



Effects of Repeated Low-Dose N-Acetylcysteine Administration on Oxidative Stress and Growth Performance in Saanen Goat Kids During Weaning

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ABSTRACT

Oxidative stress related to the weaning period in ruminants may increase susceptibility to diseases. However, information regarding the physiological changes associated with oxidative stress in goats during the weaning period is limited. In this study, twenty healthy male Saanen goat kids were randomly assigned to treatment and control groups, at approximately seventy-five days of age. N-acetylcysteine (NAC) was administered intramuscularly at a dose of 15 mg/kg to the treatment group two days before and for seven days following weaning. Blood samples were collected from both groups two days before, seven days after, and fourteen days after weaning. Total antioxidant capacity (TAC) and total oxidant status (TOS) were measured in the serum samples. Additionally, the body weights of all kids were recorded on the blood sampling days. No significant differences were found between groups in terms of body weight, TAC, TOS, and oxidative stress index (OSI). However, there were significant differences within groups in TOS, OSI, and live weight analysis. In conclusion, although low doses of NAC were not significantly effective in improving growth rates, they did temporarily reduce TOS levels.

Keywords: Goat kid, Growth rate, N-Acetylcysteine, Oxidative stress, Weaning.

ÖZ

Saanen Oğlaklarında Sütten Kesim Döneminde Tekrarlayan Düşük Doz N-Asetilsistein Uygulamasının Oksidatif Stres ve Büyüme Performansı Üzerine Etkileri

Sütten kesim dönemine bağlı oksidatif stres, gevş getiren hayvanlarda hastalıklara yatkınlığı artırabilir. Ancak, sütten kesim döneminde keçilerde oksidatif stres ile ilişkili fizyolojik değişimlere dair bilgiler sınırlıdır. Bu çalışmada, yaklaşık yetmiş beş günlük yaşta olan yirmi sağlıklı erkek Saanen oğlağı rastgele olarak tedavi ve kontrol gruplarına ayrılmıştır. Tedavi grubuna, sütten kesimden iki gün önce ve sütten kesimi takip eden yedi gün boyunca 15 mg/kg dozunda N-Asetilsistein (NAC) intramüsküler yolla uygulanmıştır. Her iki gruptan da sütten kesimden iki gün önce, sütten kesimi takiben yedi ve on dört gün sonra kan örnekleri alınmıştır. Serum örneklerinde toplam antioksidan kapasite (TAC) ve toplam oksidant kapasite (TOS) düzeyleri ölçülmüştür. Ayrıca, tüm oğlakların canlı ağırlıkları da kan örneklemeye günlerinde kaydedilmiştir. Gruplar arasında canlı ağırlık, TAC, TOS ve oksidatif stres indeksi (OSI) bakımından anlamlı bir fark bulunmamıştır. Ancak, grupların kendi iç analizlerinde TOS, OSI ve canlı ağırlık verilerinde anlamlı farklılıklar gözlemlenmiştir. Sonuç olarak, düşük dozda NAC uygulamasının büyümeye oranlarını anlamlı düzeyde artırmadığı, ancak TOS düzeylerini geçici olarak düşürdüğü belirlenmiştir.

Anahtar Kelimeler: Büyüme oranı, N-Asetilsistein, Oğlak, Oksidatif stres, Sütten kesme.

INTRODUCTION

Weaning is a transitional period in young animals, marking the shift from milk to solid feed. In ruminants, weaning is not a single event but rather a process in which milk is gradually replaced by forage, concentrates, or grain-based diets. During this period, the diet transitions from a mixture of milk-based ingredients to a more complex combination of fatty acids, glucose, and both microbial-

and milk-derived amino acids. Due to the significant changes in dietary composition, ruminants require adaptation in rumen function (Baldwin et al. 2004). Weaning can thus be considered a major metabolic event for young ruminants, whose digestive systems are not yet fully developed (Kelly and Coutts 2000). Consequently, weaning stress can negatively impact the growth performance of goat kids (Izuddin et al. 2019).

Weaning induces significant psychological stress in

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addition to physiological changes. Both psychological and physiological stress contribute to increased oxidative stress, immune system suppression, and disruptions in key host-microbe interactions. These adverse effects on goat kids may lead to substantial economic losses (Ugur et al. 2007; Liao et al. 2019; Zhang et al. 2022).

In animals, stress can be assessed through behavioral observations and various biochemical parameters, including cortisol levels, acute-phase proteins, and liver function tests (Bertoni et al. 2005; Magistrelli et al. 2013; Zobel et al. 2020). In Saanen goat kids, plasma cortisol measurement is commonly used to evaluate stress levels (Silva et al. 2021). Oxidative stress is closely linked to an animal's immune status and may serve as a risk factor for diseases affecting goat kids during weaning (Cheng et al. 2021). Furthermore, oxidative and antioxidant balance in animals can be influenced by changes in nutrition and metabolic state (Celi and Gabai 2015). The oxidative status at the time of weaning can be evaluated using total antioxidant capacity (TAC), a key oxidative biomarker (Cheng et al. 2021).

There are two main methods for weaning goat kids: abrupt and gradual weaning. Although abrupt weaning has more detrimental effects than gradual weaning, it is still widely used (Vickery et al. 2022; Vickery et al. 2023). In recent years, various strategies, such as the use of probiotics, yeast extract, and enzyme supplementation in rations, have been investigated to mitigate the adverse effects of weaning stress in goat kids (Kazemi et al. 2023; Lu et al. 2023). Reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are primarily generated by mitochondria as by-products of normal metabolism during the conversion of molecular oxygen to water (Fang et al. 2002).

N-acetylcysteine (NAC), a natural derivative of L-cysteine and a precursor to reduced glutathione (GSH), is a widely used small-molecule antioxidant that enhances intracellular GSH synthesis, increases glutathione-S-transferase activity, promotes detoxification, and acts as a free radical scavenger (Whitaker et al. 2012; Rushworth and Megson 2014). Additionally, NAC is classified as a mucolytic agent and exhibits antioxidant, antibacterial, and anti-inflammatory properties in various disease models in both human and veterinary medicine (Tenorio et al. 2021; Tieu et al. 2023).

The aim of this study was to investigate the effects of multiple NAC administered intramuscularly at a dose of 15 mg/kg on the oxidant-antioxidant balance in serum and body weight gain in weaned Saanen male kids.

MATERIAL AND METHODS

Ethics Approval

All procedures were approved by the Animal Ethics Committee (AEC) at Mehmet Akif Ersoy University: 16.04.2025/1465

Animals and Experimental Study

This study was conducted in Türkiye province of Burdur and included 20 healthy male, Saanen kids at the weaning period (70-75 days old). The animals were randomly divided into two groups treatment (n:10) and control group (n:10). In selecting the animals to be included in the groups, the criterion that goats gave birth to a single kid in the same litter was taken into account.

Animal Management

Preventive medical practices are applied to all goats in the herd. Neonatal process management is applied to newborns. During the study, kids were fed only their mother's milk in an artificial mother-milking system. Enterotoxemia (Coglavax, Ceva) and Pasteurella vaccines were administered at 2 months of age according to the manufacturer's instructions. In addition, antiparasitic treatment was applied with antiparasitic drugs containing mebendazole and closantel (Mebec, Ceva) following the manufacturer's instructions.

Blood Samples Collection

Blood samples were collected two days before weaning, seven days after weaning, and fourteen days after weaning. All samples were taken in the morning, on an empty stomach, via jugular venipuncture using coagulated tubes. The blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum and stored at -20 °C until analyzed.

Treatment Protocol

Treatment applications commenced two days before weaning and continued until seven days post-weaning. During this period, NAC (Asist, 300 mg/3 mL ampoule, Hüsnü Arsan Pharmaceuticals) was administered intramuscularly to the kids at a dose of 15 mg/kg.

Weaning and Transition to Concentrate Feeding Program in Goat Kids

After approximately 70 days of milk feeding, the goat kids underwent a gradual transition program before weaning. This transition process was initiated five days before the planned weaning date. During this period, the daily milk intake was reduced to two meals per day (a total of 1 L/kg/day), while a high-quality starter feed containing 18% crude protein was introduced. At this stage, each kid was offered approximately 50-100 g of concentrate feed per day and 20-30 g of alfalfa to stimulate rumen function. Two days before weaning, milk was reduced to one feeding per day, and the amount of concentrate feed was increased to 100-150 grams per kid. On the weaning day (day 0), milk feeding was stopped completely. On the same day, concentrate intake was increased to 150-200 g per kid per day, clean drinking water was provided ad libitum, and alfalfa intake was increased to 50 g per kid per day. Over the subsequent seven days, the concentrate intake gradually increased to 250-300 g per kid per day. No adaptation problems were observed at the end of the transition period, and all kids showed good appetite and consistent feed intake.

Body Weight Measurement

Weight measurements were conducted on days 2 days before weaning and 7 - 14 days after weaning.

Biochemical Analysis Methods

Total antioxidant capacity (TAC) levels were measured using commercially available kits via the colorimetric method (catalog number: RL0017/ Relassay, Türkiye). This method is based on the bleaching of the characteristic color of a more stable 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation by antioxidants. The results were expressed as mmol Trolox equivalent/L (Erel 2004). Total oxidant status (TOS) levels were measured using commercially available kits (catalog number: GP81170/ Relassay, Türkiye). The ratio of TOS to TAC was accepted as the OSI. For calculation, the resulting unit of TAC was converted to μ mol/L, and the OSI value was calculated according to the following formula: OSI

(arbitrary unit) = TOS ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) / TAC ($\mu\text{mol Trolox equivalent/L}$) (Erel 2005; Yumru et al. 2009). The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was converted to $\mu\text{mol/L}$, and the OSI value was calculated according to the following Formula: OSI (arbitrary unit) = TOS ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) / TAC ($\mu\text{mol Trolox equivalent/L}$).

Statistical Analysis

Normality distribution was assessed using the Shapiro-Wilk test. Differences in live weight between groups at each sampling time were analyzed using the independent samples t-test. Within-group comparisons of live weight, total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) levels across measurement times were conducted using repeated measures ANOVA. Mauchly's sphericity test and Wilks' Lambda test results were interpreted accordingly. If the data were not normally distributed, the Friedman test was applied. For post hoc analyses, differences between groups were evaluated using the Bonferroni or Wilcoxon test. A p-value of <0.05 was considered statistically significant.

RESULTS

The results of body weight analysis within and between groups are presented in Table 1. The total antioxidant stress analysis results within and between groups are provided in Table 2. The total oxidant stress analysis results within and between groups are given in Table 3. The oxidative stress index analysis results within and between groups are shown in Table 4. These are also provided in Figure 1.

Significant differences were observed in the within-group live weight analysis for both groups. In the total oxidant

status analysis, significant differences were found within the experimental group. Additionally, significant within-group differences were detected in oxidative stress analysis for both groups.

Table 1: Body Weight Changes on Sample Days

Group/Days	Pre-weaning			Post-weaning		
	Day -2	Day 7	Day 14	Day -2	Day 7	Day 14
Treatment	21.10 \pm 2.58 ^a	22.70 \pm 0.94 ^b	24.60 \pm 0.93 ^c			
Control	21.54 \pm 2.65 ^a	22.30 \pm 0.96 ^b	24.24 \pm 0.95 ^c			

Different lowercase letters within the same row indicate statistically significant differences between time points within the same group ($p<0.05$).

Table 2: Total Antioxidant Stress

Group/Days	Pre-weaning			Post-weaning		
	Day -2	Day 7	Day 14	Day -2	Day 7	Day 14
Treatment	1.43 \pm 0.08	1.26 \pm 0.05	1.26 \pm 0.07			
Control	1.45 \pm 0.13	1.20 \pm 0.07	1.17 \pm 0.04			

Different lowercase letters within the same row indicate statistically significant differences between time points within the same group ($p<0.05$).

Table 3: Total Oxidant Stress

Group/Days	Pre-weaning			Post-weaning		
	Day -2	Day 7	Day 14	Day -2	Day 7	Day 14
Treatment	6.06 \pm 0.69 ^a	3.77 \pm 0.27 ^b	6.48 \pm 0.46 ^a			
Control	5.83 \pm 0.57	4.23 \pm 0.39	6.29 \pm 0.69			

Different lowercase letters within the same row indicate statistically significant differences between time points within the same group ($p<0.05$).

Table 4: Oxidative Stress Index

Group/Days	Pre-weaning			Post-weaning		
	Day -2	Day 7	Day 14	Day -2	Day 7	Day 14
Treatment	21.10 \pm 2.58 ^a	22.70 \pm 0.94 ^b	24.60 \pm 0.93 ^c			
Control	21.54 \pm 2.65 ^a	22.30 \pm 0.96 ^b	24.24 \pm 0.95 ^c			

Different lowercase letters within the same row indicate statistically significant differences between time points within the same group ($p<0.05$).

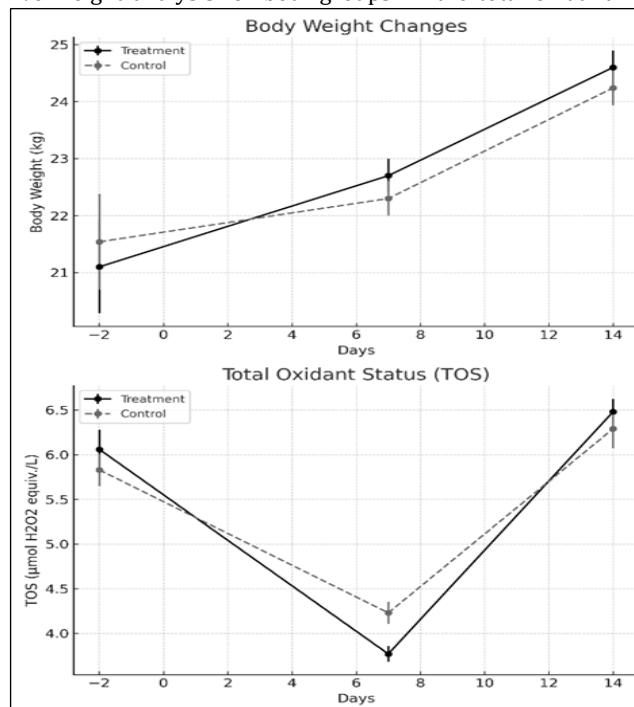


Figure 1. Body weight, Total Antioxidant Status, Total Oxidant Status and Oxidative Stress Index

DISCUSSION AND CONCLUSION

Weaning is a critical transition in young animals, shifting them from milk to solid feed. During this period, young ruminants experience growth stasis due to significant

metabolic stress (Baldwin et al. 2004; Izuddin et al. 2019). In recent years, various treatments have been explored to mitigate metabolic stress during weaning (Kazemi et al. 2023). However, no pharmacological agent or method has been identified as prominently effective in reducing this stress. In this study, N-acetylcysteine (NAC) was

administered at a dose of 15 mg/kg to goat kids to evaluate its effects on oxidative stress parameters and live weight gain.

Kazemi et al. (2023) reported a mean live weight of 15.18 \pm 0.6 kg in the control group at the beginning of the weaning period (approximately day 80). In parallel with the present study, they observed a significant increase in live weight during the process. Furthermore, they reported no significant differences in TAC levels between the groups. Similarly, Al-Sharawy et al. (2024) investigated the effects of oral NAC administration at doses of 50 mg/ml, 100 mg/ml, and 200 mg/ml, with each kid receiving 5 ml. At the end of their study, they found a significant difference in growth rate between the NAC-treated groups and the control group. However, it should be noted that they administered substantially higher NAC doses compared to the present study. Given the limited knowledge regarding NAC and its effects on growth rate, further scientific studies are needed.

Total antioxidant capacity (TAC) is one of the most commonly used biomarkers for evaluating oxidative status during the weaning period (Cheng et al. 2021; Kazemi et al. 2023). Atakisi et al. (2016) reported a significant increase in TAC levels within several hours following a single intramuscular NAC administration (100 mg/kg) in rabbits. Kaya et al. (2023) investigated the protective role of NAC in rats with kidney damage and found no significant changes in TAC levels between the control group and the healthy NAC-treated group. Similarly, Gunturk et al. (2019) reported no significant differences in TAC levels between healthy controls and NAC-treated groups in rats. Kazemi et al. (2023) also found no significant effect on TAC levels in goat kids in their study examining the effects of yeast extract and probiotic supplementation on oxidative stress, inflammatory response, and growth. In contrast, Bozkurt et al. (2023) reported significant changes in TAC levels in goats suffering from papillomatosis, both with and without ivermectin administration. Moreover, in line with our study, they observed fluctuating TAC levels throughout the treatment process. Previous studies have been conducted on various animal species, often using higher NAC doses than in the present study. Accordingly, the findings of the present study are consistent with those reported in previous research.

These results are further supported by our observations on total oxidant status (TOS). We found a significant decrease in TOS levels only in the experimental group at the second sampling time compared to other time points. In contrast, Bozkurt et al. (2023) reported significant increases in their control group; however, it is important to note that these goats were infected with papillomavirus and received no treatment. Similarly, Atakisi et al. (2016) observed significantly reduced TOS levels in rabbits following a single NAC administration, while Kaya et al. (2023) found similar TOS levels between the control and healthy NAC groups, with slightly lower levels in the NAC group—a trend that aligns with our findings. On the other hand, Gunturk et al. (2019) reported higher TOS levels in the NAC-treated group compared to the healthy control group. Notably, in our study, the decrease in TOS levels coincided with the NAC administration period, and this significant reduction was observed only in the experimental group. Thus, it can be inferred that the observed changes are attributable to the antioxidant properties of NAC.

Gokce et al. (2022) reported a mean OSI of 4.28 \pm 0.29 in healthy lambs on day 28 and observed that OSI levels

generally decrease from parturition to day 28. In contrast, Tekeli et al. (2024) found a mean OSI of 2.80 \pm 0.52 in healthy goats, with goats aged 0 to 6 months. They also noted lower OSI levels in goats older than six months, which is consistent with the findings of Gokce et al. (2022). In the present study, the mean OSI levels of the groups were found to align with those reported by Gokce et al. (2022). However, the OSI levels of the experimental group on the 7th day were lower than those observed on other days. This difference can be attributed to the antioxidant effects of NAC. Consequently, a significant difference in OSI levels between the 7th and 28th-day sampling times was only observed in the experimental group. Similar to previous studies, OSI levels in both groups tended to increase toward the end of the period.

The low number of goat kids included in the study, the exclusive use of intramuscular administration, the 15 mg/kg dosage, and the investigation of a limited number of biochemical parameters are some limitations of the present study. On the other hand, NAC is a commonly used and well-known effective antioxidant molecule. In previous studies, yeast extract and probiotics were found to be ineffective in reducing oxidative stress in goat kids. The relationship between oxidative stress, growth rate, and low-dose NAC administration represents a strength of this study. Future research should investigate different dosages and administration routes of NAC to better manage oxidative stress in goats during the weaning period.

In conclusion, although a low dose of NAC may have limited effectiveness in enhancing growth rate, it can temporarily decrease total oxidative stress (TOS) levels.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: FK, GB
Supervision / Consultancy: FK, GB
Data Collecting and Processing: GB
Analysis and Interpretation: FK, GB
Writing the Article: FK
Critical Review: FK, GB

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