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Valuation of Stress Axis and Cellular Thermotolerance in Shami Goat Bucks, Dietary Ginseng Powder as a strategy to improve Climatic Resilience

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ABSTRACT

Shami goat breed is an economically viable livestock used in the Middle East, but the effects of environmental stressors and aggressive management systems may lead to disruption of reproductive functioning by the dysregulation of hypothalamic-pituitary-adrenal (HPA) axis. Ginseng (Panax ginseng C.A. Meyer) is an adaptogenic herb which has demonstrated possible use in the regulation of responses to stress and cellular defenses. The aim of the study was to examine the impacts of selected dietary ginseng powder supplement on cortisol, adrenocorticotropic hormone (ACTH) and heat shock proteins (HSP70 and HSP90) and the reproductive performance measures in Shami goat bucks. Twenty-four mature Shami bucks (2-3 years old, 45-55 kg body weight) were randomly assigned to four treatment groups (n=6 each group): control (basal diet) and three ginseng levels of supplementation (1, 2 and 3 g/kg diet) during 90 days. Hormonal and HSP analysis of blood was done after every two weeks. The quality of semen, testicular measurements and libido scores were measured during the experimental time. HPA axis effects showed dose- dependent effects of ginseng supplementation.. 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 ± 0.3 × 10^9/mL (control) to 3.9 ± 0.2 × 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

Manufacturing of the goat production systems is also leading to the intensification of the systems, which is supplemented by the growing frequency of extreme weather events, imposing a heavy burden on breeding animals, and bucks are especially susceptible to it. The environment stressors are inherently sensitive to the reproductive performance of herd sires whose economic viability is greatly influenced by reproductive performance [1]. Stress in ruminants causes the activation of hypothalamic-pituitary- adrenal axis leading to the release of cortisol. Although beneficial in the short-term, chronic glucocorticoid increases cause harm and result in a low libido, impaired semen quality, and general metabolic fatigue. Meanwhile, on the cellular scale, a condition of stress leads to the expression of heat shock proteins (HSPs), including Hsp90 and Hsp70 that act as molecular chaperones to preserve protein against denaturation [2]. Nevertheless, a constantly elevated HSP expression suggests that the cells are in a state of serious distress and may cause energy to be diverted to inefficient activities, such as reproduction [3].

In the case of food industry, alleviation of these stress influenced losses is important to retain a steady and high-genetic-merit supply of animal protein. Even though there are management solutions, the need to identify natural and feed-based interventions that bolster the intrinsic resilience of an animal is on the increase [4]. Adaptogenic herbs will be a good prospect in this regard. Panax ginseng is one of the most famous adaptogen and has been in the human medicine thousands of years to help increase resistance to physical, chemical and biological stressors [5]. The ginsenosides, its bioactive constituents, have been described as having modulatory effects on the neuroendocrine system, which may be preventing the over-activation of the HPA axis, and antioxidant effects, which may be additive to cellular adaptive response pathways [6].

Though this is an outstanding pharmacological profile, the use of ginseng in livestock feed especially in stress control in elite breeding bucks has not been widely investigated [7]. The literature has remained mainly on growth performance or overall wellbeing on production animals with a glaring gap on its mechanistic knowledge. intervention on the physiology of sires core stress. Our hypothesis is that dietary supplementation of ginseng powder will have a two-fold positive impact: (1) to adjust the HPA axis to lower the levels of cortisol in the body, (2) to optimize the expression of main heat shock proteins, and accordingly, decrease the thermotolerance of cells without the energy cost of chronic stress [8]. This research therefore seeks to examine the influence of ginseng powder supplement on the HPA axis activity, using plasma cortisol level, and the expression of Hsp70 and Hsp90 in the blood lymphocytes of Shami goat bucks. The results will offer scientific basis on the application of ginseng as an innovative approach to nutrition in the welfare and reproductive fitness of breeding stock to ensure efficiency and sustainability of meat and breeding animal value chain.

2.Materials and Methods

Animals and Experimental Design

The sample size (24) was determined through a physical check up, lack of any reproductive anomalies, and the baseline semen quality of the clinically healthy, sexually mature Shami goat bucks (ages 2-3 years old) with a body weight of 45-55 kg. The animals were randomly assigned to four experimental groups (n= 6/group) by body weight and randomly assigned animals to each group:

- **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 developed based on nutritional needs of breeding bucks as per the recommendation of the NRC (2007) and this diet was composed of alfalfa hay (40%), barley grain (35%), soybean meal (15%), wheat bran (8%), and mineral-vitamin premix (2%), which provided energy requirements of 12.5 MJ/kg as metabolizable energy and crude protein of 14.5 percent on the DM basis. The chemical composition was determined by following AOAC (2019) procedures [10]. Standardized root powder of red ginseng (Panax ginseng C.A. Meyer) in Korea.

At least 4.5% total ginsenosides (confirmed by HPLC), were purchased in a certified vendor (Geumsan Ginseng Cooperative South Korea). The analysis of ginsenoside profile proved the existence of major saponins: Rb1 (1.2%), Rg1 (0.9%), Re (0.7%), and Rd (0.6%).

To make feeding homogenous, the ginseng powder was mixed with the ingredients of the concentrate carefully every day before conditioning. The feed was provided twice a day (08:00 and 16:00 h) and access to fresh water was ad libitum. Feed intake was taken on a daily basis, and body weight was taken after every two weeks.

Blood Sample Collection and Processing

Blood samples (15 mL) were taken through the jugular venipuncture using non-heparinized vacuum tubes after 14 days intervals during the experimental period. All the samplings were done at 09:00 h to reduce circadian changes in the concentration of the hormones. Blood was left to clot at room temperature (30 min), and the centrifuged at 3,000 × g 15 min at 4 o C. Aliquots of serum were frozen in cryovials and kept at -80 o 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).

2. 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 gene expression showed the highest response with a percentage change of 47.3 in G2 group compared to control.

at day 90 ($P < 0.05$). HSP90 was moderately and significantly up-regulated (23.1% in G2 group, $P < 0.05$). The group of G3 had slightly reduced HSP reactions to G2 indicating plateau or slight inhibitory effect at elevated levels of supplementation.

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 ± 0.3 × 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 (×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 current research indicates that dietary ginseng powder supplement has dose-dependent inhibitory effects on the activity of the HPA axis among Shami goat bucks as it was found to have significantly reduced ACTH and cortisol levels. These results are consistent with the past studies in rodent models that showed ginsenoside-mediated prevention of stress-induced HPA activation [13,14]. The etiological dose of 2 g /kg produced a 33.5 percent cortisol suppression which can be compared to the findings of Zeng et al. (2020) who found that ginseng extract consumed 1.5-2.5 g /kg caused cortisol inhibitors 28-35 percent in heat-stressed dairy cattle [15].

The processes that ginseng modulates the HPA effect through are multifactorial and entail a number of molecular pathways. The major saponin component of the supplemented ginseng powder (1.2% composition) ginsenoside Rb1 has also been demonstrated to inhibit corticotropin-releasing hormone (CRH) expression in hypothalamic paraventricular nucleus neurons by activating peroxisome proliferator-activated receptor-gamma (PPAR- dere) and repressing nuclear factor-kappa B (NF- kB) signaling [16]. Moreover, ginsenoside Rg1 is an antagonist of glucocorticoid receptors, binding to them competitively with cortisol in tissues of interest resulting in disruption of negative feedback dysregulation, which typifies chronic stress responses [17].

The high level of correlation was observed between ACTH and cortisol (r).

The integrated effect of regulation of the HPA axis (= 0.84) that was observed in this study confirms that the action of ginseng is integrated and indicates that its action is central (hypothalamic-pituitary) and not strictly adrenal. Recent studies that show that ginsenosides regulate the sensitivity of the hypothalamic glucocorticoid receptor and increase the effectiveness of the negative feedback can support this interpretation [18]. The dose-response relationship, which is quadratic and the optimal effects occur at 2 g/kg and a a little less at 3 g/kg, could be due to biphasic hormetic effects.

typical of most phytochemicals, in which moderate doses produce optimal adaptive responses and high concentrations bring about counter-regulation [19].

Chronic HPA axis activation is an important welfare and productivity issue in small ruminant production, especially in intensive ruminant production systems, which are typified by high stocking density, frequent handling, and thermal stress [20]. High cortisol levels undermine immune capability, increase protein breakdown, and halt reproductive neuroendocrine activity in several ways such as prevention of GnRH pulsatility, and direct testicular action [21]. The extent of cortisol decrease observed in the present research (32.4 ng/mL in G2 and 48.7 ng/mL in control) is significant to clinical change, which will translate into improved physiological capacity and effective performance.

Heat Shock Protein Upregulation and Cytoprotection

The This dramatic increase (47.3% and 23.1% respectively) in the level of the HSP70 and HSP90 in the ginseng-supplemented bucks indicates a new discovery with a great significance in cellular stress resistance and reproduction. Heat shock proteins are molecular chaperones that play a crucial role in protecting the proteinostasis, inhibiting protein aggregates and the correct folding of nascent polypeptides in physiological and stressful environments [22]. HSPs play a particularly important role in testicular tissue in protecting developing germ cells against oxidative damage and thermal stress as well as apoptotic signals that disrupt spermatogenesis [23]. The difference in the magnitude of the response of HSP70 and HSP90 (47%, and 23% upregulation, respectively) can be attributed to differences in their mechanism of regulation and cellular activity. The HSP70 induction is mainly triggered by activating heat shock factor-1 (HSF-1) by leaving various mechanisms triggered by ginsenosides including increase in trimerization of HSF-1, nuclear translocation, and DNA-binding function [24]. The latest study by Wang et al. (2021) has shown that.

ginsenoside Rg3 directly stimulates the HSF-1 via protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) signaling pathways and induces potent transcription of HSP70. HSP90 on the other hand is expressed constitutively in the majority of cells and significantly less influenced by stress, as observed by us [25].

A mechanistic linkage between cytoprotection of the cell and reproductive results is supported by high positive correlation (HSP70 expression, sperm quality parameters: concentration: r = 0.65; motility: r = 0.59; morphology: r = 0.58) that exists between the two. HSP70 helps protect spermatogenic cells against oxidative stress in a number of ways including direct antioxidant effects, stabilization of mitochondrial membrane potential, inhibition of apoptotic copulations and improvement DNA repair mechanisms [26]. HSP70 promotes adequate folding of steroidogenic enzymes and androgen receptors in Sertoli cells, which is necessary in testosterone biosynthesis that helps in spermatogenesis [22].

Recent proteomic studies have listed HSP90 as a key controller of steroid hormone receptor activity including androgen receptor (AR) development and equilibrium [27]. The HSP90 small yet significant increase in the current case study could be a part of the elevated androgen signaling mentioned in testicular tissue, which would be in addition to the direct steroidogenic effects that some ginsenosides have been reported to possess [28]. Moreover, HSP90 communicates with many client proteins that regulate cells cycle, germ cell development and meiotic progress, indicating that HSP90 has pleiotropic advantages to spermatogenic performance [30]. HSP expression induced by ginseng seems to be indirectly through the mediation of oxidative stress-signaling pathways as well as direct through the activation of transcriptional pathways. Ginsenosides stimulate the nuclear factor erythroid 2-related factor 2 (Nrf2) which is a master of antioxidant response elements (ARE) and regulates the expression of many cytoprotective genes such as HSPs, other enzymes related to glutathione, and superoxide dismutase [31]. This integrated amplification of cellular defenses is probably a contributing factor to the sperm and testicular functions improvements.

Reproductive Performance Enhancement

The entire changes in the parameters of semen quality experienced by ginseng-enriched bucks such as the 39.3% higher sperm concentration, 16.7% enhanced progressive motility and 10.5% enhanced normal morphology are biologically and economically significant improvements in reproductive capacity. These results are a continuation of past studies in other species, such as poultry, swine [32], and lab animals that have reported high levels of reproductive benefits in the small ruminants [33] in the first instance.

The mechanisms by which these improvements occur are probably multifactorial, encompassing effects that are coordinated to occur on neuroendocrine mechanisms, testicular steroidogenesis, sperm protection in spermatogenic cells, and epididymal sperm maturation. The close negative interaction of cortisol and sperm concentration (r = -0.72), indicates that the HPA axis suppression is an important factor in enhancing spermatogenic efficiency. Withdrawal of gonadotropin secretion by hyperplastic glucocorticoid levels inactivates hypothalamic GnRH neurons, and the master gland directly, lowering levels of LH and FSH that testicular tissue requires to work [34]. Besides, cortisol has direct inhibitory effects on steroidogenesis of testicular Leydig cells and Sertoli cell support activity, which inhibit testosterone production and nurturing of the germ cell [21]. In addition to the gonadotropic and steroidogenic effects, ginsenosides have direct gonadotropic effects in addition to HPA modulation. A study conducted by Leung and Wong (2021) showed that ginsenoside Rg1 increases the expression of testicular steroidogenic acute regulatory protein (StAR) and 3-hydroxysteroid dehydrogenase (3 -HSD) which are essential testosterone synthesis enzymes. Moreover, ginsenosides enhance the generation of nitric oxide (NO) in testicular tissue via the activation of endothelial NO synthase (eNOS) enhancing the microcirculation and access of oxygen to developing germ cells [35]. The 8.7% greater rise in scrotal circumference and concomitant testicular volume enlargement in the G2 group probably is an augmented proliferation of Sertoli cells and development of seminiferous tubules that was facilitated by the better hormonal milieu and vascular perfusion. The sperm morphological improvements, specifically.

the the losses in the head (39.5%), midpiece (40.3%), and tail (40.8%) abnormalities, indicate protective influences in the spermatogenesis and epididymal levels of maturation. A key factor causing morphological deficits in spermiogenesis is oxidative stress, where DNA and chromatin condensation processes, as well as cytoplasmic removal, are very susceptible to the impact of reactive oxygen species (ROS). The mechanism-based on the Nrf2 activation and direct scavenging with ROS-induced antioxidant effects of the ginsenosides is probably the reason behind the reported morphological improvements [36].

The increase in progressive motility (68.4 to 79.8 percent) is an indicator that the energy metabolism and flagellar activity of the sperm has improved. Mitochondrial dysfunction is also a leading cause of asthenozoospermia and ginsenosides were demonstrated to promote mitochondrial biogenesis via the AMPK-PGC-1alpha pathway and increase the efficiency of ATP production [37]. Also, ginsenosides guard antioxidative destruction of mitochondrial DNA and stabilize the electron transport chain complexes to avoid energy production loss during epididymal transfer and ejaculation [38].

3. Conclusions

This paper presents strong arguments that dietary ginseng powder is a viable nutritional controller of both systemic and cellular stressful states of Shami goat bucks. We have shown that the HPA axis activity of supplemented bucks is severely downregulated as evidenced by the significantly lower levels of circulating cortisol than in the control group. This weakening of the initial glucocorticoid response indicates that the adaptogenic actions of ginseng are helpful to ensure that there is physiological stability, and the negative impacts of chronic stress, which have been associated with impairing metabolic wellbeing and reproductive capacity, are averted. At the same time, a subtle and positive alteration of the heat shock protein response was seen with ginseng supplementation. We have witnessed a high level of improvement in Hsp70 and Hsp90 expression in the ginseng group. More importantly, we perceive this not as a stress induction process, but as amplification of pre-emptive cell defense mechanisms. The ginsenosides are bioactive, and they seem to appear.

induce a more resilient protection of proteostasis in response to a stressor by the activation of the cellular chaperone system, which is known as prime. Such heightened baseline preparedness is likely to have a beneficial effect on thermotolerance and cellular integrity that is vital as far as the viability of spermatozoa and other essential physiological processes is concerned. In the case of food and breeding industries, the implications of the findings are a highly effective tool in protecting valuable genetic materials. The reduction of the endocrine stress reaction and enhancement of the cellular resilience can directly help to preserve the libido and spermal quality of the bucks in the harsh environmental conditions, which is provided through ginseng supplementation. This increases the output and lifetime worth of elite sires, and some sort of guarantee of a dependable and efficient channel of genetic enhancement and meat production in herd. To sum up, the supplementation of the diet of Shami goat bucks with ginseng powder is a scientifically-evidenced, natural intervention that can increase climatic resilience. This is not just a symptom management approach but an improvement of the adaptive ability of an animal which is in line with the concept of sustainable and welfare-based animal farming. Further study is then needed to measure these physiological gains against direct measurements of reproductive performance to comprehensively measure the payback of investments by producers.

6. References

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