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Modulating the Stress Axis and Cellular Thermotolerance in Shami Goat Bucks: Dietary Ginseng Powder as a Strategy to Enhance Climatic Resilience

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ABSTRACT

The Shami goat breed represents an economically important livestock resource in the Middle East, yet environmental stressors and intensive management systems can compromise reproductive performance through dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Ginseng (Panax ginseng C.A. Meyer), recognized for its adaptogenic properties, has shown promise in modulating stress responses and cellular protective mechanisms. This study investigated the effects of dietary ginseng powder supplementation on cortisol, adrenocorticotropic hormone (ACTH), heat shock proteins (HSP70 and HSP90), and reproductive performance parameters in Shami goat bucks. Twenty-four mature Shami bucks (2-3 years old, 45-55 kg body weight) were randomly allocated to four treatment groups (n=6 per group): control (basal diet), and three ginseng supplementation levels (1, 2, and 3 g/kg diet) for 90 days. Blood samples were collected bi-weekly for hormonal and HSP analysis. Semen quality, testicular measurements, and libido scores were evaluated throughout the experimental period. Ginseng supplementation demonstrated dose-dependent effects on HPA axis regulation. The 2 g/kg treatment group showed significantly reduced serum cortisol (32.4 ± 2.1 ng/mL vs. 48.7 ± 3.4 ng/mL in control, $P < 0.01$) and ACTH levels (28.6 ± 1.8 pg/mL vs. 42.3 ± 2.9 pg/mL in control, $P < 0.01$). HSP70 expression increased by 47% ($P < 0.05$) while HSP90 showed moderate elevation (23%, $P<0.05$) in the optimal treatment group. Sperm concentration improved from $2.8 \pm 0.3 \times 10^9$/mL (control) to $3.9 \pm 0.2 \times 10^9$/mL (2 g/kg group, $P < 0.01$), with corresponding improvements in motility (68.4 ± 3.2% vs. 79.8 ± 2.1%, $P<0.01$) and morphology.

1.Introduction

The intensification of goat production systems, coupled with the increasing frequency of extreme weather events, places significant stress on breeding animals, with bucks being particularly vulnerable. The economic viability of a herd is intrinsically linked to the reproductive performance of its sires, which is highly sensitive to environmental stressors [1]. In ruminants, stress activates the hypothalamic-pituitary-adrenal (HPA) axis, culminating in the secretion of cortisol. While adaptive in the short term, chronic elevation of glucocorticoids is detrimental, leading to suppressed libido, reduced semen quality, and overall metabolic strain. Concurrently, at the cellular level, stress triggers the expression of heat shock proteins (HSPs), such as Hsp70 and Hsp90, which function as molecular chaperones to protect proteins from denaturation [2]. However, a perpetually high HSP expression indicates significant cellular distress and can divert energy from productive processes, including reproduction [3].

For the food industry, mitigating these stress-induced declines is crucial for maintaining a consistent and high-genetic-merit supply of animal protein. While management solutions exist, there is a growing imperative to find natural, feed-based interventions that enhance an animal's intrinsic resilience [4]. In this context, adaptogenic herbs offer a promising avenue. *Panax ginseng*, one of the most renowned adaptogens, has been used for centuries in human medicine to bolster resistance to physical, chemical, and biological stressors [5]. Its bioactive components, ginsenosides, have been shown to exert modulatory effects on the neuroendocrine system, potentially blunting the HPA axis over-activation, and possess antioxidant properties that may synergize with cellular stress response pathways [6].

Despite this compelling pharmacological profile, the application of ginseng in livestock nutrition, particularly for managing stress in elite breeding bucks, remains largely unexplored [7]. The existing literature has primarily focused on growth performance or general health in production animals, with a critical gap in understanding its mechanistic action on the core stress physiology of sires. We hypothesize that dietary supplementation with ginseng powder will exert a dual beneficial effect: (1) modulating the HPA axis to reduce circulating cortisol levels, and (2) optimizing the expression of key heat shock proteins, thereby enhancing cellular thermotolerance without the associated energy penalty of chronic stress [8]. This study therefore aims to investigate the effect of ginseng powder supplementation on the HPA axis activity, through plasma cortisol measurement, and the expression of Hsp70 and Hsp90 in the blood lymphocytes of Shami goat bucks. The findings will provide a scientific foundation for using ginseng as a novel nutritional strategy to improve the welfare and reproductive robustness of breeding stock, thereby securing the efficiency and sustainability of meat and breeding animal supply chains.

2.Materials and Methods

Animals and Experimental Design

Twenty-four clinically healthy, sexually mature Shami goat bucks aged 2-3 years with body weights ranging from 45-55 kg were selected based on physical examination, absence of reproductive abnormalities, and baseline semen quality assessment (progressive motility >70%, normal morphology >80%). Animals were stratified by body weight and randomly allocated to four experimental groups (n = 6 per group) using a completely randomized design:

- **Group 1 (Control):** Basal diet without supplementation
- **Group 2 (G1):** Basal diet + 1 g ginseng powder/kg diet
- **Group 3 (G2):** Basal diet + 2 g ginseng powder/kg diet
- **Group 4 (G3):** Basal diet + 3 g ginseng powder/kg diet

The experimental period comprised a 14-day adaptation phase followed by a 90-day treatment period, consistent with recommendations for studying reproductive responses in male goats [9].

Dietary Treatments and Ginseng Preparation

The basal diet was formulated to meet nutritional requirements for breeding bucks according to NRC (2007) recommendations, consisting of alfalfa hay (40%), barley grain (35%), soybean meal (15%), wheat bran (8%), and mineral-vitamin premix (2%), providing 12.5 MJ/kg metabolizable energy and 14.5% crude protein on dry matter basis. Chemical composition was analyzed according to AOAC (2019) methods [10]. Standardized Korean red ginseng (*Panax ginseng* C.A. Meyer) root powder, containing ≥4.5% total ginsenosides (verified by HPLC), was procured from a certified supplier (Geumsan Ginseng Cooperative, South Korea). Ginsenoside profile analysis confirmed the presence of major saponins: Rb1 (1.2%), Rg1 (0.9%), Re (0.7%), and Rd (0.6%). The ginseng powder was thoroughly mixed with concentrate ingredients daily before feeding to ensure homogeneous distribution. Feed was offered twice daily (08:00 and 16:00 h) with ad libitum access to fresh water. Daily feed intake was recorded, and body weight was measured bi-weekly.

Blood Sample Collection and Processing

Blood samples (15 mL) were collected via jugular venipuncture into non-heparinized vacuum tubes at 14-day intervals throughout the experimental period. All samplings occurred at 09:00 h to minimize circadian variation in hormone concentrations. Blood was allowed to clot at room temperature (30 min), then centrifuged at 3,000 × g for 15 min at 4°C. Serum was aliquoted into cryovials and stored at -80°C until analysis.

Hormonal Assays

Cortisol Analysis

Serum cortisol concentrations were quantified using a commercially available competitive enzyme-linked immunosorbent assay (ELISA) kit (Cortisol ELISA Kit, Demeditec Diagnostics GmbH, Germany) validated for caprine samples. The assay sensitivity was 2.5 ng/mL, with intra-assay and inter-assay coefficients of variation (CV) of 4.2% and 7.8%, respectively. All samples were analyzed in duplicate, and absorbance was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific).

ACTH Measurement

Adrenocorticotropic hormone was measured using a solid-phase, two-site sequential chemiluminescent immunometric assay (Immulite 2000 ACTH, Siemens Healthcare Diagnostics). Samples were processed according to manufacturer instructions with analytical sensitivity of 5 pg/mL and functional sensitivity of 9 pg/mL. Intra-assay and inter-assay CVs were 5.3% and 8.9%, respectively.

Heat Shock Protein Analysis

Peripheral Blood Mononuclear Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples using density gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare). Cell viability (>95%) was confirmed by trypan blue exclusion. Cells were washed twice with phosphate-buffered saline (PBS) and pelleted by centrifugation.

Protein Extraction and Western Blot Analysis

Total protein was extracted using RIPA buffer supplemented with protease inhibitor cocktail (Sigma-Aldrich). Protein concentration was determined by Bradford assay. Equal amounts of protein (40 μg) were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk in Tris-buffered saline with Tween-20 (TBST) for 1 h at room temperature, then incubated overnight at 4°C with primary antibodies: anti-HSP70 (1:1000, Cell Signaling Technology, #4872), anti-HSP90 (1:1000, Cell Signaling Technology, #4877), and anti-β-actin (1:2000, loading control). After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000) for 2 h. Protein bands were visualized using enhanced chemiluminescence (ECL) substrate and quantified by densitometry using ImageJ software (NIH). Results were normalized to β-actin expression.

Semen Collection and Evaluation

Semen Collection

Semen was collected bi-weekly using an artificial vagina (42°C) with teaser females. Two consecutive ejaculates were obtained from each buck with a 30-minute interval, and the second ejaculate was used for analysis to ensure consistency [11].

Semen Quality Assessment

- **Volume:** Measured using graduated collection tubes
- **Concentration:** Determined using a hemocytometer (Neubauer chamber) after dilution in formal-citrate solution
- **Mass motility:** Evaluated on a warm stage (37°C) using a 0-5 scale
- **Progressive motility:** Assessed using computer-assisted sperm analysis (CASA, IVOS II, Hamilton Thorne) analyzing ≥500 spermatozoa per sample
- **Morphology:** Evaluated in eosin-nigrosin stained smears, examining 200 spermatozoa per sample under 1000× magnification
- **Viability:** Determined by eosin-nigrosin staining (live sperm remain unstained)

Testicular Measurements and Libido Assessment

Scrotal circumference was measured at the widest point using a flexible measuring tape at 28-day intervals. Testicular volume was calculated using the formula: Volume = $4/3\pi \times (\text{length}/2) \times (\text{width}/2)^2$. Libido was evaluated using a standardized scoring system (0-10 scale) based on reaction time to female, mounting attempts, and sustained sexual interest during a 10-minute observation period with an estrus-synchronized female [12].

Statistical Analysis

Data were analyzed using SAS software (version 9.4, SAS Institute Inc.). Normality was assessed using Shapiro-Wilk tests, and homogeneity of variance was verified by Levene's test. Repeated measures data (hormones, semen parameters) were analyzed using mixed model ANOVA with treatment, time, and their interaction as fixed effects, and animal as a random effect. Post-hoc comparisons among treatment groups were performed using Tukey's HSD test. Correlation analyses between HPA hormones, HSPs, and reproductive parameters were conducted using Pearson correlation coefficients. Dose-response relationships were evaluated using polynomial regression. Statistical significance was declared at $P < 0.05$, with trends noted at $0.05 \leq P < 0.10$. Results are presented as least squares means ± standard error of the mean (SEM).

3.Results

Feed Intake and Body Weight

No significant differences were observed in dry matter intake among treatment groups throughout the experimental period (Table 1). Average daily feed consumption ranged from 1.42 to 1.48 kg DM/day ($P = 0.64$). Body weight gain showed a positive trend in ginseng-supplemented groups, with the G2 group achieving numerically higher final body weights compared to control (52.8 ± 1.4 vs. 50.2 ± 1.3 kg, $P = 0.08$), though differences did not reach statistical significance.

Table 1. Feed Intake and Body Weight Parameters (ADG/DMI)

Parameter	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
Initial BW (kg)	49.8	50.1	49.6	50.3	1.2	0.95
Final BW (kg)	50.2^{ab}	51.4^{ab}	52.8^{a}	51.9^{ab}	1.4	0.08
ADG (g/day)	44.4^{b}	64.4^{ab}	88.9^{a}	72.2^{ab}	12.3	0.04
DMI (kg/day)	1.44	1.46	1.48	1.42	0.08	0.64
Feed efficiency	0.031^{b}	0.044^{ab}	0.060^{a}	0.051^{ab}	0.009	0.03

BW = body weight; ADG = average daily gain; DMI = dry matter intake. Different superscripts within rows indicate significant differences (P < 0.05).

Serum Cortisol Concentrations

Ginseng supplementation significantly reduced serum cortisol levels in a dose-dependent manner (treatment × time interaction: $P < 0.01$). By day 60, the G2 group exhibited the lowest cortisol concentrations (32.4 ± 2.1 ng/mL) compared to control (48.7 ± 3.4 ng/mL), representing a 33.5% reduction ($P < 0.01$). The G1 and G3 groups showed intermediate reductions of 18.7% and 26.3%, respectively (Table 2).

Table 2. Serum Cortisol Concentrations (ng/mL) Throughout Experimental Period

Day	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
0	47.2	46.8	47.5	46.9	2.8	0.99
14	48.3^{a}	44.6^{ab}	42.1^{b}	43.8^{ab}	2.4	0.04
28	49.1^{a}	42.8^{b}	38.7^{c}	40.2^{bc}	2.2	<0.01
42	48.5^{a}	41.3^{b}	35.9^{c}	38.1^{bc}	2.1	<0.01
60	48.7^{a}	39.6^{b}	32.4^{c}	35.9^{bc}	2.1	<0.01
90	47.9^{a}	38.8^{b}	31.8^{c}	34.7^{c}	2.0	<0.01

Different superscripts within rows indicate significant differences (P < 0.05).

Plasma ACTH Levels

ACTH concentrations paralleled cortisol responses, with significant treatment effects evident from day 28 onwards ($P < 0.01$). The G2 group demonstrated optimal ACTH suppression, achieving 32.4% lower concentrations than control by day 90 (28.6 ± 1.8 vs. 42.3 ± 2.9 pg/mL, $P < 0.01$). Quadratic regression analysis revealed an optimal dose-response relationship, with maximal ACTH reduction occurring at 2.1 g/kg ginseng supplementation ($R^2 = 0.89$, $P < 0.001$).

Table 3. Plasma ACTH Concentrations (pg/mL) Throughout Experimental Period

Day	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
0	41.8	42.1	41.6	42.3	2.6	0.98
14	42.5^{a}	39.7^{ab}	37.2^{b}	38.9^{ab}	2.3	0.06
28	42.9^{a}	37.4^{b}	33.8^{c}	35.6^{bc}	1.9	<0.01
42	42.6^{a}	36.2^{b}	31.4^{c}	33.7^{bc}	1.8	<0.01
60	42.8^{a}	35.6^{b}	29.7^{c}	32.1^{bc}	1.9	<0.01
90	42.3^{a}	34.9^{b}	28.6^{c}	31.4^{bc}	1.8	<0.01

Different superscripts within rows indicate significant differences (P < 0.05).

Heat Shock Protein Expression

Western blot analysis revealed significant upregulation of both HSP70 and HSP90 in ginseng-supplemented groups (Figure 1, Table 4). HSP70 expression exhibited the most pronounced response, increasing by 47.3% in the G2 group relative to control at day 90 ($P < 0.05$). HSP90 showed moderate but significant elevation (23.1% in G2 group, $P < 0.05$). The G3 group demonstrated slightly attenuated HSP responses compared to G2, suggesting a plateau or mild inhibitory effect at higher supplementation levels.

Table 4. Relative Heat Shock Protein Expression (Fold Change Relative to Control)

Parameter	Day	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
HSP70	30	1.00	1.18[b]	1.32[a]	1.24[ab]	0.08	0.02
	60	1.00	1.26[b]	1.43[a]	1.35[ab]	0.09	0.01
	90	1.00	1.29[b]	1.47[a]	1.38[ab]	0.09	<0.01
HSP90	30	1.00	1.09	1.16[a]	1.12[ab]	0.05	0.08
	60	1.00	1.12[b]	1.21[a]	1.17[ab]	0.05	0.04
	90	1.00	1.14[b]	1.23[a]	1.19[ab]	0.06	0.03

Different superscripts within rows indicate significant differences (P < 0.05).

Semen Quality Parameters

Ginseng supplementation substantially improved multiple semen quality characteristics (Table 5). Sperm concentration increased progressively in supplemented groups, with the G2 group achieving 39.3% higher concentration than control by day 90 (3.9 ± 0.2 vs. $2.8 \pm 0.3 \times 10^9$/mL, $P < 0.01$). Progressive motility improved from 68.4 ± 3.2% in control to 79.8 ± 2.1% in G2 group ($P < 0.01$). Normal sperm morphology exhibited dose-dependent enhancement, reaching 87.6 ± 1.4% in G2 compared to 79.3 ± 2.1% in control ($P < 0.01$).

Table 5. Semen Quality Parameters at Day 90

Parameter	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
Volume (mL)	1.04	1.12	1.18	1.14	0.09	0.32
Concentration ($\times 10^9$/mL)	2.8[c]	3.3[b]	3.9[a]	3.6[ab]	0.2	<0.01
Mass motility (0-5)	3.2[c]	3.8[b]	4.3[a]	4.0[ab]	0.2	<0.01
Progressive motility (%)	68.4[c]	74.2[b]	79.8[a]	76.9[ab]	2.1	<0.01
Normal morphology (%)	79.3[c]	83.7[b]	87.6[a]	85.4[ab]	1.4	<0.01
Viability (%)	82.1[b]	85.9[ab]	88.7[a]	86.8[ab]	1.8	0.02
Abnormal heads (%)	8.6[a]	6.9[b]	5.2[c]	6.1[bc]	0.7	<0.01
Abnormal midpiece (%)	7.2[a]	5.8[b]	4.3[c]	5.1[bc]	0.6	<0.01
Abnormal tail (%)	4.9[a]	3.6[b]	2.9[b]	3.4[b]	0.5	0.01

Different superscripts within rows indicate significant differences (P < 0.05).

Testicular Measurements and Libido

Scrotal circumference increased significantly in ginseng-supplemented groups, with the G2 group showing 8.7% greater circumference than control at day 90 (29.8 ± 0.6 vs. 27.4 ± 0.7 cm, $P < 0.01$). Calculated testicular volume paralleled these changes. Libido scores improved progressively, with the G2 group achieving scores of 8.4 ± 0.3 compared to 6.8 ± 0.4 in control ($P < 0.01$), indicating enhanced sexual behavior and reduced reaction time.

Table 6. Testicular Measurements and Libido Scores

Parameter	Control	G1 (1 g/kg)	G2 (2 g/kg)	G3 (3 g/kg)	SEM	P-value
Scrotal circumference (cm)	27.4[c]	28.6[b]	29.8[a]	29.1[ab]	0.6	<0.01
Testicular volume (cm^3)	312.4[c]	341.7[b]	368.9[a]	352.3[ab]	12.8	<0.01
Libido score (0-10)	6.8[c]	7.6[b]	8.4[a]	8.0[ab]	0.3	<0.01
Reaction time (sec)	48.3[a]	36.7[b]	28.4[c]	32.1[bc]	3.6	<0.01

Different superscripts within rows indicate significant differences (P < 0.05).

4.Discussion

Ginseng Modulation of the HPA Axis

The present study demonstrates that dietary ginseng powder supplementation exerts dose-dependent inhibitory effects on HPA axis activity in Shami goat bucks, as evidenced by significant reductions in both ACTH and cortisol concentrations. These findings align with previous research in rodent models demonstrating ginsenoside-mediated attenuation of stress-induced HPA activation [13,14]. The optimal dose of 2 g/kg achieved 33.5% reduction in cortisol levels, comparable to results reported by Zeng et al. (2020) who observed 28-35% cortisol suppression in heat-stressed dairy cattle receiving 1.5-2.5 g/kg ginseng extract [15].

The mechanisms underlying ginseng's HPA-modulatory effects are multifaceted and involve several molecular pathways. Ginsenoside Rb1, the predominant saponin in the supplemented ginseng powder (1.2% composition), has been shown to inhibit corticotropin-releasing hormone (CRH) expression in hypothalamic paraventricular nucleus neurons through activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and suppression of nuclear factor-kappa B (NF-κB) signaling [16]. Furthermore, ginsenoside Rg1 demonstrates glucocorticoid receptor antagonistic properties, competing with cortisol for receptor binding in target tissues and thereby attenuating negative feedback dysregulation that characterizes chronic stress states [17].

The strong correlation between ACTH and cortisol (r = 0.84) observed in this study confirms the integrated nature of HPA axis regulation and suggests that ginseng's primary mechanism involves central (hypothalamic-pituitary) rather than purely adrenal effects. This interpretation is supported by recent work demonstrating that ginsenosides modulate hypothalamic glucocorticoid receptor sensitivity and enhance negative feedback efficiency [18]. The quadratic dose-response relationship, with optimal effects at 2 g/kg and slightly diminished benefits at 3 g/kg, may reflect biphasic hormetic effects characteristic of many phytochemicals, wherein moderate doses optimize adaptive responses while excessive concentrations trigger compensatory counter-regulation [19].

In the context of small ruminant production, chronic HPA axis activation represents a significant welfare and productivity challenge, particularly in intensive systems characterized by high stocking densities, frequent handling, and thermal stress [20]. Elevated cortisol impairs immune function, promotes protein catabolism, and suppresses reproductive neuroendocrine function through multiple mechanisms including inhibition of GnRH pulsatility and direct testicular effects [21]. The magnitude of cortisol reduction achieved in this study (32.4 ng/mL in G2 vs. 48.7 ng/mL in control) represents a clinically meaningful improvement likely to translate into enhanced physiological resilience and productive performance.

Heat Shock Protein Upregulation and Cytoprotection

The significant upregulation of HSP70 (47.3% increase) and HSP90 (23.1% increase) in ginseng-supplemented bucks represents a novel finding with important implications for cellular stress resistance and reproductive function. Heat shock proteins function as molecular chaperones essential for maintaining proteostasis, preventing protein aggregation, and facilitating proper folding of nascent polypeptides under both physiological and stress conditions [22]. In testicular tissue, HSPs are particularly crucial for protecting developing germ cells against oxidative damage, thermal stress, and apoptotic signals that compromise spermatogenesis [23]. The differential response magnitudes between HSP70 and HSP90 (47% vs. 23% upregulation) reflect their distinct regulatory mechanisms and cellular functions. HSP70 induction is primarily mediated through heat shock factor-1 (HSF-1) activation, which ginsenosides promote through multiple pathways including enhanced HSF-1 trimerization, nuclear translocation, and DNA-binding activity [24]. Recent research by Wang et al. (2021) demonstrated that ginsenoside Rg3 directly activates HSF-1 through protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) signaling cascades, leading to robust HSP70 transcription. Conversely, HSP90 expression is constitutively high in most cells and shows more modest stress-induced elevation, consistent with our observations [25].

The strong positive correlation between HSP70 expression and sperm quality parameters (concentration: r = 0.65; motility: r = 0.59; morphology: r = 0.58) supports a mechanistic link between cellular cytoprotection and reproductive outcomes. HSP70 protects spermatogenic cells against oxidative stress through multiple mechanisms including direct antioxidant effects, stabilization of mitochondrial membrane potential, inhibition of apoptotic signaling, and enhancement of DNA repair mechanisms [26]. In Sertoli cells, HSP70 facilitates proper folding of androgen receptors and steroidogenic enzymes, thereby supporting testosterone biosynthesis essential for spermatogenesis [22].

Recent proteomic analyses have identified HSP90 as a critical regulator of steroid hormone receptor function, including androgen receptor (AR) maturation and stabilization [27]. The modest but significant HSP90 upregulation observed in this study may contribute to enhanced androgen signaling in testicular tissue, complementing the direct steroidogenic effects reported for certain ginsenosides [28]. Furthermore, HSP90 interacts with numerous client proteins involved in cell cycle regulation, germ cell differentiation, and meiotic progression, suggesting pleiotropic benefits for spermatogenic efficiency [30]. The activation of HSP expression by ginseng appears to involve both direct transcriptional mechanisms and indirect effects mediated through oxidative stress reduction. Ginsenosides activate nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of antioxidant response elements (ARE) that controls expression of numerous cytoprotective genes including HSPs, glutathione-related enzymes, and superoxide dismutase [31]. This coordinated upregulation of cellular defense systems likely contributes to the observed improvements in sperm quality and testicular function.

Reproductive Performance Enhancement

The comprehensive improvements in semen quality parameters observed in ginseng-supplemented bucks—including 39.3% increased sperm concentration, 16.7% improved progressive motility, and 10.5% enhanced normal morphology—represent biologically and economically significant enhancements in reproductive capacity. These findings extend previous research in other species, including poultry, swine [32], and laboratory animals, demonstrating substantial reproductive benefits in small ruminants for the first time reproductive benefits in small ruminants [33].

The mechanisms underlying these improvements are likely multifactorial, involving coordinated effects on neuroendocrine regulation, testicular steroidogenesis, spermatogenic cell protection, and epididymal sperm maturation. The strong negative correlation between cortisol and sperm concentration (r = -0.72) suggests that HPA axis suppression contributes significantly to improved spermatogenic efficiency. Chronic glucocorticoid elevation suppresses gonadotropin secretion through inhibition of hypothalamic GnRH neurons and direct pituitary effects, reducing circulating LH and FSH concentrations essential for testicular function [34]. Additionally, cortisol exerts direct inhibitory effects on testicular Leydig cell steroidogenesis and Sertoli cell support function, impairing both testosterone production and germ cell nurturing [21]. Beyond HPA modulation, ginsenosides demonstrate direct gonadotropic and steroidogenic effects. Research by Leung and Wong (2021) demonstrated that ginsenoside Rg1 enhances testicular steroidogenic acute regulatory protein (StAR) expression and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity, key enzymes in testosterone biosynthesis. Furthermore, ginsenosides stimulate nitric oxide (NO) production in testicular tissue through endothelial NO synthase (eNOS) activation, improving microcirculation and oxygen delivery to developing germ cells [35]. The 8.7% increase in scrotal circumference and corresponding testicular volume expansion observed in the G2 group likely reflects enhanced Sertoli cell proliferation and seminiferous tubule development, supported by improved hormonal milieu and vascular perfusion. The improvements in sperm morphology, particularly the reductions in head (39.5%), midpiece (40.3%), and tail (40.8%) abnormalities, suggest protective effects during spermatogenesis and epididymal maturation. Oxidative stress during spermiogenesis represents a major cause of morphological defects, as DNA packaging, chromatin condensation, and cytoplasmic elimination processes are highly vulnerable to reactive oxygen species (ROS) damage. The antioxidant properties of ginsenosides, mediated through Nrf2 activation and direct ROS scavenging, likely contribute to the observed morphological improvements [36].

Progressive motility enhancement (from 68.4% to 79.8%) reflects improved sperm energy metabolism and flagellar function. Mitochondrial dysfunction represents a primary cause of asthenozoospermia, and ginsenosides have been shown to enhance mitochondrial biogenesis through AMPK-PGC-1α signaling and improve ATP production efficiency [37]. Additionally, ginsenosides protect mitochondrial DNA from oxidative damage and stabilize electron transport chain complexes, preserving energy generation capacity during epididymal transit and ejaculation [38].

5. Conclusions

This study provides compelling evidence that dietary ginseng powder serves as an effective nutritional modulator of systemic and cellular stress responses in Shami goat bucks. Our results demonstrate a significant downregulation of HPA axis activity in supplemented bucks, as indicated by markedly lower circulating cortisol levels compared to the control group. This attenuation of the primary glucocorticoid response suggests that ginseng's adaptogenic properties help maintain physiological homeostasis, preventing the detrimental effects of chronic stress that are known to compromise metabolic health and reproductive function. Concurrently, ginseng supplementation led to a nuanced and beneficial modulation of the heat shock protein response. We observed a significant increase in the expression of Hsp70 and Hsp90 in the ginseng group. Crucially, we interpret this not as an induction of stress, but as an enhancement of pre-emptive cellular defense mechanisms. The bioactive ginsenosides appear to "prime" the cellular chaperone system, enabling a more robust protection of proteostasis upon encountering a stressor. This elevated baseline preparedness likely contributes to improved thermotolerance and cellular integrity, which is paramount for maintaining the viability of spermatozoa and other critical physiological functions. For the food and breeding industries, these findings translate into a powerful strategy for safeguarding valuable genetic material. By mitigating the endocrine stress response and fortifying cellular resilience, ginseng supplementation can directly contribute to sustaining libido and semen quality in bucks under challenging environmental conditions. This enhances the productivity and lifetime value of elite sires, ensuring a more reliable and efficient genetic pipeline for herd improvement and meat production. In conclusion, the incorporation of ginseng powder into the diet of Shami goat bucks represents a scientifically-supported, natural intervention to enhance climatic resilience. This approach moves beyond mere symptom management to fundamentally improve an animal's adaptive capacity, aligning with the principles of sustainable and welfare-oriented animal agriculture. Future research should focus on correlating these physiological improvements with direct reproductive performance metrics to fully quantify the return on investment for producers.

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