Volume 59, January-December 2025, ISSN: 2079-3480

ANIMAL SCIENCE

CUBAN JOURNAL OF AGRICULTURAL SCIENCE

Journal site: https://cjascience.com Cu-ID: https://cu-id.com/1996/v59e12

EFFECT OF THE INCLUSION OF EFFICIENT MICROORGANISMS ADIZOOT 1® ON DIETS FOR FEMALE PELIBUEY SHEEP

EFECTO DE LA INCLUSIÓN DE MICROORGANISMOS EFICIENTES ADIZOOT 1® EN LA DIETA DE OVINOS PELIBUEY HEMBRAS

D. RODRÍGUEZ ACOSTA[1*], YOANDRA MARRERO RODRÍGUEZ[2], NIURCA GONZÁLEZ IBARRA[2], AVELINA LEÓN GOÑI[2], ILIANA SOSA TESTÉ[2]

[1]*Centro de Investigaciones de Plantas Proteicas y Productos Bionaturales (CIPB), La Habana, Cuba*
[2]*Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB), La Habana, Cuba*

[*]*E-mail: duniesky838@gmail.com*

To evaluate the effect of including the zootechnical additive Adizoot 1® in the diet for Pelibuey ewe on their productive and health indicators, an experiment was designed with 26 animals, with an average age of 120 days, belonging to the herd of the ram colony from CENPALAB. Two groups of 13 animals were formed. Treatments consisted in the inclusion or not of the product Adizoot 1® at a dose of 150 mL/animal per day. The product was administered mixed with the concentrate in the morning hours. The diet consisted of 400 g/animal/day and bulky feed at will. Body weight was measured monthly to calculate daily mean gain, and voluntary intake was determined three times a week. Additionally, blood samples were taken for hematological studies. The incidence of diarrhea and other typical diseases of this species was studied. The inclusion of the additive decreased feed conversion compared to the control ($p<0.05$) and improved hematological parameters of hematocrit and erythrocytes ($p<0.05$) at the end of the experiment, compared to animals that did not consume the additive. It is concluded that the inclusion of 150 mL/d/animal of the additive Adizoot 1® improved feed conversion and hematological indicators in Pelibuey ewe. It is recommended to encourage studies in other categories with the application of other doses.

Keywords: *feed conversion, health, zootechnical additive*

Para evaluar el efecto de la inclusión en la dieta del aditivo zootécnico Adizoot 1® en los indicadores productivos y de salud de hembras ovino Pelibuey, se diseñó un experimento con 26 animales, con edad promedio de 120 d, pertenecientes al rebaño de la colonia de carneros de CENPALAB. Se conformaron dos grupos de 13 animales. Los tratamientos consistieron en la inclusión o no del producto Adizoot 1® en dosis de 150 mL/animal al día. El producto se administró mezclado con el concentrado en el horario de la mañana. La alimentación consistió en 400 g/animal/d y alimento voluminoso a voluntad. Se midió peso corporal mensualmente para calcular la ganancia media diaria y se determinó el consumo voluntario tres veces a la semana. Además, se tomaron muestras de sangre para estudios hematológicos. Se estudió la incidencia de diarreas y otras enfermedades propias de esta especie. La inclusión del aditivo disminuyó la conversión alimentaria con respecto al control ($p<0.05$) y mejoró los indicadores hematológicos de hematocritos y eritrocitos ($p<0.05$) al final del experimento, con respecto a los animales que no consumieron el aditivo. Se concluye que la inclusión de 150 mL/d/animal del aditivo Adizoot 1® mejoró la conversión alimentaria en hembras ovinas de la raza Pelibuey y los indicadores hematológicos. Se recomienda encaminar estudios en otras categorías con la aplicación de otras dosis.

Palabras clave: *aditivo zootécnico, conversión alimentaria, salud*

Received: December 19, 2024
Accepted: April 02, 2025
Conflict of interests: The authors of this study declare that there is not conflict of interest among them
CRediT Authorship Contribution Statement: D. Rodríguez Acosta: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft. Yoandra Marrero Rodríguez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project management, Writing-original draft. Niurca González Ibarra: Conceptualization, Data curation, Investigation, Methodology, Writing-original draft. Avelina León Goñi: Data curation, Formal analysis. Iliana Sosa Teste: Data curation, Formal analysis, Investigation, Methodology, Writing-original draft.

Introduction

In the 1980s, Agricultural Engineer Dr. Teuro Higa, professor of Horticulture at the University of Ryukyus in Okinawa, Japan, created a technology related to the use of efficient microorganisms. The technology aimed to provide information about groups of benevolent microorganisms that are physiologically compatible with each other: lactic acid bacteria, phototropic bacteria, actinomycetes, yeasts, and fungi within natural ecosystems.

Efficient microorganisms, such as microbial inoculants, restore soil microbial balance, improve its physical-chemical conditions, increase crop production and protection, conserve natural resources, and generate more sustainable agriculture and environment. They can be used in livestock for animal breeding and increasing productive variables and waste management in facilities (Calero *et al*. 2019, González-Ramírez 2021 and Valdés Suárez *et al*. 2022).

Efficientective microorganisms (EM) currently have multiple applications in environmental (De la Cruz-Balón *et al.* 2019), agricultural (Abreu *et al.* 2024), and livestock areas. Various experiences demonstrate that their use in animal feeding improves the yields of several species, for example in birds (Gelvez Esteban 2022) and pigs (Quintero Rincón 2021) and others.

Adizoot 1® is a registered product for pig feeding. Due to the good results for this species in the studies for its registration and the composition of the product, it could also have beneficial effects in ruminant animals. Therefore, its evaluation in sheep was considered.

The costs of balanced feed in sheep rearing are relatively high, allowing the search for strategies based on the use of alternative feeds or supplements that help reduce feeding costs. In this sense, the goal is to understand the effect of introducing effective microorganisms in the diet of sheep to analyze the possibility of achieving greater profitability, improving health, and consequently, reducing feeding costs. The objective of this study was to evaluate the effect of including the zootechnical additive Adizoot 1® in the diet for Pelibuey ewes, on their productive and health indicators.

Materials and Methods

Experimental treatments: A total of 26 female animals of Pelibuey breed were used, divided into two treatments, with an average age of 120 days, belonging to the herd of the National Center for Laboratory Production, CENPALAB (initials in Spanish), located in Havana, Cuba. Treatments consisted of the inclusion or not of 150 mL/day of the product Adizoot 1® to determine the advantages or disadvantages of its application. Batches of Adizoot 1® were delivered by the Biotechnology Board, batches 22,008 and 22,010, along with their quality certification. Product composition is reflected, namely molasses, whey, and the concentrations of the microorganisms established in the specification. The experiment lasted 120 days.

Experimental procedure: Animals were housed in 4 x 3 m cubicles in a covered building with a cement floor, with access to a 5x3 m sun patio. Feeding consisted of 400 g/animal/day of concentrate and forage, composed of protein plants and grasses (*Tithonia diversifolia*, *Morus alba*, and *Chenchrus purpureus*) *ad libitum.* Adizoot 1® was administered homogeneously mixed with the meal concentrate, EMO 1703 formula, a multipurpose sterilizable diet, in the morning hours. Water was provided at will water throughs. At the beginning of the evaluation, a good veterinary condition of the animal lot was ensured through deworming and health control at the entrance of outside personnel to the unit and daily cleaning of the facilities.

Weighings were carried out during the early hours of the morning, every month, using a digital platform scale, with an accuracy of ± 0.5 kg before food supply. Body weight was measured to calculate daily mean gain (DMG) and food conversion and intake were determined twice a week.

The incidence of digestive, respiratory and other diseases of the species that could occur was studied. For the hematology studies, 3.0 mL were collected by puncturing the jugular vein at the beginning and end of the treatments. Samples were collected in 3.0 mL plastic tubes with a purple cap containing 10 % EDTA (ethylenediaminetetraacetic acid) for subsequent hematological processing. Hematological parameters were determined using a MICROS ABX ESV 60 hematology analyzer (Horiba Medical). The following hematological parameters were recorded: hemoglobin (HB), hematocrit (HTO), erythrocytes (ETO), and leukocyte differential (including neutrophils (N)). A MICROS ABX cell counter (Roche Diagnostic Systems) was used for this, except for the differentiated cells, which were observed using a Carl Zeiss microscope (EQ-06.124). The differential white blood cell count (Leuk. Diff) was performed through a blood smear. They were fixed with May-Grunwald, stained with Giemsa, and observed under a Carl Zeiss phase microscope with a 100x immersion lens, where morphology of blood cells was verified and the differential count was performed using a counting chamber. Neutrophils (N), as differentiated cells, were expressed in percentage. All these techniques were carried out according to the operational procedures established in the clinical laboratory of the Department of Toxicology and Animal Experimentation.

Experimental design and statistical analysis: A completely randomized design was applied for the study of productive performance and a completely randomized design with factorial arrangement (2 x 2), two experimental treatments x two sampling times for the studies of hematological analysis. The statistical program INFOSTAT, proposed by Di Rienzo *et al*. (2012), was used for processing results. In the necessary cases, Duncan (1955) multiple range test was used for $P<0.05$.

Results and Discussion

Nowadays, the use of efficient microorganisms as a zootechnical additive has been reported in different species to achieve greater efficiency in their productive performance (Quintero Rincón 2021 and Henao e*t al.* 2023).

In the current experiment, there were no alterations in the health status of animals. There were no digestive or respiratory processes, which are the most frequently observed. Table 1 shows the performance of daily mean gain (DMG) and conversion in Pelibuey ewes, supplemented or not with the zootechnical additive Adizoot 1®. As can be observed, the inclusion of the product had no effects on the DMG or on dry matter (DM) intake. However, the addition of Adizoot 1® in the diet of Pelibuey ewes increased food use efficiency by decreasing feed conversion ratio.

This increase in feed utilization efficiency was previously reported by Rodríguez *et al.* (2013), who, in an *in vitro* ruminal fermentation study, obtained greater degradability of DM and neutral detergent fiber (NDF) when a microbial additive was included to a diet of *Chenchrus purpureus*. This may be related to the contribution of growth factors from the cellulose-degrading microorganisms that are provided, as these supplements are rich in nitrogenous compounds and organic acids. This same study also allowed for greater microbial biomass synthesis, as well as its utilization efficiency at 120 hours after the additive was supplied.

Gutiérrez *et al*. (2012) proposed that the inclusion of microbial additives, in the ruminal medium, causes modifications in synthesis efficiency, due to changes in the relative proportions of specific groups of microorganisms in total populations, as well as an increase in short-chain fatty acids and bacterial biomass.

These results, which are related to the greater efficiency in the use of bulky feed, were also demonstrated in studies by Iraola *et al*. (2017), who used two doses of inclusion of beneficial microorganisms, activated in bulls grazing in a silvopastoral system supplemented with sugar cane, in which, for the same dry matter intake in the different groups studied, an increase in live weight was obtained. When conducting the feed balance, lower excesses of protein and energy were found as the dose of microbial additive increased. When a carrier supplement of lactic acid bacteria was used in the diet of sheep, beneficial effects were obtained in *in vivo* intake and *in situ* estimated digestibility, and possibly, it promoted microbial protein synthesis in the rumen (considering the indirect evidence provided by the formation and disappearance of NH_3) (Franco *et al*. 2009).

In the results of blood analysis obtained in females (table 2), there was interaction among the factors for the evaluated indicators. When Adizoot 1® was added to the diet, hemoglobin values increased compared to those at the beginning of the experiment. Similarly, erythrocyte and hematocrit values increased at the end of the experiment when Adizoot 1® was administered, compared to control treatment ($P<0.05$). These data fall within the ranges obtained by Torres *et al*. (2023) and Rodríguez *et al*. (2024) for Pelibuey sheep.

Erythrocytes are the most abundant cells in blood and are responsible for carrying oxygen to different parts of the body from hemoglobin, which is a protein present inside them. The fact that the use of Adizoot 1® has increased their values is an element that should be considered in the studied animals, in addition to suggesting that sheep improve their overall health status.

It is important to maintain or improve the health status of animals to provide a product with the required quality and ensure the subsequent recovery of biomodels.

Chacón-Martén (2021) determined the quality of defibrinated blood and hemovin. This author suggested that there is a directly proportional relationship between the hemoglobin levels of the biomodel and those of the products obtained from its blood. According to the quality control established for hemovin, values are rejected if it is found that the product has hemoglobin lower than 9 g/L. This is justified by the interrelation between the hemoglobin level of this supplement and the observation of hemolysis reactions on plates. It can be inferred that if the hemoglobin is low, the expression of hemolysis will be poor or absent, which can lead to a false interpretation of results.

From the higher concentration of erythrocytes, a greater percentage of hematocrit was also obtained, considering that this indicator is the solid phase of blood, once separated from plasma and that its main component is erythrocytes. It is logical to infer its greater ($p>0.05$) volume in relation to control treatment (Bermeo-Perez 2024).

Table 1. Performance of DMG and conversion in Pelibuey ewes, supplemented or not with Adizoot 1®

Indicators	Forage of legumes and grasses + concentrate	Forage of legumes and grasses + concentrate + Adizoot 1® zootechnical additive	SE ±
DMG, kg/animal/d	0.05	0.06	0.01
Fresh matter intake, kg/animal/d	1.56	1.54	0.06
Food conversion, kg DM/kg LW	29.41	24.1	1.05*

*a,b Means with different letters differ ($P<0.05$) (Duncan 1955)

Table 2. Hematological results in Pelibuey females, supplemented, or not, with Adizoot 1® zootechnical additive, determined at the beginning and end of the experiment

Indicators	Forage of legumes and grasses + concentrate	Forage of legumes and grasses + concentrate + Adizoot 1® zootechnical additive
Initial hemoglobin	9.58[a]	9.32[a]
Final hemoglobin	10.48[ab]	11.04[b]
	SE± 0.4*	
Initial ETO 10^6 /mm^3	10.42[a]	10.53[a]
Final ETO	10.52[a]	12.1[b]
	SE± 0.46*	
Initial hematocrit, %	30.76[ab]	31.54[ab]
Final hematocrit, %	28.78[a]	34.44[b]
	SE± 1.66*	
Initial neutrophil, %	27.8[a]	35.6[b]
Final neutrophil, %	28.8[a]	29.6[ab]
	SE ± 2.14*	

* a,b Means with different letters differ (P<0.05) (Duncan 1955)

The complete blood count is a basic diagnostic element, where the number, proportion, and variation of blood elements (erythrocytes, leukocytes, and platelets) are expressed. The evaluation and interpretation of the different data provides very important information about the general state of the animal and serves as guidance to request other complementary tests, determine a possible diagnosis, and, in some cases, assess the effects of applied products on the hematological system (Herrera Arias and Unda-Lopez 2021). Like all tissues in the body, blood fulfills multiple functions necessary for life, including defense against infections, gas exchanges, and nutrient distribution. To fulfill all these functions, it has different cell types suspended in the plasma. A blood analysis or sampling is a powerful diagnostic tool for identifying the physiological responses of an animal, as it can reveal important information about its health, well-being, and nutritional status (Paucar-Arcos 2018).

Neutrophils are abundant in mammals. They are 60-75 % of white blood cells in carnivores and humans, and only 30-40 % in ruminants. Neutrophils are the main defense against the invasion of tissues by microorganisms and bacteria removal, as well as being able to damage or participate in the destruction of fungi, algae, or virus.

When analyzing the results of the neutrophils (table 2), at the beginning of the experiment, values in the animals of the group supplemented with Adizoot 1® were higher. However, the values of these cells that were determined are within normal ranges (Herrera-Arias and Unda-Lopez 2021), so the differences found do not seem to have biological significance. Nevertheless, by the end of the study, these values decreased, so there were no differences among treatments.

Beneficial microorganisms of natural origin, traditionally used in food, when they come into contact with organic matter, secrete beneficial substances, such as vitamins, organic acids, chelated minerals, and antioxidants. These microorganisms are in a latent state, and, therefore, are used for making other secondary products of efficient microorganisms.

The use of probiotics and zootechnical additives based on beneficial microorganisms in ruminants leads to an increase in apparent voluntary intake, higher digestibility of the cell walls of forages, an increase in ruminal pH, and a higher concentration of ammonium and SCFAs in the rumen, likely due to a greater number of bacteria in the animals. This would allow for better performance by optimizing the ruminal environment (and, consequently, digestive processes) and not merely by improving food quality (Elías and Herrera 2008 and Galina *et al*. 2009). These products consist of a pool of microorganisms: bacteria, yeasts, and their metabolites, for which the necessary conditions are provided to create beneficial effects. In the case of Adizoot 1®, beneficial effects were also found in this ruminant species.

Conclusions

It is concluded that the inclusion of 150 mL/d of the additive Adizoot 1® improved the feed conversion in female sheep of the Pelibuey breed and improved the hematological health parameters. It is recommended to conduct studies on other categories and use other doses.

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