

Effect of two levels of AD3EC on the rumen environment of local lambs.

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Abstract

This study aimed to investigate the effect of two levels of AD3EC on the rumen environment of local lambs during the period, from, 2\1\2025 to 1\4\2025. The study was conducted at the Agricultural Station affiliated with the College of Agriculture and Marshes. Twelve local sheep lambs at weaning age were purchased and divided into three treatments, with four lambs per treatment, having an average weight of 13.00–13.55 kg. The first treatment served as the control and was not administered AD3EC, left for comparison. The second treatment received 3 ml of AD3EC, and the third treatment received 4 ml of AD3EC. All lambs were fed the same diet. The results showed that in the first, second, and third months of the study, there was a significant increase in pH values for the treatments receiving AD3EC compared to the control treatment. Additionally, regarding total bacteria, in the first, second, and third months, there was a significant increase in total bacterial counts for the treatments that received AD3EC, compared to the control treatment.

I. Introduction

The rumen environment is home to many microorganisms, fungi, bacteria, protozoa, and anaerobic fungi. These organisms live symbiotically within the rumen of ruminants, digesting roughage and converting it into protein, called microbial protein, through anaerobic fermentation in the rumen. They also produce soluble sugars, proteins, fats, and other nutrients Gruninger et al., (2019). Vitamins A3 and E3 work to improve the pH of the rumen fluid and create a suitable environment for these microorganisms within the rumen, improving their function and increasing their numbers digestion and absorption in the rumen environment, and vitamin A maintains the integrity of the epithelial tissue lining the rumen and prevents inflammation (Moise et al., 2007). Vitamin D3 aids in the metabolism and absorption of phosphorus and calcium in the intestine, which are important for skeletal growth (Mora et al., 2008). Vitamin E acts as an antioxidant, protecting rumen cell membranes from damage. In addition, it plays an important immunological role in preventing inflammation, which increases rumen health (Lewis et al., 2019). Vitamin C protects cells from damage by acting as an antioxidant and helping to neutralize free radicals. It is considered a powerful reducing agent and free radical scavenger (Rice, 2000). This study aimed to investigate the effect of adding two levels of AD3EC to the rumen environment of local lambs

II. Materials and Methods

The experiment consisted of a preliminary period, which took place within the 90-day period, from, 2\1\2025 to 1\4\2025. The experiment was conducted in the animal field of the College of Agriculture and Marshlands, University of Thi Qar. It was divided into four groups: the first group, serving as the control group, did not receive AD3EC vitamins. The second group was administered 3 ml of AD3EC, diluted with distilled water. The third group received 4 ml of AD3EC, also diluted with distilled water, and was administered twice every ten days. The lambs were fed the same concentrated feed, comprising 40% crushed barley, 25% wheat bran, 12% crushed yellow

corn, 7% flour, 13% soybeans, and 3% salts. Veterinary care was provided, including a preventive program covering all necessary vaccinations to protect the animals from diseases. Additionally, the animals were vaccinated against intestinal and liver flukes. The animals were treated with albendazole at a dose of 1 ml/10 kg (600 ml/mg) and were injected with an antiparasitic drug. Rumen fluid samples were collected monthly at the end of each month throughout the experimental period, both before and several hours after feeding. The rumen fluid was collected by inserting a gastric tube through the lambs' mouths, down the esophagus, and into the rumen. Subsequently, the rumen fluid was observed flowing down the tube, which was then transferred in to a stomach tube and sealed tightly. The samples were promptly taken to the laboratory for pH measurement and bacterial counting, as described below:

1- pH Measurement: 2-

The rumen fluid, collected in the stomach tube, was extracted directly and tested using a pH meter. Prior to utilization, the device was calibrated to ensure a more precise reading. A probe was immersed in a buffer solution for calibration. Following calibration, the probe, devoid of air bubbles and fully submerged, was placed in to the rumen fluid, and the pH reading was allowed to stabilize on the device's screen, after which it was recorded.

2-2-Calculation of Total Bacterial Counts with Rumen Fluid:

This process involves the plate count method. Initially, 1 ml of the rumen fluid sample containing bacteria is taken and deposited in a tube with 9 ml of sterile water, which is then shaken (1:10 dilution). Subsequently, 1 ml from the former tube is transferred, via a pipette, to another tube containing 9 ml of sterile water, which is again shaken (1:100 dilution). This process is reiterated multiple times to achieve dilutions up to 1:1,000,000. Following this, 1 ml from the last three dilutions is transferred to well-sterilized Petri dishes. Two dishes are allocated for each dilution. Nutrient agar, onco-liquefied at 45°C, is added and poured into the dishes, which are gently swirled to ensure medium dispersion, cooled, and allowed to solidify. Subsequently, the dishes are inverted and placed in an incubator at 37°C for 48 hours. Post-incubation, the number of live bacterial cells in 1 ml of the original culture is computed using the subsequent formula: Number of bacterial colonies/cm³ of the original sample = Number of colonies in the dish × Reciprocal of the dilution utilized in the colony count method on culture plates.

III. Results and discussion:

pH

The results showed a significant increase in the pH values of the rumen fluid in the experimental treatments throughout the study period. In the first month of the experiment, there was a significant increase in the values of the treatments that received AD3EC, with averages of (6.36 and 5.80), compared to the control treatment, which averaged (5.34). Similarly, in the second month of the experiment, there was a significant increase in the treatments that received AD3EC, with averages of (6.54 and 6.50), compared to the control treatment, which averaged (5.38). Also, in the third month of the experiment, there was a significant increase in the treatments that received AD3EC, with averages of (6.63 and 5.91), compared to the control treatment, which averaged (5.37). The AD3EC vitamins, as shown in Table 1, improved the rumen environment and brought pH levels within the ideal limits for bacterial growth, as microbes in the sheep rumen interact with the ratios of food and vitamins to produce acids. Volatile fatty acids, in turn, affect the pH level, which is very important in regulating the activity of the numbers and proportions of microorganisms within the rumen environment Dunn et al., (1979).

Table 1: pH values of the rumen fluid of sheep in the control group and the two treatment groups with two levels of AD3EC throughout the study period.

Experimental treatment	PH		
	The first month	The second month	The third month
Control treatment	5.34± 0.14b	5.38± 0.03 b	5.37± 0.04 b
Second treatment	5.80± 0.19a	6.50±0.04 a	5.91±0.05 a
Third treatment	6.36± 0.07 a	6.54± 0.04a	6.63 ±0.05 a
Significant	Significant 0.05	Significant 0.05	Significant 0.05

Different letters indicate significant differences between treatments. The first treatment, the control, was not given AD3EC. The second treatment was given 3 ml of AD3EC. The third treatment was given 4 ml of AD3EC.

Total Bacteria Count

The results of the study indicated a significant superiority in the average values of total bacterial counts between the experimental treatments. In the first month of the experiment, there was a significant increase in the second and third treatments, which were dosed with AD3EC, with averages of (281.56 and 260.23) $\times 10^8$ CFU/ml, compared to the control treatment with an average of (40.00) $\times 10^8$ CFU/ml. In the third month of the study, there was a significant increase in the treatments treated with AD3EC, with an average of (564.18 and 576.24) $\times 10^8$ CFU/ml, compared to the control treatment with an average of (80.37) $\times 10^8$ CFU/ml. In the second month, the treatments treated with AD3EC outperformed with an average of (157.00 and 134.20) $\times 10^8$ CFU/ml, compared to the control treatment with an average of (33.49) $\times 10^8$ CFU/ml. Table (2). AD3EC vitamins maintained the rumen environment within the ideal levels of pH and bacterial counts. In a study conducted by Zhang et al. (2015), it was shown that adding vitamin E improved the digestibility of dry matter and protein. Adding vitamin E at an amount of 30 IU/kg improved the rumen environment and increased the bacterial counts. In a study conducted by Zhang et al. (2018), on vitamins given to goats, it was found that when administered over a period of 50 days, the number of rumen bacteria increased.

Table (2): Number of bacteria $\times 10^8$ in the rumen fluid of lambs in the control group and the group that received two levels of AD3EC during the study period.

Experimental treatment	Total bacteria		
	The first month	The second month	The third month
Control treatment	40.00±7.45 b	33.49±12.37 b	80.37±102.00 b
Second treatment	260.23 ±18.28 a	134.20±45.31a	564.18±323.15a
Third treatment	281.56 ±51.93 a	157.00±40.24 a	576.24±275.94 a
Significant	Significant 0.05	Significant 0.05	Significant 0.05

The first treatment, the control, was not given AD3EC. The second treatment was given 3 ml of AD3EC. The third treatment was given 4 ml of AD3EC. *Different letters indicate significant differences between treatments.*

IV. References

Dunn, B. H., Emerick, R. J., Enbry, L. B. (1979) Sodium benzoate and sodium bicarbonate in high-concentrate diets for lambs and steers. J. Anim. Sci. 48: 764-769

Rice, M.E. (2000): Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci., 23(5): 209-216

Gruninger, R.J.; Ribeiro, G.O.; Cameron, A.; McAllister, T.A. (2019) Invited review: Application of meta-omics to understand the dynamic nature of the rumen microbiome and how it responds to diet in ruminants. Animal, 13, 1843-1854.

Lewis, E. D., Meydani, S. N., & Wu, D. (2019). Regulatory role of vitamin E in the immune system and inflammation. IUBMB Life, 71(4), 487-494.

Moise, A. R., Noy, N., Palczewski, K., & Blaner, W. S. (2007). Delivery of retinoid-based therapies to target tissues. Biochemistry, 46(15), 4449-4458.

Mora Immunol Iwata, M. and VonAndrin, U. H. (2008). Vitamin effect on the immune system: vitamins A and D take center stage. Nat. 8: 685-698., J.R.:

Wei, C., Lin, S. X., Wu, J. L., Zhao, G. Y., Zhang, T. T., & Zheng, W. S. (2015). Effects of supplementing vitamin E on in vitro rumen gas production, volatile fatty acid production, dry matter disappearance rate, and utilizable crude protein. Czech Journal of Animal Science, 60(8), 335-341.

Zhang, R.Y.; Jin, W.; Feng, P.F.; Liu, J.H.; Mao, S.Y. 2018 High-grain diet feeding altered the composition and functions of the rumen bacterial community and caused damage to the laminar tissues of goats. Animal, 12, 2511-2520