$5.026^{b} \pm 0.441$	$5.287^{c} \pm 0.623$	$5.321^{c} \pm 0.550$
C	$4.352^{a} \pm 0.236$	$4.736^{c} \pm 0.424$	$4.392^{a} \pm 0.313$	$4.928^{c} \pm 0.431$	$5.015^{c} \pm 0.442$
D	$4.709^{b} \pm 0.411$	$4.923^{c} \pm 0.393$	$5.184^{a} \pm 0.492$	$5.867^{d} \pm 0.592$	$6.801^{c} \pm 0.394$
E	$4.403^{b} \pm 0.375$	$4.794^{a} \pm 0.284$	$5.069^{a} \pm 0.401$	$5.837^{d} \pm 0.423$	$6.239^{c} \pm 0.483$
F	$3.896^{d} \pm 0.193$	$4.029^{c} \pm 0.385$	$4.848^{a} \pm 0.331$	$6.487^{e} \pm 0.595$	$8.928^{e} \pm 0.624$
G	$4.301^{a} \pm 0.352$	$4.846^{c} \pm 0.385$	$5.729^{f} \pm 0.378$	$7.027^{d} \pm 0.628$	$8.184^{b} \pm 0.388$
H	$4.148^{e} \pm 0.420$	$5.823^{b} \pm 0.332$	$7.218^{d} \pm 0.417$	$8.639^{d} \pm 0.491$	$9.029^{c} \pm 0.596$
I	$4.352^{a} \pm 0.391$	$5.938^{d} \pm 0.284$	$6.839^{e} \pm 0.433$	$8.478^{g} \pm 0.783$	$9.882^{f} \pm 0.652$
J	$4.037^{b} \pm 0.283$	$5.892^{f} \pm 0.384$	$7.039^{e} \pm 0.482$	$8.932^{c} \pm 0.682$	$10.984^{d} \pm 0.835$

Table 13: Malondialdehyde (MDA) concentration (µmol/L) over time in the experimental groups. Results are expressed as mean ± S.E.M, (n = 6). ANOVA analysis was carried out using Duncan's post hoc test for multiple comparisons. Superscripts with different letters show statistical differences. Values were considered statistically significant at $p \leq 0.05$ (confidence level = 95%).

The stability in the serum testosterone levels observed in Groups A, B, and C suggests minimal impact under non-stressful conditions, aligning with previous research indicating that stable housing and a balanced diet are crucial for maintaining hormonal homeostasis [12]. The rise in testosterone levels that escalated significantly in the caffeine-administered group D was also reported by Park., *et al*. [13]. The sharp rise in testosterone levels observed in the group subjected to sorghum and caffeine is consistent with studies by [14], who reported that caffeine could enhance testosterone levels when accompanied by adequate dietary support. However, the long-term effects were not assessed. Van Cauter., *et al*. [15] who highlighted similar disruptions in hormonal balance under sleep-deprived conditions. The observed decline in testosterone levels among caffeine-administered and tryptophan-deficient groups aligns with findings by Eliot [16], who reported that caffeine and sleep deprivation can impair testosterone synthesis, resulting in behavioral and physiological changes such as aggression, muscle atrophy, and hair loss.

The significant drop in IGF-1 levels in the sleep-deprived groups corroborates findings from Spiegel [17], who underscored the negative impact of chronic stress on IGF levels. This decrease in serum IGF-1 in sleep-deprived groups echoes research by Wan., *et al*. [18], which found that sleep deprivation impairs growth factor production, compounded by caffeine's antagonistic effects on growth hormone secretion. Interestingly, the increasing trend in serum IGF-1 levels observed in rats from Groups D and E as the duration of caffeine administration lengthened, challenges the previously reported antagonistic effect of caffeine on growth hormone secretion noted by Wan [18].

These fluctuations in serum melatonin and serotonin levels observed in this study suggest that the combination of sleep deprivation and caffeine intake disrupts normal circadian rhythms, as similarly noted by Jenkins., *et al*. [19], where melatonin suppression and serotonin depletion were linked to mood disorders and cognitive deficits.

The increase in the pro-inflammatory and oxidative stress markers observed in the sleep-deprived groups is consistent with previous studies indicating that chronic sleep deprivation can lead to systemic inflammation and increased oxidative stress, as reported by Irwin., *et al*. [20]. The combination of these stressors, evidenced by elevated MDA levels, suggests significant oxidative damage that could explain the severe behavioral and physiological deterioration, aligning with models of neuroinflammation and oxidative stress described by Bellesi., *et al*. [21].

Interestingly, lower doses of caffeine have been shown to exhibit neuroprotective effects, enhancing cognitive function and reducing oxidative stress markers. In contrast, higher doses, like those used in this study (20 mg/kg), are more likely to exacerbate

stress responses and contribute to neuroinflammation [22]. This highlights the importance of dosage when considering caffeine's effects on physiological and psychological health.

Moreover, studies focusing on the neuroprotective effects of antioxidant-rich diets, such as the Mediterranean diet or those high in omega-3 fatty acids, suggest that these dietary patterns can significantly buffer the negative effects of stress and high caffeine intake. For example, dietary supplementation with omega-3 fatty acids has been shown to reduce levels of inflammatory cytokines (such as TNF-α and IL-1β) and oxidative stress markers that were elevated in this study under conditions of high caffeine and sleep deprivation. Additionally, diets rich in polyphenols, found in fruits and vegetables, have been shown to enhance brain-derived neurotrophic factor (BDNF) expression, providing neuroprotection even under chronic stress conditions [23].

Conclusion

This study highlights the pivotal role of sleep quality in maintaining hormonal balance, demonstrating that while caffeine can enhance testosterone levels under non-stressful conditions, its effects become detrimental in the context of sleep deprivation. The findings offer significant insights into the interactions between dietary and environmental factors and their influence on hormonal regulation during sleep deprivation, particularly emphasizing the roles of tryptophan intake and housing conditions. Overall, this research elucidates the complex relationships among sleep, dietary factors, and hormonal and inflammatory responses in rats. It underscores the potential neurotoxic effects associated with caffeine consumption when coupled with sleep deprivation. These results underscore the urgent need for further investigation into dietary strategies that could alleviate these adverse effects and promote better hormonal and overall health. By advancing our understanding of these interactions, we can pave the way for more effective interventions aimed at enhancing sleep quality and optimizing hormonal health.

Future Directions

Future research should explore the mechanistic pathways underlying the combined effects of sleep deprivation and caffeine on hormonal and inflammatory regulation, with a focus on neurotransmitter modulation and circadian rhythm disruption. Longitudinal studies assessing the chronic impact of dietary interventions, such as tryptophan supplementation, on sleep quality and hormonal balance are warranted. Additionally, investigations into sex-specific responses, different doses of caffeine, and varied environmental conditions could provide a more comprehensive understanding of these interactions. Translational studies in humans would also be valuable to determine the clinical relevance of these findings and to inform dietary and lifestyle recommendations aimed at mitigating the neuroendocrine consequences of sleep deprivation.

Ethical Statement

The Ethical Committee approved the study on the Use of Laboratory Animals, Department of Biochemistry, Kwara State University, Malete, Nigeria, with approval reference: case number KWASU/FPAS/AEC/2021/012.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Credit Authorship Contribution Statement

Lutfat A. Usman: Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. Lutfat A. Usman: Resources, Investigation, and Data curation. Rasheed B. Ibrahim, Hammed A. Madandola, Emmanuel A. Irondi: Investigation, Data curation. Abdulhakeem O. Sulyman, Mutiu A. Alabi, Raliat A. Aladodo, Asiat Na'Allah, Hassan T. Abdulameed: Resources, Investigation. Emmanuel O. Ajani: Writing – review and editing. Afolabi C. Akinmoladun: Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Consent for Publication

All authors have reviewed and approved the manuscript for publication.

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