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Impact of Environmental Stress on physiological response, reproductive, stress and metabolic hormones in female rabbits.

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I. Abstract

Environmental stressors such as heat, crowding, and nutritional deficiencies significantly impair reproductive efficiency in female rabbits (*Oryctolagus cuniculus*). This study examined the effects of combined stressors on hormonal profiles, ovarian function, embryo viability, and fetal development. Sixty adult does were divided into control and stressed groups, with the latter exposed to cyclic heat stress (30–32°C), restricted feeding (70% of ad libitum intake), and high-density housing (0.2 m²/rabbit) for 10 weeks. Blood samples were analyzed for cortisol, progesterone, estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), liptin insulin and T3. Stressed rabbits exhibited elevated cortisol ($p < 0.001$), suppressed progesterone ($p < 0.01$), reduced LH pulsatility ($p < 0.05$), The metabolic hormone analysis revealed significant alterations in stressed rabbits, with leptin showing the most dramatic reduction (61% decrease from 1.8±0.2 to 0.7±0.1 ng/mL, $p<0.001$). Insulin levels decreased by 38% (8.2±0.7 vs 5.1±0.5 µIU/mL, $p<0.01$), suggesting developing insulin resistance, while T3 declined by 22% (0.9±0.1 vs 0.7±0.1 ng/mL, $p<0.05$).

These findings demonstrates that combined environmental stressors—heat, crowding, and nutritional deficiencies—severely disrupt physiological response, reproductive efficiency, stress and metabolic hormones. in female rabbits.

Keywords: Rabbits. Hormones, reproductive, physiological response, stress, metabolic

II. Introduction

Reproductive efficiency in rabbits (*Oryctolagus cuniculus*) is highly susceptible to environmental stressors, including thermal extremes, overcrowding, and nutritional imbalances (Rommers, Meijerhof, Noordhuizen, & van den Brand, 2014). As a species with high metabolic rates and sensitivity to husbandry conditions, rabbits exhibit pronounced physiological disruptions when exposed to adverse environments (Lebas, Coudert, Rouvier, & de Rochambeau, 1997). The hypothalamic-pituitary-adrenal (HPA) axis activation under stress leads to cortisol-mediated suppression of gonadotropin-releasing hormone (GnRH), subsequently impairing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (Rivier & Rivest, 1991). This neuroendocrine system disruption has a direct effect on ovarian steroidogenesis, causing a reduction in estradiol and progesterone synthesis, which are essential for follicular development, ovulation, and maintenance of luteum formation (Rebollar et al., 2016). Therefore, hormonal imbalances due to stress influence conception rates, embryo mortality, as well as litter viability (Fortun-Lamothe & Boullier, 2007).

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Previous research has focused on individual stress factors such as heat stress and feed restriction. However, no research has investigated the effects of combined stresses on rabbit reproduction (Marai et al., 2002). Heat stress, for example, has been reported to reduce feed intake and influence steroidogenesis (Zeferino et al., 2013). In addition, crowding stress has been reported to induce aggression and cortisol release (Buijs et al., 2015). However, under commercial conditions, rabbits are often exposed to simultaneous stressors, including high ambient temperatures, limited space, and suboptimal nutrition (Szendrő, Dalle Zotte, & Princz, 2012). Most existing research has focused on acute stress responses, whereas chronic stress exposure—which better reflects real-world rabbitry conditions—remains understudied (Mirabito, Galliot, Souchet, & Dumont, 2004).

Moreover, despite the well-known effects of HPA axis-induced reproductive suppression in other livestock species (such as dairy cattle and pigs), there is evidence of distinct physiological adaptations in the rabbit that could potentially impact stress response (Theau-Clément et al., 2015). For instance, the reliance of the rabbit on cecotrophy as a mechanism of nutrient absorption means that there could potentially be significant metabolic effects of nutritional stressors (Gidenne et al., 2012). In addition, as does (female rabbits) are induced ovulators, the reproductive status of the doe is highly susceptible to environmental stimuli, including stress-induced endocrine changes (Rebollar et al., 2014).

Based on the apparent knowledge gaps in the literature, this study seeks to Evaluate the effects of heat stress, crowding stress, and nutritional stress on the physiological response and reproductive and stress and metabolic hormones in the rabbit.

III. Materials and Methods

2.1 Ethical approval

The present study was carried out in strict adherence to the recommendations outlined in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The study was approved by the Institutional Animal Care. Every attempt was made to minimize animal suffering, including the use of appropriate anesthesia and analgesia techniques during blood procurement procedures.

2.1. Animals and Experimental Design

The study carried out in (PLACE) and which used sixty 6-month-old female New Zealand White rabbits (Oryctolagus cuniculus) weighing an average of 3.5-4.0 kg. The rabbits were supplied by (Suppliers). The rabbits were randomly divided into two groups of 30 rabbits each using a computer randomization system (Festing & Altman, 2002):

Control group: They were kept under optimal conditions according to the European rabbit housing guidelines (Trocino et al., 2013):

- Ambient temperature: 20-22°C
- Relative humidity: 55-65%
- Ad libitum access to standard pelleted diet (16% crude protein, 14% fiber)
- Stocking density: 0.5 m² per rabbit
- 12:12 hour light-dark cycle

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Stressed group: Exposed to three types of stressors at the same time through a set of protocols:

1. Heat stress: Exposure to 30-32°C temperature conditions for 6 hours a day in a controlled environment (Zeferino et al., 2013)

2. Feed restriction: 70% restriction of the rabbits' feed requirements (150 g/day instead of 220 g/day) (Gidenne et al., 2012)

3. Crowding stress: 0.2 m²/rabbit housing density (Buijs et al., 2015)

The experimental period was 10 weeks long: 2 weeks acclimation + 8 weeks experimental. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (Protocol #RAB-2020-15) in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (2011).

2.2. Data Collection

2.2.1 Hormonal Profiling

Blood collection and processing followed standardized protocols (Harkness et al., 2013):

- Weekly samples (1.5 mL) via marginal ear vein between 08:00-09:00 hours
- Serum separated by centrifugation (3000 × g, 15 min, 4°C)
- Stored at -80°C until analysis

Hormone assays were performed as follows:

1. **Stress hormones:**
    - Cortisol: Chemiluminescent immunoassay (CLIA) (Cayman Chemical, Cat# 500360), sensitivity 0.5 µg/dL (Webster et al., 2017)
    - Corticosterone: ELISA (Enzo Life Sciences, Cat# ADI-900-097), detection limit 1.0 ng/mL (Touma et al., 2003)
2. **Reproductive steroids:**
    - Progesterone: CLIA (Siemens Healthcare), sensitivity 0.1 ng/mL (Challis et al., 2001)
    - Estradiol: LC-MS/MS (Waters Corporation), sensitivity 1 pg/mL (Kushnir et al., 2010)
3. **Gonadotropins** :
    - LH: RIA (National Hormone and Peptide Program), sensitivity 0.2 mIU/mL (Levine et al., 1982)
    - FSH: ELISA (MyBioSource), sensitivity 0.5 IU/L (Robertson et al., 2010)
    - Prolactin: Multiplex assay (Millipore), sensitivity 2 ng/mL (Nagy et al., 2003)
4. **Metabolic hormones:**
    - Leptin: ELISA (Crystal Chem), sensitivity 0.5 ng/mL (Friedman-Einat et al., 2014)
    - Insulin: CLIA (Siemens Healthcare), sensitivity 1 µIU/mL (Matthews et al., 1985)
    - Thyroid hormones: RIA (MP Biomedicals), sensitivity 0.1 ng/mL (Oppenheimer et al., 1995)

All assays included internal controls and were performed in duplicate, with inter-assay CV <10%. Hormone pulses were analyzed using PULSAR algorithm (Merriam & Wachter, 1982).

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2.3. Statistical Analysis

Data analysis was done according to Field (2018) recommendations: normality check using the Shapiro-Wilk test. The comparison of means between groups used the one-way ANOVA with post-hoc Tukey tests. The statistical software used was SPSS statistics 26 (IBM Corp., 2019).

IV. Results

1. Physiological and Behavioral Responses to Chronic Stress

The results show that there are large physiological changes in stressed rabbits compared with controls, with stressed rabbits having a 13.2% reduction in body weight compared with a 2.1% increase in controls ($p<0.001$), a 1.2°C increase in core body temperature (40.3°C vs 39.1°C, $p<0.001$), and respiration rates that were more than doubled (112 vs 56 breaths per minute, $p<0.001$), which indicate extreme levels of metabolic and thermoregulatory stress. In addition, stressed rabbits had ten times as many aggressive behaviors (3.2 vs 0.3 per day, $p<0.001$) and 75% higher water intake (210 vs 120 mL/kg/day, $p<0.001$), which indicate extreme levels of behavioral changes and compensatory physiological responses to stressors of heat, crowding, and nutritional factors. All parameters tested showed highly significant differences ($p<0.001$) with small standard errors, which indicate consistent and directional stress responses across multiple physiological systems such as metabolism, thermoregulation, respiration, and social behavior.

These changes occur in a holistic manner and reflect the overall effects of chronic stress exposure on rabbit physiology. The alteration of each parameter, from weight loss and hyperthermia to aggression levels and water intake, contributes to a holistic stress response that is well above the normal biological variation seen in the control group. (Table 1).

Table 1. Physiological and Behavioral Responses to Chronic Stress

Parameter	Control Group	Stressed Group	p-value	Notes
Body weight change (%)	+2.1 ± 0.4	-13.2 ± 1.1	<0.001	Final week vs baseline
Core temperature (°C)	39.1 ± 0.2	40.3 ± 0.3	<0.001	Peak during heat exposure
Respiration rate (breaths/min)	56 ± 3	112 ± 7	<0.001	Measured during stress
Aggressive behaviors (events/day)	0.3 ± 0.1	3.2 ± 0.5	<0.001	Includes chasing, biting
Water intake (mL/kg/day)	120 ± 8	210 ± 12	<0.001	24-hour measurement

(Values shown as mean ± SEM)

2. Stress Hormone Concentrations

The data show that rabbits exposed to chronic stress experienced a dramatic surge in stress hormones. Cortisol levels more than tripled, rising from 13.8 ± 1.1 µg/dL in the control group to 42.7 ± 3.2 µg/dL in stressed animals—a 209% increase ($p<0.001$). Corticosterone also rose sharply, climbing from 2.1 ± 0.3 µg/dL to 5.9 ± 0.6 µg/dL, which represents a 181% increase ($p<0.001$).

These rapid changes clearly point to an intense activation of the stress response system. It is evident that cortisol had the greatest change, while the change in corticosterone was more variable, as indicated by its larger error margins. It is noteworthy that these two hormones increased by such large amounts, which indicates that multiple neuroendocrine pathways were activated, leading to a wide stress response.

It is crucial to note that these changes, especially that of cortisol, which tripled, are beyond those that occur when experiments are conducted with single stressors. It is apparent that there is a synergistic effect of multiple

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environmental challenges that put pressure on the hypothalamic-pituitary-adrenal axis. These differences in response (209% vs. 181%) may be because of the unique functions of these hormones, with that of cortisol being more intense.

These changes in hormonal responses point to a heavy allostatic load that is bound to impact the metabolism, immune response, and reproductive health of the rabbits. (Table 2).

Table-2. Stress Hormone Concentrations in Serum
(All values in µg/dL, mean ± SEM)

Hormone	Control	Stressed	% Change	p-value
Cortisol	13.8 ± 1.1	42.7 ± 3.2	+209%	<0.001
Corticosterone	2.1 ± 0.3	5.9 ± 0.6	+181%	<0.001

3. Reproductive Hormone Profiles

The findings reveal a marked suppression of reproductive hormones in stressed rabbits. Progesterone levels fell sharply, dropping by 64% (from 8.9 ± 0.7 to 3.2 ± 0.4 ng/mL, $p<0.001$), while estradiol declined by 45% (52.3 ± 4.1 to 28.6 ± 3.2 pg/mL, $p<0.01$). Gonadotropins showed a similar pattern: luteinizing hormone (LH) decreased by 59% (2.7 ± 0.3 to 1.1 ± 0.2 mIU/mL, $p<0.001$), and follicle-stimulating hormone (FSH) dropped by 44% (4.1 ± 0.4 to 2.3 ± 0.3 IU/L, $p<0.01$).

The pronounced reduction in progesterone highlights a particular vulnerability of the luteal phase, while the parallel declines in LH and FSH point to disruption at the level of the hypothalamic–pituitary–gonadal axis. All changes were statistically robust ($p<0.01$ or better), with progesterone and LH showing the most dramatic suppression. Collectively, these alterations would be expected to severely compromise reproductive capacity (Table 3).

Table 3. Reproductive Hormone Profiles
(Mean ± SEM concentrations)

Hormone	Control	Stressed	Units	p-value
Progesterone	8.9 ± 0.7	3.2 ± 0.4	ng/mL	<0.001
Estradiol	52.3 ± 4.1	28.6 ± 3.2	pg/mL	<0.01
LH	2.7 ± 0.3	1.1 ± 0.2	mIU/mL	<0.001
FSH	4.1 ± 0.4	2.3 ± 0.3	IU/L	<0.01

4. Metabolic Hormone Alterations

Stress had a clear impact on the rabbits' metabolism. The most striking change was in leptin, which dropped by 61% (from 1.8 ± 0.2 to 0.7 ± 0.1 ng/mL, $p<0.001$). This sharp decline signals a severe energy deficit and is especially important because low leptin can disrupt reproductive function by weakening hypothalamic kisspeptin signaling. Insulin also fell by 38% (8.2 ± 0.7 vs. 5.1 ± 0.5 µIU/mL, $p<0.01$), pointing to emerging insulin resistance. Meanwhile, T3 decreased by 22% (0.9 ± 0.1 vs. 0.7 ± 0.1 ng/mL, $p<0.05$), reflecting a slowdown in metabolic activity. Taken together, these reductions in leptin, insulin, and T3 show that the stressed animals entered a coordinated catabolic state, with energy metabolism and nutrient partitioning significantly altered (Table 4).

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Table 4. Metabolic Hormone Alterations

Hormone	Control	Stressed	Units	p-value
Leptin	1.8 ± 0.2	0.7 ± 0.1	ng/mL	<0.001
Insulin	8.2 ± 0.7	5.1 ± 0.5	µIU/mL	<0.01
Triiodothyronine (T3)	0.9 ± 0.1	0.7 ± 0.1	ng/mL	<0.05

V. Discussion

4.1 Physiological and Behavioral Stress Responses

The physiological parameters used in this study provide valuable information regarding how rabbits cope with multiple environmental stressors. It is known that body weight is one of the most fundamental physiological parameters of metabolic status; when body weight is stable, it is considered that adaptation has been successfully achieved (Gidenne et al., 2012). In our study, it was found that rabbits under stress conditions lost 15.3% of body weight, which is more than that found by Marai et al. (2002) under single stress conditions (8-10% body weight loss). It is believed that voluntary feed intake is reduced by 30% and that energy expenditure is increased because of thermoregulatory demands, which is confirmed by an increase of 75% in water consumption (210 vs. 120 mL/kg/day), which is a well-known phenomenon in lagomorphs (Lebas, 2004).

Thermoregulation in rabbits is also compromised as they have fewer sweat glands, relying mainly on ear vasodilation for heat dissipation (Zeferino et al., 2013). In our study, a rise in body temperature of 1.2°C (from 39.1°C to 40.3°C) was noted, which is higher than that seen in heat stress alone (0.8°C; Marai et al., 2008). This is a critical factor as reproductive performance is compromised when body temperatures rise above 39.5°C, with conception rate reduced by 5% for every 0.5°C above this (Theau Clément et al., 2015).

The respiration rate almost doubled (112 vs. 56 breaths/min), which indicates that rabbits were trying to compensate for heat loss through evaporation. This is in line with previous reports of heat stress-induced respiration rate (Finzi, 2005), although this mechanism is energetically expensive for rabbits.

4.2 Glucocorticoid Activation and Stress Response

The endocrine stress response, as seen in our study, indicates a complex neuroendocrine response to chronic stress challenges. Cortisol, which is a major component of the class of steroids known as glucocorticoids in mammals, increased by 209% (42.7 vs. 13.8 µg/dL in controls), which is a much higher percentage increase compared to those seen in studies that utilized a single type of stress challenge, as seen in rabbit stress physiology studies done by Rebollar et al. (2014). This much higher increase in cortisol levels is most likely a result of the synergistic effect of the HPA axis, which is a key component of the cumulative stress model that was originally described by Moberg (2000), as seen in his seminal paper on biological stress response. This much higher increase in cortisol levels indicates that rabbits have a lower threshold for HPA axis activation compared to other domesticated species when exposed to a cumulative effect of environmental challenges.

Our results also highlight the unique characteristic of the corticosteroid response in lagomorphs. Though corticosterone is a secondary glucocorticoid in most mammalian species, the 181% increase in the hormone level in our study (5.9 vs. 2.1 µg/dL) confirms its importance in rabbit stress biology, as first revealed by Touma and Palme (2005). The simultaneous increase in the levels of both cortisol and corticosterone is responsible for the additive effect on reproductive suppression through different pathways. At the hypothalamic level, the activity of glucocorticoid receptors is responsible for the inhibition of GnRH activity, as revealed by Dallman et al. (2003). At the level of the

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pituitary gland, the increase in the level of glucocorticoids is responsible for the reduced sensitivity of the gonadotropes to GnRH stimulation, a fact investigated by Dobson et al. (2003). In addition, ovarian steroidogenesis is also directly affected through the inhibition of the activity of key enzymes through the glucocorticoid receptor, as revealed by Michael et al. (2003).

4.3 Reproductive Hormone Suppression and Fertility Impacts

The reproductive hormone profile in this study demonstrates a significant disruption in the hypothalamic-pituitary-gonadal (HPG) axis in the presence of a chronic stress environment (Rebollar et al., 2014; Theau-Clément et al., 2015). The most interesting result was the dramatic drop of 64% in the concentration of progesterone, decreasing from 8.9 ng/mL in the control group of rabbits to as low as 3.2 ng/mL in the rabbits subjected to a chronic stress environment. This drop in progesterone concentration was more dramatic than the 40-50% drop in dairy cows subjected to heat stress environments (Wolfenson et al., 2000). This demonstrates a particular vulnerability in the reproductive system of rabbits. The extreme sensitivity of the rabbit luteal function in the face of environmental stressors could be attributed to their unique reproductive biology. The fact that rabbits display an induced ovulation process (Fortun-Lamothe, 2006) and a short luteal phase duration (Pérez-Marín et al., 2009) could be factors in this extreme sensitivity. Unlike other species that display spontaneous ovulation and rely on other factors for their reproductive process, rabbits rely entirely on coitus-induced neuroendocrine signals for their reproductive process (The

The simultaneous inhibition of LH (59% reduction) and FSH (44% reduction) release indicates that there is a central effect on gonadotropin release at the hypothalamic-pituitary axis (Rivier & Rivest, 1991). These results are consistent with well-established models of glucocorticoid-induced inhibition of the HPG axis (Whirledge & Cidlowski, 2013). In these models, elevated cortisol levels disrupt various components of the reproductive axis. In the hypothalamus, glucocorticoids reduce GnRH neuron activity (Kinsey-Jones et al., 2008). In the pituitary gland, glucocorticoids reduce responsiveness to GnRH stimulation (Breen et al., 2005). In addition, glucocorticoids induce changes in gene expression patterns within gonadotrope cells (Oakley et al., 2009).

Notably, the ovarian steroidogenic response exhibited compartment-specific effects. Estradiol was reduced by 45%, in contrast to the 64% reduction in progesterone. These data may indicate different sensitivities of ovarian cell types. The granulosa cell compartment may still harbor partial protective mechanisms, possibly through local growth factor-mediated signaling (Sirotkin, 2010), which could explain the maintenance of aromatase activity and subsequent estradiol production. Conversely, the corpus luteum appears to be a highly sensitive compartment following stress-mediated tissue damage (Agarwal et al., 2012). This may be explained by the high metabolic activity of the corpus luteum and its susceptibility to oxidative stress. The theca cell layer may also represent a sensitive compartment because the production of androgens is required for the subsequent production of estradiol (Shi et al., 2018).

These hormonal alterations are accompanied by considerable functional impairments during the entire process. The ultrasonographic monitoring indicated that there are considerable declines in preovulatory follicular development (Rommers et al., 2014). In vitro studies showed that oocyte quality and development are impaired (Sánchez et al., 2023). In practice, only 68% of induced LH surges resulted in ovulation among stressed does, while 92% succeeded in controls (Theau-Clément et al., 2020). The high percentage of ovulation failure indicates that chronic stress has a considerable effect on basic reproductive processes.

4.4 Metabolic Consequences and Energy Regulation

The profile of metabolic hormones in rabbits that were subjected to stress indicates that there was a significant catabolic response, which was associated with marked changes in the levels of key regulatory hormones. Among the most significant changes was the 61% fall in the levels of leptin, from 1.8 ng/mL in the normal rabbits to 0.7 ng/mL in the rabbits that were subjected to stress. This significant fall in the levels of leptin, or hypoleptinemia, indicates a significant change in the energy balance and serves as an important biomarker of the impact of the combined effects

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of environmental stressors on the metabolic system of rabbits. The impact of the suppression of leptin levels extends to multiple physiological processes. In the hypothalamus, there is a suppression of kisspeptin, which affects the pulsatile release of GnRH that is essential for reproductive processes (Smith et al., 2006). At the same time, there was significant suppression of the thyroid axis, indicated by a 22% fall in T3 levels, which suggests that there were significant adaptations to conserve energy in response to stress (Blake et al., 1991; Silva, 2006).

Collectively, these hormonal changes represent a coordinated metabolic response to chronic stress, in accordance with the 'adaptive thermogenesis' hypothesis, in which the conservation of energy is prioritized over non-essential physiological processes such as reproduction (Silva, 2006; López-Luna et al., 2021). The reduction in insulin levels by 38% in conjunction with the reduction in leptin and thyroid hormone levels indicates the onset of a metabolically resistant state, thereby exacerbating the poor state of the subjects (González-Mariscal et al., 2010; Rebollar et al., 2014). This represents a vicious cycle in that the metabolic changes induced by stress result in inefficient nutrient metabolism, thereby enhancing the effects of stress. This cycle is detrimental to the subjects' health. From the cellular perspective, the liver displays decreased insulin sensitivity, and the muscles display decreased glucose uptake potential, both of which contribute to inefficient metabolism (González-Mariscal et al., 2010).

These metabolic changes have particularly severe implications for reproductive function in rabbits. The low leptin and insulin levels create an unfavorable endocrine environment for follicular development and ovarian steroidogenesis (Sirotkin, 2010; Theau-Clément et al., 2015). The hypothalamus receives conflicting signals about the energy balance and responds inappropriately in regulating appetite and energy expenditure (Friedman-Einat et al., 2014). At the same time, the suppressed thyroid axis contributes to a generalized slowing of all the body's metabolic processes, resulting in a reduction in the energy available for reproduction (Ortiga-Carvalho et al., 2023). This explains the reproductive dysfunction in stressed rabbits and points to a particularly sensitive reproductive system in rabbits in response to changes in their energy balance and metabolic status (Fortun-Lamothe, 2006; Zeferino et al., 2013).

5. Conclusions and Future Directions

This study proves that rabbits subjected to combined environmental stresses, including heat, crowding, and feed restriction, experience severe physiological disruption via three major mechanisms: (1) hyperactivation of glucocorticoid signaling, as evidenced by a 209% increase in cortisol; (2) disproportionate inhibition of reproductive hormone secretion, with a 64% reduction in progesterone; and (3) metabolic disruption, as indicated by a 61% reduction in leptin. These physiological consequences of combined stresses were found to be more severe compared to those subjected to individual stresses, thus proving that rabbits experience synergistic detrimental effects when exposed to multiple environmental stresses. These results underscore the pressing need to develop effective rabbit management strategies to ensure their welfare and productivity. Future studies should be directed toward developing effective strategies to mitigate stresses in rabbits, as well as exploring their genetic resilience to ensure sustainability in rabbit production systems.

Future research should investigate:

- Genetic selection for stress resilience
- Hormonal supplementation strategies
- Precision environmental control systems
- Early biomarkers of stress susceptibility

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