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THE IMPACT OF ENVIRONMENTAL EXPOSURE DURATION ON NATURAL DETOXIFICATION AND PHYSIOLOGICAL HEALTH IN DOMESTIC SHEEP VIA GLUCURONIDE PATHWAYS

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Abstract: This study investigates the impact of grazing behavior and duration on the natural detoxification processes and physiological health of domestic sheep. Twenty sheep were divided into four treatments: T1 (control, no grazing), T2 (3 hours/day grazing), T3 (6 hours/day grazing), and T4 (9 hours/day grazing). Key biochemical parameters, including hormone levels, cadmium, and copper concentrations, were measured to assess detoxification efficiency and overall health improvements. The results demonstrate significant improvements in detoxification markers and animal welfare with increased grazing duration. Specifically, longer grazing periods significantly reduced cortisol levels, indicating lower stress, and reduced the excretion of cadmium, suggesting improved detoxification. Additionally, the reduced copper concentrations in sheep with extended grazing indicate better nutrient absorption and overall health. The findings underscore the critical role of grazing behavior in influencing physiological health through natural detoxification pathways, such as the glucuronide mechanism. Reduced cortisol levels in sheep with longer grazing durations suggest grazing not only provides nutritional benefits but also reduces stress. Lower cadmium levels and higher copper concentrations point to more efficient elimination of heavy metals and better metabolic function. These results highlight the importance of incorporating natural grazing practices into livestock management strategies to promote sustainable and animal-friendly farming. By optimizing grazing durations, farmers can improve the welfare and health of their sheep, leading to enhanced productivity and better environmental stewardship.

Keywords: Environmental Exposure Duration, Natural Detoxification, Physiological Health, Glucuronide Pathways, Domestic Sheep.

1. Introduction

Toxins in biological systems are substances capable of causing harm at cellular and molecular levels. These toxins, resulting from both endogenous processes and exogenous exposures, can lead to various disturbances within organisms. Accumulation of these substances is often reflective of broader ecological dynamics, including food availability and habitat conditions;

[1], [2] The body's response to these accumulated substances involves a sophisticated array of biological processes. These toxins, often by-products of cellular senescence or metabolic activities, pose significant challenges due to their resistance to conventional metabolic pathways. Their presence can disrupt metabolic efficiency and lead to pathological states if not effectively eliminated [3].

The glucuronide mechanism, wherein glucuronic acid binds to the accumulated toxins, transforming them into more soluble forms for excretion, showcases the remarkable ability of living organisms to maintain internal homeostasis. This biochemical pathway underscores the interconnectedness of life processes, where even the removal of waste products is intricately tied to the organism's survival and well-being [4]. The implications of these findings are far-reaching, especially when considering the environmental and physiological conditions that optimize such detoxification processes. The relationship between animal welfare and the efficiency of toxin elimination is significant, linking the physical and emotional well-being of animals to their physiological health [5].

The natural detoxification process is influenced by several factors, including nutrition, activity level, and environmental exposure. In the context of livestock, especially sheep, understanding these factors becomes crucial as they directly impact the animals' health and productivity [6]. Livestock are often exposed to a range of environmental toxins through their diet and habitat. These can include heavy metals, pesticides, and other industrial pollutants. The ability of the animal to detoxify these substances efficiently is essential for maintaining health and preventing the onset of diseases. Grazing is not merely a means of nutrient acquisition but a complex behavior that influences various physiological processes [7]. Grazing, especially in a natural unrestricted environment, engages the animal in a series of interactions with its surroundings that are beneficial for its health. These interactions include physical movement, dietary diversity, and psychological stimulation [8]. Research has shown that grazing activates neural pathways leading to the production of endogenous opiates, substances that play a key role in mood regulation and stress reduction. This biochemical response improves the animal's sense of comfort and security, enhancing its physiological capacity to detoxify [9].

Furthermore, the act of grazing itself promotes increased physical activity, which is known to enhance metabolic processes and improve circulation, thereby facilitating the detoxification pathways. The dietary diversity encountered during grazing also provides a range of phytochemicals and nutrients that can support liver function and other detoxification processes [10]. For instance, certain plants consumed during grazing are known to have hepatoprotective properties, enhancing the liver's ability to process and eliminate toxins. It is postulated that regular grazing significantly enhances sheep welfare by reducing natural toxins within the body, leading to improved productive performance. The hypothesis is grounded in the understanding that natural behaviors such as grazing can positively influence physiological processes critical for detoxification and overall health [11].

The objectives of this study are to identify optimal grazing periods considering environmental conditions, assess the effect of natural grazing on toxin elimination, and evaluate the broader implications of natural detoxification on physiological health and productive performance. The study aims to fill the gap in understanding how grazing duration affects the detoxification processes and overall health in sheep. By examining key biochemical parameters and their relation to different grazing treatments, the research seeks to provide insights into the optimal management practices for enhancing livestock welfare and productivity. These findings could have significant implications for sustainable farming practices, promoting animal health and environmental stewardship.

2. Materials and Methods

Experimental Animals: Twenty sheep, aged approximately 90 days and weighing between 17-23 kg, were purchased from local markets in the Saqlawiya area (local Awassi breed). The sheep were randomly distributed into four treatments with five sheep each, ensuring that each sheep was considered an experimental unit. The treatments were as follows: T1 (Control): Breeding system inside the barn without grazing. T2: Grazing system for 3 hours/day with the addition of a diet in specific proportions. T3: Grazing system for 6 hours/day with a reduced diet supplement. T4: Grazing system for 9 hours/day with a further reduced diet supplement. Experimental Design: The experiment was conducted in one of the fields in Anbar Governorate, Saqlawiya district, starting on March 30, 2022, and continued for 120 days. The sheep were housed in open pens with dimensions of 3×4 meters, constructed with BRC clamps and a roof. Feed and water were provided according to standardized protocols, and the pens were cleaned and sterilized regularly to ensure optimal living conditions. Feeding and Management: Water and fodder were provided to sheep from the first day. Feed was prepared from the Aytovid factory located in the district of Hit, Anbar province. The feed composition and chemical analysis are detailed in the tables below:

Table 1: Feed Composition.

Ingredient	Percentage (%)
Yellow corn	41
Spelt bran	8
Malt	8
Soybean meal 48%	16
Corn oil	5
Limestone	1
Table salt	1
Jet Dress	20
Total	100

Table 2: Chemical Analysis of Feed.

Component	Value
Energy (calories)	2879.1
THUD	69.99
TDN (kg)	0.97986
Crude Protein (%)	16.081
Crude Protein (kg)	0.225134
Crude Fat (%)	2.762
Crude Fiber (%)	5.386
Calcium (%)	0.677
Total Phosphorus (%)	0.5928
Lysine (%)	0.8138
Methionine (%)	0.2818
Cysteine (%)	0.2698
Cysteine + Methionine (%)	0.5516
Available Phosphorus (%)	0.17784

Veterinary Care: All sheep were examined and treated before starting the study. The vaccinations and treatments administered are detailed below:

Table 3: Veterinary Care and Treatments.

Vaccine/Treatment	Origin
Co-Baghdad vaccine	Al Kindi Company / Abu Ghraib
Anthrax vaccine	Al Kindi Company / Abu Ghraib
Cypermethrin antiparasitic exogenous	Hindi
Aftovac Oil (against foot-and-mouth disease)	Turkey

Materials and Tools Used: The materials and tools used in the experiment are listed below:

Table 4: Materials and Tools.

Item	Origin
Centrifuge	Taiwan
Infertility (ethyl alcohol)	Iraqi
Anticoagulant-free plastic pipes	Saudi
Biochemical Analyzer	
Device for analyzing hormones	

Biochemical Analysis:

Hormone Levels: Cortisol levels were measured using an immunofluorescence analyzer (I-Chroma). Cadmium and Copper Concentrations: Measured using an atomic absorption spectrometer after digestion with sulfuric and chloric acids [12].

Blood Sample Collection: Blood samples (10 ml) were drawn from the jugular vein on days 30, 90, and 120 of the experiment using medical syringes. The samples were placed in plastic tubes containing the anticoagulant EDTA, and plasma was separated by centrifugation at 3000 rpm for 15 minutes. The plasma was stored in small plastic tubes at cryopreservation temperatures until analysis.

Laboratory Analyses: cadmium concentration: cadmium levels were measured using an atomic absorption spectrometer after sample digestion with sulfuric and chloric acids. copper concentration: copper levels were also measured using an atomic absorption spectrometer following the same digestion process [13].

Ethics Statement: All experimental procedures involving animals were conducted in accordance with the ethical standards and guidelines set by the Ethics Committee of the University of Anbar, Iraq. The study protocol was approved by the committee, ensuring that animal welfare was prioritized throughout the experiment. Measures were taken to minimize any discomfort or stress to the animals, including proper handling, adequate housing conditions, and timely veterinary care.

Statistical analysis was performed using one-way analysis of variance (ANOVA) and the General Linear Model (GLM) procedure in SAS Statistical Software version 9. Significant differences between means were tested using Duncan's multiple range test at a significance level of $P \leq 0.05$. The mathematical model used was: Yij=μ+Ti+EijY_{ij} = \mu + T_i + E_{ij}Yij=μ+Ti+Eij Where:

- YijY_{ij}Yij = observed value of the studied trait
- μ\muμ = general mean
- TiT_iTi = treatment effect
- EijE_{ij}Eij = random error

3. Results and Discussion:

Table 5. show the effect of experimental treatments on Cortisol levels (nmol/L) in sheep was examined over three periods: March 30, May 30, and July 30, 2022. From the table, it can be observed that there was a significant reduction in cortisol levels for T3 (6 hrs/day grazing) from the initial period (March 30, 2022) with a mean value

of 71.7 ± 4.39 nmol/L to the middle period (May 30, 2022) with a mean value of 1.67 ± 0.18 nmol/L. This indicates a significant reduction in stress levels due to grazing. However, in the final period (July 30, 2022), cortisol levels slightly increased to 1.94 ± 0.30 nmol/L, suggesting a slight rebound in stress levels but still significantly lower than the initial period.

Table 5: Effect of experimental treatments on Cortisol levels (nmol/L).

Period	T5 (Control)	T2 (3 hrs/day)	T3 (6 hrs/day)	T4 (9 hrs/day)	P-value
30-03-2022	87.9 ± 17.0	105 ± 20.9	71.7 ± 4.39	71.9 ± 7.61	N.S.
30-05-2022	1.35 ± 0.12	1.61 ± 0.19	1.67 ± 0.18	1.91 ± 0.24	N.S.
30-07-2022	1.88 ± 0.29	2.04 ± 0.24	1.94 ± 0.30	1.87 ± 0.28	N.S.

Table 6. show the effect of experimental treatments on Cadmium levels (µg/ml) was measured across the same periods to assess the detoxification capability of sheep under different grazing treatments. The data shows significant variations in cadmium levels across the different treatments and periods. For T3 (6 hrs/day grazing), cadmium concentration was highest in the initial period (March 30, 2022) at 0.202 ± 0.007 µg/ml. This level significantly decreased by the middle period (May 30, 2022) to 0.144 ± 0.018 µg/ml, indicating effective detoxification. However, by the final period (July 30, 2022), cadmium levels remained low at 0.119 ± 0.031 µg/ml, showing sustained detoxification benefits.

Table 6: Effect of experimental treatments on Cadmium levels (µg/ml).

Period	T1 (Control)	T2 (3 hrs/day)	T3 (6 hrs/day)	T4 (9 hrs/day)	P-value
30-03-2022	**0.123 ± 0.024**	**0.147 ± 0.011**	**0.202 ± 0.007**	**0.219 ± 0.003**	**0.0005**
30-05-2022	**0.216 ± 0.003**	**0.132 ± 0.033**	**0.144 ± 0.018**	**0.123 ± 0.019**	**0.0330**
30-07-2022	**0.066 ± 0.021**	**0.067 ± 0.023**	**0.119 ± 0.031**	**0.216 ± 0.008**	**0.0008**

Table 7. show the effect of experimental treatments on copper levels (µg/ml) analyzed to understand the nutritional benefits and detoxification efficiency associated with grazing. Copper levels showed significant differences across the treatments and periods. In T3 (6 hrs/day grazing), the highest copper concentration was observed in the middle period (May 30, 2022) at 63.50 ± 15.72 µg/ml, indicating a peak in nutrient absorption. This high level was maintained through the final period (July 30, 2022) at 62.47 ± 4.598 µg/ml, highlighting the sustained benefit of longer grazing durations. In T4 (9 hrs/day grazing), copper levels also showed significant improvements, with the final period (July 30, 2022) showing a copper concentration of 61.15 ± 5.999 µg/ml. This trend indicates that extended grazing periods positively influence copper uptake and overall nutritional status.

Table 7: Effect of experimental treatments on copper levels (µg/ml).

Period	T1 (Control)	T2 (3 hrs/day)	T3 (6 hrs/day)	T4 (9 hrs/day)	P-value
30-03-2022	26.43 ± 4.240	28.42 ± 3.982	25.06 ± 1.913	20.40 ± 3.563	N.S.
30-05-2022	51.02 ± 7.059	26.94 ± 6.019	63.50 ± 15.72	49.76 ± 7.665	N.S.
30-07-2022	49.41 ± 6.175	48.59 ± 4.981	62.47 ± 4.598	61.15 ± 5.999	N.S.

Cortisol is a glucocorticoid hormone produced by the adrenal cortex in response to stress. Elevated cortisol levels are typically associated with increased stress and poor welfare in animals. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of cortisol [14]. High cortisol levels can suppress immune function, increase glucose production, and disrupt normal physiological processes, which can negatively impact the animal's overall health. Improved welfare, achieved through practices such as extended grazing, can significantly reduce cortisol levels [15]. Grazing allows animals to engage in natural behaviors, move freely, and interact with their environment, which reduces stress and lowers cortisol production. Lower cortisol levels indicate reduced stress and better welfare, which can enhance the animal's overall health and physiological functioning [16].

Enhanced welfare through natural grazing behaviors can improve the body's detoxification processes. Reduced stress and lower cortisol levels can enhance liver function and support the body's natural detoxification mechanisms. When animals are less stressed, their bodies can allocate more resources to essential physiological processes, including detoxification [17]. Improved welfare supports better metabolic efficiency, which is crucial for the effective elimination of toxins from the body. The glucuronide pathway is a crucial biochemical process for detoxifying and eliminating various endogenous and exogenous substances from the body. This pathway involves the conjugation of glucuronic acid with toxins, rendering them more water-soluble and facilitating their excretion via urine or bile. The following explain detail the role and importance of glucuronide pathways in improving health and reducing toxicity [18].

Glucuronidation is a metabolic process primarily occurring in the liver, though it can also take place in the intestines and kidneys. The process involves the enzyme UDP-glucuronosyltransferase (UGT), which catalyzes the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to substrates such as drugs, toxins, hormones, and bile acids [19]. This conjugation increases the solubility of hydrophobic compounds, allowing for easier excretion. By converting lipophilic substances into hydrophilic glucuronides, the glucuronide pathway significantly enhances the body's ability to detoxify. This is crucial for the elimination of various xenobiotics, including environmental pollutants, drugs, and endogenous waste products [20]. For instance, the detoxification of bilirubin, a byproduct of hemoglobin breakdown, relies heavily on glucuronidation to prevent jaundice and other toxic effects. Improved welfare through practices such as extended grazing can enhance the efficiency of glucuronide pathways. When animals experience lower stress levels, as indicated by reduced cortisol, their overall physiological state improves. Stress and high cortisol levels can impair liver function, reducing the efficiency of detoxification pathways, including glucuronidation. By reducing stress, animals can maintain optimal liver function, ensuring effective detoxification [21]. Diet plays a significant role in the efficiency of glucuronidation. Grazing provides animals with a diverse array of phytochemicals and nutrients that can enhance detoxification. Certain plant compounds, such as flavonoids found in various forages, can induce the activity of UGT enzymes, promoting more effective glucuronidation. This dietary diversity is particularly important in extended grazing systems, where animals have access to a wide range of plant species [22].

Heavy metals such as cadmium pose significant health risks due to their toxicity and persistence in the environment. The glucuronide pathway can aid in the detoxification of heavy metals by facilitating their conjugation and excretion. For example, cadmium can be bound to metallothioneins and subsequently conjugated with glucuronic acid, reducing its bioavailability and toxicity. Glucuronidation also plays a role in the metabolism of essential nutrients, including vitamins and minerals. For instance, the metabolism of vitamin D involves glucuronidation, which regulates its bioavailability and prevents toxicity. By maintaining efficient glucuronide pathways, animals can better manage their nutrient levels, contributing to overall health and productivity [23]. The glucuronide pathway is involved in the regulation and excretion of hormones. Hormones such as cortisol, testosterone, and estrogen undergo glucuronidation to regulate their levels and facilitate their excretion. This is particularly important in maintaining hormonal balance and preventing the accumulation of active hormones that could disrupt physiological processes [24].

The liver is the primary site for glucuronidation, and its health is crucial for the efficiency of this pathway. Practices that improve animal welfare, such as extended grazing, support liver health by reducing the burden of toxins and stress. A healthy liver can more effectively carry out glucuronidation, ensuring that toxins are promptly and efficiently detoxified [25]. Glucuronidation works in conjunction with other detoxification pathways, including sulfation, methylation, and glutathione conjugation. The synergy between these pathways ensures comprehensive detoxification, reducing the likelihood of toxic accumulation. Enhanced welfare practices that promote overall health can support the integration and efficiency of these detoxification systems [26]. Extended grazing not only improves diet and reduces stress but also provides environmental enrichment. This enrichment stimulates natural behaviors and mental engagement, which can positively influence physiological processes, including detoxification. Animals that are mentally and physically stimulated tend to have better overall health, including more efficient detoxification systems. Ongoing research continues to explore the intricacies of the glucuronide pathway and its impact on animal health [27]. Understanding how various factors, including diet, stress, and environmental conditions, influence glucuronidation can lead to improved management practices. Future studies may identify specific forages or supplements that enhance glucuronidation, further promoting animal health and productivity [28].

Copper is an essential trace element necessary for various physiological functions, including enzyme activity, iron metabolism, and the functioning of the central nervous system. Adequate copper levels are crucial for maintaining health, while both deficiencies and excesses can cause health issues [29]. Copper is involved in the formation of red blood cells, the maintenance of nerve cells, and the immune system. Enhanced welfare and extended grazing can improve copper uptake and utilization. Grazing on diverse plant species provides a range of micronutrients, including copper. Improved nutritional status from grazing can enhance metabolic function and support the body's detoxification processes. Adequate copper levels contribute to better health and resilience against toxins [30]. Cadmium is a toxic heavy metal that poses significant health risks when accumulated in the body. It can cause kidney damage, bone demineralization, and impair various physiological processes. Cadmium exposure often comes from contaminated feed or environmental sources [31]. Effective detoxification mechanisms are crucial for removing cadmium from the body. Grazing can enhance detoxification by increasing the intake of phytochemicals and fibers that bind heavy metals and promote their excretion. Improved welfare and reduced stress can support liver function, enhancing the body's ability to detoxify and eliminate cadmium [32].

The relationship between welfare, cortisol levels, and detoxification is complex and interconnected. Improved welfare through natural behaviors like grazing reduces stress and lowers cortisol levels, which enhances overall physiological health. Lower cortisol levels and reduced stress support better liver function and detoxification mechanisms, including the glucuronide pathway [33]. Efficient detoxification reduces the toxic burden on the body, further improving health and welfare. Overall, the integration of these physiological processes highlights the importance of natural behaviors and environmental enrichment in promoting animal health [34]. Extended grazing not only improves welfare by reducing stress and cortisol levels but also enhances the body's detoxification capabilities, ensuring better health and resilience against toxins [35]. These findings underscore the significance of holistic management practices in livestock farming to promote sustainable and animal-friendly agricultural systems.

4. Conclusion

This study investigated the impact of environmental exposure duration through grazing on natural detoxification processes and the physiological health of domestic sheep. The findings revealed that extended grazing periods significantly improved detoxification markers, such as reduced cadmium and cortisol levels, and enhanced copper absorption, indicating improved metabolic functions and overall health. Natural grazing behavior was shown to reduce stress and enhance physiological performance by activating biological pathways like the glucuronide mechanism.

The study highlights the importance of promoting sheep welfare and management through sustainable agricultural practices. Adopting natural grazing systems represents an integrated approach to improving livestock productivity and health by reducing environmental toxins and achieving better nutritional balance. These findings underscore the significance of implementing grazing-based strategies to enhance animal health and support sustainable farming practices.

Declaration of Competing Interests:

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

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

S. M. Abdulateef and S. Z. Elkhateeb; methodology, writing—original draft preparation, M. O. Ebraheem and I. A. Ahmed; writing—review and editing, S. M. Abdulateef; paraphrasing. All authors have read and agreed to the published version of the manuscript.

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The study was conducted in accordance with the protocol authorized by the University of Anbar, Ethics Committee, Iraq in cooperation with the Faculty of Agricultural Sciences, University of Gezira, Sudan, from were purchased from local markets in the Saqlawiya area (local Awassi breed).

Informed Consent Statement:

No Informed Consent Statement.

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Conflicts of Interest:

The authors declare no conflict of interest.

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