

**Research Article**

# The Association of Oxidative Stress and Inflammatory Response Parameters on Saanen Goat Kids Supplemented with Probiotic and Yeast Extract

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**ABSTRACT**

**Introduction:** The weaning process is a stressful phase for goat kids, impacting energy metabolism, antioxidant levels, and inflammatory responses, potentially hindering health and growth. Supplementing probiotics and yeast extracts may help mitigate these effects by improving gut health and immune responses. This study evaluated the relationship between oxidative stress markers and inflammatory parameters in Saanen goat kids during weaning.

**Materials and methods:** The study involved 40 female Saanen goat kids with closely aligned birth dates ( $\pm 2$  days), randomly divided into four groups, Group 1 (n=10) received 1g/day probiotics (Pr), Group 2 (n=10) received 3g/day yeast cell wall extract (YC), Group 3 (n=10) received both supplements (1g Pr + 3g YC), and Group 4 (n=10) served as the control. Key parameters were cortisol, ferritin, nitric oxide (NO), total antioxidant capacity (TAC), triglycerides, and adenosine deaminase (ADA) and were measured to assess their associations and correlations during the weaning period under supplementation.

**Results:** The results showed a significant negative correlation between ferritin and cortisol, as well as between nitric oxide (NO) and cortisol. While ADA was not directly associated with NO, a significant positive correlation was observed between them. TAC demonstrated significant negative correlations with both NO and triglycerides, with the latter showing a quadratic relationship.

**Conclusion:** This study highlights the intricate interplay between stress parameters (cortisol, TAC, and NO), inflammatory markers (ferritin and ADA), and energy metabolism (triglycerides) during the weaning phase in goat kids. Supplementation with probiotics and yeast extract demonstrated potential benefits in modulating these physiological traits, reducing stress, and supporting overall health.

## 1. Introduction

The early developmental months of goat kids, particularly during the milk-feeding period and the transition to weaning, are critical phases marked by high vulnerability and elevated mortality rates<sup>1</sup>. This period involves a significant dietary transformation, shifting from a diet rich in casein, lactose, and triglycerides to a more complex nutritional intake requiring microbial involvement for efficient digestion<sup>2</sup>. These changes impact nutrient absorption patterns and digestive efficiency. Research highlights that introducing rumen fluid during early life can foster microbial colonization in the rumen and accelerate the weaning process. However, the stress associated with weaning

has been shown to disrupt critical host-microbe interactions and alter immune responses in young goats<sup>3-5</sup>.

Probiotic microorganisms are frequently employed as dietary supplements to optimize productivity, boost performance, and prevent diseases by promoting a healthy gastrointestinal environment<sup>6</sup>. Their inclusion in the diet has been shown to enhance the survival of beneficial rumen microbial populations, improve feed efficiency, and support growth. Yeast-based and bacterial probiotics offer additional benefits through mechanisms such as immunostimulants<sup>7</sup>. Notably, yeast cell wall supplements and probiotics have been

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found to mitigate the effects of mycotoxins like aflatoxins, which are associated with immunosuppression in blood and milk. They also enhance the utilization of ruminal protein and reduce blood urea nitrogen (BUN) levels, contributing to overall animal health<sup>8-10</sup>.

While most studies on probiotics in small ruminants have focused on productivity outcomes, there remains a gap in understanding their effects on immune mechanisms and oxidative stress parameters<sup>11</sup>. The stress induced by weaning can negatively influence growth performance, antioxidant levels, and the expression of acute-phase proteins. Plasma cortisol concentration is often used as an indicator of stress in young goat kids, particularly in Saanen breeds. Oxidative stress, which correlates with an animal's immune status, can increase susceptibility to diseases during the weaning period. This stress is influenced by dietary changes and the animal's metabolic state, which can disrupt the balance between oxidative and antioxidant activities<sup>12-14</sup>.

The total antioxidant capacity (TAC) serves as a valuable biomarker for assessing oxidative stress during weaning, providing insights into the animal's oxidation status. Nitric oxide (NO), a multifunctional molecule involved in physiological processes like hemostasis, has been studied for its potential role in linking oxidative stress and innate immunity<sup>15</sup>. Adenosine deaminase (ADA), an enzyme integral to the breakdown of adenosine into inosine, plays a vital role in lymphocyte function and immune response, particularly in lymphoid tissues. Its activity is significant in the context of viral diseases and immune regulation<sup>16</sup>.

Given these insights, monitoring oxidative stress biomarkers during the weaning phase is essential for understanding and mitigating the physiological challenges faced by goat kids. Supplements, including probiotics and yeast derivatives, offer promising strategies to modulate oxidative stress and support immune function, thereby improving health outcomes during this critical developmental stage. The objective of this research was to assess the association between oxidative stress and inflammatory response parameters in Saanen goat kids supplemented with probiotics and yeast extract.

## 2. Materials and Methods

### 2.1. Ethical approval

All the Saanen kids used in this experiment were handled in accordance with the technical regulations and the guidelines set out by the committee of animal ethics of Shiraz University, Iran (IACUC no: 4687/63).

### 2.2. Study design and animals

**Table 1.** Composition of the ration in goat kids

Basal diet		Goat milk		Weaning mixture	
Component	DM (%)	Component	DM (%)	Component	DM (%)
CP	20.53	Cp	26.2	Cp	19.9
Total carbohydrate	19.72	Fat	31.4	EE	5.3
Total fat	7.91	Lactose	37.9	Starch	17.7
Total fiber	39.24				
Vitamin and mineral supplement	6.3				

CP: Crude protein, EE: Ether extract

This study was conducted on a goat farm in Ardakan-e Yazd, Iran, involving a sample of 60 first-freshener Saanen goats selected randomly from a population of 200 parturient pregnant goats. These goats were chosen based on uniform health status, consistent diet, no prior history of diseases, similar body weights, body condition scores, and confirmation of carrying a single offspring. The goats, aged 1.5 to 2 years, were identified and selected two weeks before the expected parturition date. Prior to their inclusion in the study, a thorough veterinary assessment confirmed their overall health. However, 20 goats were excluded from the final selection, either because they carried twins (n=12) or because of health complications in their newborn kids (n=8).

Following parturition, 40 female goat kids with closely aligned birth dates ( $\pm 2$  days) were separated from their mothers and assigned randomly to one of four experimental treatments. A qualified veterinarian conducted physical examinations of the newborn kids in adherence to established farm health protocols. The experimental groups were housed in four separate, straw-bedded indoor pens with natural ventilation. The indoor environment maintained a temperature range of 16.1 to 27.7°C to ensure animal comfort and minimize environmental stress. The study comprised four groups including Group 1 (n = 10) received probiotics (Pr) supplements along with a basal diet; Group 2 (n = 10) received yeast cell wall extract (YC) supplements with a basal diet; Group 3 (n = 10) received both probiotics and yeast extract (Pr+YC) supplements alongside a basal diet; and Group 4 (n = 10) served as the control group.

All goat kids were bottle-fed colostrum from their mothers for the first three days of life. From the fourth day onward, milked maternal milk was bottle-fed twice daily, constituting 10% of the kids' body weight per feeding session at 8:00 a.m. and 6:00 p.m. Supplements for the experimental groups were dissolved in water and added to the milk beginning at 21  $\pm$  2 days before the initiation of the weaning program (at 80  $\pm$  2 days). Supplementation continued until 21 days post-weaning. Solid feed was introduced to all kids at 10 days of age, and uneaten portions were removed daily.

The weaning program commenced at 80  $\pm$  2 days of age with a gradual reduction in milk feeding alongside increased access to solid feed. Between 80 and 86 days, milk volume was limited to 9% of the kids' body weight daily. From 86 to 95 days, milk was reduced by 1% per day until completely withdrawn by day 96. Fresh feed was provided at 8:00 a.m. and 6:00 p.m. daily, with any uneaten feed removed.

The dietary composition of the weaning feed mixture, detailed in Table 1, included a variety of nutritional components: grass hay, dehydrated alfalfa hay, steam-flaked maize, whole soybean flakes, wheat straw, dried sugar beet pulp, maize gluten meal, soybean meal, sunflower seeds, sugarcane molasses, minerals, and

vitamin supplements. The average body weight of the goat kids at the first sampling point, 21 days before weaning, was  $11.48 \pm 0.41$  kg. Daily dry matter intake (DMI) for each group was recorded. To mitigate potential health risks and confounding factors, rectal temperature and behavioral observations were conducted twice daily, ensuring close monitoring for infectious diseases and other concerns.

### 2.3. Diet

The quantities of probiotic (Probiocin, Pharmed Daroo BIOTECH company, Shiraz, Iran) and yeast extract (ROBIOSYN, Pharmed Daroo BIOTECH company, Shiraz, Iran) administered were 1 gram and 3 grams per day for each animal, respectively, following the manufacturer's recommendations. The group that received both yeast and probiotic extracts was given the combination of these two substances in the same manner. Probiocin is composed of four natural probiotic strains, specifically BRY60, which includes *Bacillus subtilis*, *Bacillus lechiformis*, *Streptococcus thermophilus*, and *Enterococcus faecium*. ROBIOSYN is derived from the natural yeast cell wall of *Saccharomyces cerevisiae*.

### 2.4. Sampling

Blood samples (5 ml) were obtained at four distinct time points including 21 days prior to weaning (ST1), 2 days after weaning (ST2), which coincides with the weaning period, 7 days post-weaning (ST3), and 21 days post-weaning (ST4). The samples were drawn from the jugular vein at noon using 22-gauge, 1-inch-long needles, and were collected into plain evacuated tubes. These tubes were subsequently placed on ice until centrifugation, which was performed at  $1,200 \times g$  for 15 minutes at a temperature of  $4^{\circ}\text{C}$  to facilitate plasma separation. The resulting plasma was then aliquoted into microcentrifuge tubes and stored at  $-32^{\circ}\text{C}$  until further analysis. Serum cortisol levels were assessed using a solid phase sandwich ELISA method, utilizing an American Monobind ELISA kit.

The Goat Nitric Oxide (NO) levels were measured using an ELISA kit from the Bioassay Technology Laboratory, Shanghai, China. The concentration of Adenosine Deaminase (ADA) was assessed through a colorimetric-enzymatic kit from Diazyme Laboratories, Gregg Court, California, USA. Ferritin levels were evaluated using a Ferritin ELISA kit from Biozol Diagnostica Vertrieb GmbH, Eching, Germany. The total antioxidant capacity (TAC) was measured with a commercial kit from Zell Bio Company, Germany. Measurements for triglycerides (TG) were conducted using commercial kits from Pars Azmoon Co. in Tehran, Iran, and analyzed with a biochemical autoanalyzer (Alpha Classic AT++, Sanjesh Company, Iran).

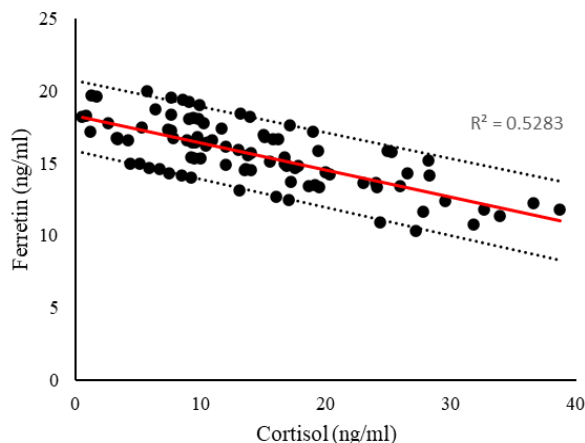
### 2.5. Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Multivariable models were developed with the PROC MIXED procedure to assess the associations between oxidative stress biomarkers and

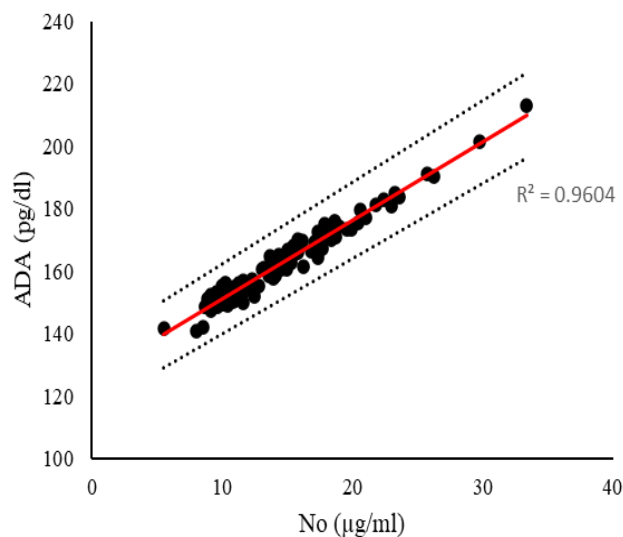
metabolic parameters. The models included fixed effects for treatment, sampling time, and their interaction, along with linear and quadratic terms for the explanatory metabolic variable. Treatment, sampling time, and the linear effect of the metabolic variable were retained in all models, while the interaction term and quadratic effect were excluded when the P-value was  $\geq 0.05$ . Residuals were visually inspected to confirm normality and homogeneity of variance. Animals were included as a random effect nested within treatment, and repeated measures were accounted for by, including the day of blood sample collection as a repeated factor. Significant associations identified from these analyses were further examined using Pearson correlation coefficients, calculated with the PROC CORR procedure. Statistical significance was set at  $P \leq 0.05$ , with trends reported for  $0.05 < P \leq 0.10$ .

## 3. Results

Ferritin was associated with sampling time ( $P = 0.04$ ) and cortisol ( $P < 0.001$ ), but treatment, the interaction of



**Figure 1.** The relationship between adenosine deaminase (ADA) and cortisol in Saanen goat kids receiving probiotic and yeast extract supplementation during the weaning phase

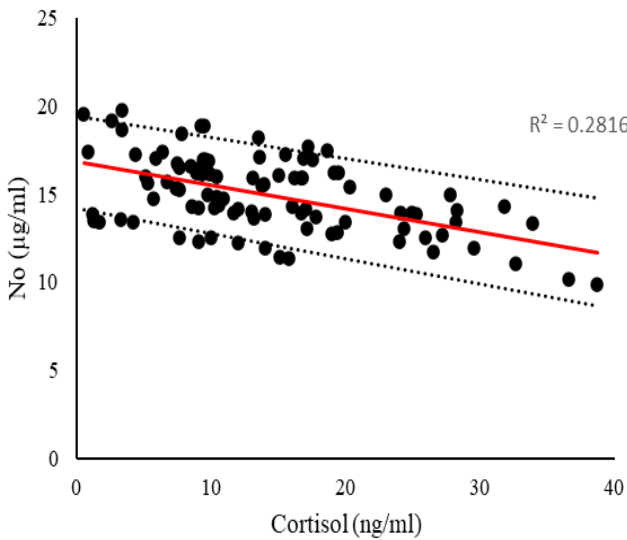


**Figure 2.** The relationship between deaminase (ADA) with nitric oxide (No) in Saanen goat kids receiving probiotic and yeast extract supplementation during the weaning phase

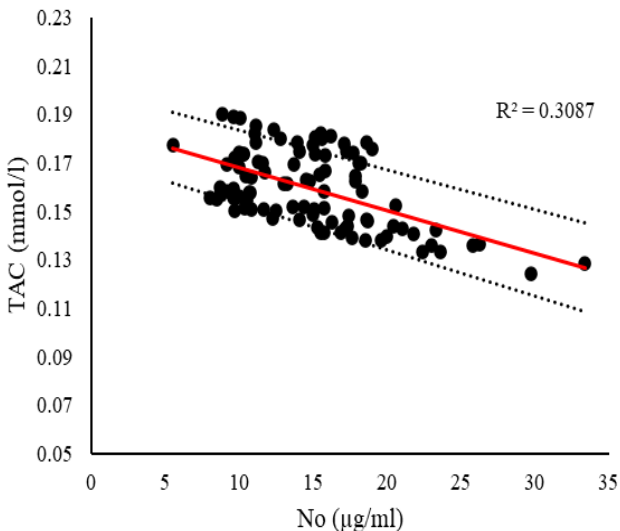
treatment and sampling time, and the quadratic term were not associated with Ferritin concentration. Correlation analysis showed a significant negative correlation between Ferritin and Cortisol  $R^2 = 0.53$  (Figure 1).

ADA was not associated with sampling time ( $P < 0.001$ ), treatment ( $P < 0.001$ ), interaction of treatment and sampling time, and quadratic effect of No, but No was associated with ADA concentration ( $P < 0.001$ ). Correlation analysis showed a significant positive correlation between ADA and No ( $R^2 = 0.96$ , Figure 2).

No was associated with sampling time ( $P = 0.04$ ) and cortisol ( $P = 0.02$ ), but treatment interaction of treatment and sampling time and the quadratic term was not associated with No concentration. Correlation analysis



**Figure 3.** The relationship between nitric oxide (No) with cortisol in Saanen goat kids receiving probiotic and yeast extract supplementation during the weaning phase



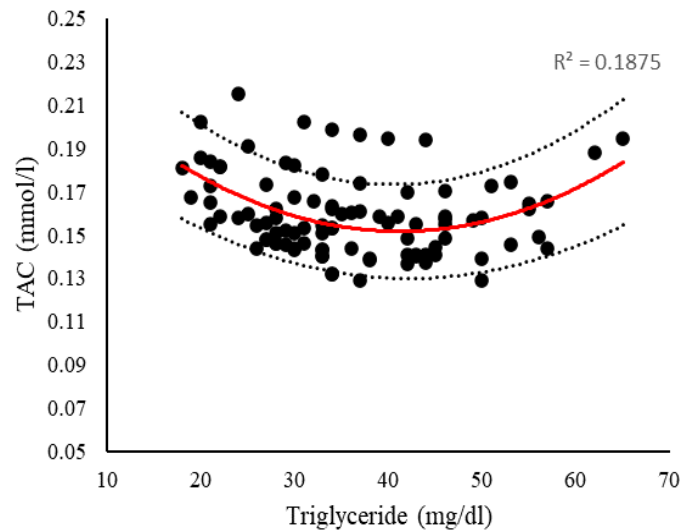
**Figure 4.** The relationship between total antioxidant capacity (TAC) with nitric oxide (No) in Saanen goat kids receiving probiotic and yeast extract

supplementation during the weaning phase

showed a significant negative correlation between No and Cortisol  $R^2 = 0.28$  (Figure 3).

TAC was associated with sampling time ( $P < 0.001$ ) and No ( $P = 0.04$ ), but treatment, the interaction of treatment and sampling time, and the quadratic term were not associated with No concentration. Correlation analysis showed a significant negative correlation among TAC and No  $R^2 = 0.31$  (Figure 4).

TAC was associated with sampling time ( $P < 0.001$ ) and triglyceride ( $P = 0.03$ ), but treatment was not associated with No concentration. The quadratic effect of triglyceride was associated with TAC ( $P = 0.04$ ). Correlation analysis showed a significant quadratic correlation between TAC and triglyceride  $R^2 = 0.19$  (Figure 5).



**Figure 5.** The relationship between total antioxidant capacity total antioxidant capacity (TAC) with triglyceride in Saanen goat kids receiving probiotic and yeast extract supplementation during the weaning phase

#### 4. Discussion

The blood parameters of small ruminants are influenced by numerous factors, including breed, sex, nutrition, environmental conditions, and the animal's overall health. Additionally, cortisol levels in goats can fluctuate due to diurnal and seasonal variations<sup>14-17</sup>. This study focuses on the relationship between oxidative stress and inflammatory response markers in Saanen goat kids supplemented with probiotics and yeast extract. However, since the study was conducted on a single farm, the findings should be interpreted cautiously, and further studies are recommended to enhance their generalizability.

In a study by Kabirian Moghadam et al.<sup>18</sup>, calves receiving probiotics showed reduced ferritin levels during the weaning phase compared to a control group. The current study also revealed a negative correlation between cortisol and ferritin, suggesting that as cortisol levels rise due to stress, ferritin levels tend to decrease. This aligns with findings that animals experiencing inflammatory conditions often exhibit lower serum iron levels<sup>19</sup>. However, Orino et al.<sup>20</sup> highlighted that ferritin levels might increase in response to oxidative stress.

The current study suggests a complex interplay where probiotics may reduce ferritin levels during elevated cortisol states, although dietary variations could also influence ferritin dynamics over time.

The study also identified a negative correlation between nitric oxide (NO) and cortisol, indicating that NO levels decrease as cortisol levels rise during stress conditions such as weaning. This stress-induced fluctuation in cortisol concentrations may persist for about a week post-weaning, although individual differences, sampling times, and other stress-related factors could also contribute to these variations. NO, a key oxidative stress marker, appears to decrease under heightened stress, which aligns with cortisol's role in modulating stress responses. Furthermore, cortisol levels are influenced by factors such as circadian rhythms, seasonal changes, dietary composition, and adrenal gland activity.

Dietary interventions significantly impact oxidative stress biomarkers. Research by Hosoda et al.<sup>21</sup>, Casamassima et al.<sup>22</sup>, and Di Trana et al.<sup>23</sup> underscores the importance of diet in modulating oxidative stress. *In vitro* analyses by Kim et al.<sup>6</sup> demonstrated that probiotics from various species possess antioxidant properties, possibly due to their ability to bind metal ions, enhance the host's antioxidant defenses, and synthesize antioxidant enzymes like glutathione. Probiotic metabolites, such as butyrate, also exhibit antioxidant effects by inhibiting harmful bacteria that generate endotoxins contributing to oxidative stress<sup>25</sup>. This study found a positive correlation between NO and adenosine deaminase (ADA), indicating that as NO levels rise, inflammatory markers like ADA also increase. This connection suggests a link between oxidative stress and inflammatory responses.

Oxidative stress significantly affects apoptosis, with antioxidants potentially mitigating cell death<sup>26</sup>. Increased NO levels observed post-weaning may result from reduced energy availability in goat kids unaccustomed to dry feed. The study also found a negative correlation between total antioxidant capacity (TAC) and NO, suggesting an inverse relationship between these oxidative stress markers. Previous studies have linked food deprivation to elevated NO levels in rats, supporting the need for further research on dietary supplements and their effects on oxidative stress during weaning<sup>27,28</sup>.

Additionally, a positive correlation was observed between TAC and triglyceride levels, indicating that lipid metabolism may be disrupted during stress. Probiotic supplementation has been shown to improve lipid metabolism, reduce triglycerides, NEFA, and low-density lipoproteins in small ruminants<sup>29</sup>. However, during stressful periods like weaning, increased TAC might coincide with elevated triglyceride levels due to metabolic imbalances. These findings highlight the importance of addressing oxidative stress and lipid metabolism during critical growth phases to improve the welfare and performance of goat kids. Future research should explore dietary strategies and supplements to mitigate oxidative stress and enhance the resilience of small ruminants during weaning.

## 5. Conclusion

The findings suggest that under stress conditions, such as weaning in goat kids, the supplementation of probiotics (1 gr

per animal) and yeast extract (3 gr per animal) in Saanen goat kids demonstrated a correlation between stress parameters, including cortisol, nitric oxide, and total antioxidant capacity, and inflammatory markers such as adenosine deaminase and ferritin, as well as energy metabolites like triglycerides. Additionally, further research is warranted to explore the effects of probiotics and yeast extract in different stress conditions, including vaccination and dehorning.

## Declarations

### *Competing interests*

The author declares that there is no conflict of interest.

### *Authors' contributions*

Ali Hajimohammadi was responsible for designing the experiment, performing the statistical analysis, and revising the final manuscript. Sajjad Kazemi undertook the sampling and authored the article. Additionally, Ali Hajimohammadi conducted a statistical analysis. Saeed Nazifi carried out the laboratory work, while Sajjad Kazemi was involved in writing the manuscript. All authors read and approved the final version of the manuscript.

### *Authors' relationships and activities*

The authors declare that there is no relationship and activities.

### *Funding*

This study was conducted without financial support and this study was a part of a thesis that was published before.

### *Availability of data and materials*

All relevant data and materials used in this study are available from the authors upon reasonable request to ensure transparency and facilitate further research.

### *Ethical considerations*

The authors confirm that ethical guidelines were thoroughly reviewed and adhered to during the study. This includes compliance with principles related to plagiarism, obtaining consent for publication, and avoiding data fabrication or falsification. The integrity and accuracy of the research were prioritized at all stages.

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