Review Form 3

Journal Name:	**Annual Research & Review in Biology**
Manuscript Number:	**Ms_ARRB_120784**
Title of the Manuscript:	**Relationship of Udder, Flank and Leg Hygiene Scores with Elevated Somatic Cell count and Subclinical Mastitis in Dairy cow**
Type of the Article	

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PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write few sentences regarding the importance this manuscript for scientific community. Why do you like (or dislike) this manuscript? Minimum 3-4 sentences may be required for this part.		
Is the title of the article suitable? (If not please suggest an alternative title)		
Is the abstract of the article comprehensive? Do you suggest addition (or deletion) of some points in this section? Please write your suggestions here.		
Are subsections and structure of the manuscript appropriate?		
Please write few sentences regarding the scientific correctness of this manuscript. Why do think that this manuscript is scientifically robust and technically sound? Minimum 3-4 sentences may be required for this part.		
Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.		

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Minor REVISION comments **Is language/English quality of the article suitable for scholarly communications?**		
Optional/General comments	Dear Editor, I have reviewed Manuscript Number 2024_ARRB_120784 and am pleased to provide my comments. The manuscript presents a thorough analysis with well-supported findings. I believe it meets the standards for publication in the Annual Research & Review in Biology. Please find my detailed review attached. In material and methods: 1. Is the specific procedure for using the Somaticell Kit and the criteria for interpreting the results described in detail? 2. Were the cows maintained in a tail-to-tail tie-stall management system with access to free grazing for 3-4 hours daily, and were details provided on how consistency was maintained in grazing conditions (e.g., pasture quality and availability) as well as whether housing conditions (e.g., ventilation, bedding) were monitored and kept consistent throughout the study period? 3. Was the milking schedule (twice daily at 6:00 AM and 5:00 PM) standardized to avoid variability, and were the types of commercial concentrates and green fodders used, along with their nutrient composition, specified to ensure reproducibility? 4. Were all relevant data (e.g., SCC, milk yield, health parameters) collected systematically, and were any potential confounding factors controlled? In Milk Sample Collection: 5. Specify the antiseptic solution used for washing and wiping the udders and teats. 6. Ensure that the amount of foremilk discarded is consistent across all samples. 7. Mention if any training was provided to personnel collecting the samples to ensure consistency and avoid contamination. Sample Analysis: 8. Provide more information about the Lactoscan SCC (e.g., calibration procedures, maintenance of the device). 9. Mention if any quality control measures were in place to ensure the accuracy and precision of SCC measurements. Data Reporting: 10. Specify how frequently the SCC data were recorded and any statistical methods used to analyze the data over time. 11. Was the temperature at which the milk samples were frozen specified, and were any measures taken to avoid multiple freeze-thaw cycles, which could affect bacterial viability? 12. What were the specific thawing conditions (e.g., temperature, duration) used to ensure consistency across milk samples? 13. Was any specific type of blood agar (e.g., sheep blood agar) used for bacterial culture, and was this detail documented? 14.	

	For Pseudomonas spp., what type of agar was used for the pigment production test, and how was the test performed? 15. For Staphylococcus aureus, were the mannitol fermentation and coagulase tests conducted using standard kits or specific protocols? 16. For Streptococcus agalactiae, what reagents were used in the agglutination test, and were they detailed? 17. What was the threshold for marking samples as contaminated (e.g., more than two bacterial species per sample), and were there any exceptions or additional notes provided for specific pathogens like Streptococcus agalactiae? 18. What was the rationale for combining environmental and contagious pathogens into a "major bacteria" category for some analyses, and how does this categorization align with the study's objectives? 19. Were Kappa statistics used to assess the reliability and repeatability of udder, flank, and lower leg hygiene scores, and was the agreement between duplicated scores documented? 20. 21. the method for achieving normal distribution and minimizing heterogeneity of variance clearly outlined? 22. Was the General Linear Model (univariate) used for analyzing the effects of hygiene scores on SCC described in detail, and were the results expressed as means and standard errors of the mean? 23. Was the Bonferroni test employed for mean separation to stratify the effects of hygiene scores on SCC, and were the procedures for this analysis detailed? 24. Was Pearson's correlation analysis used to examine the relationship between udder, flank, and lower leg cleanliness and udder SCC, and were the methods and results reported? 25. hygiene scores analyzed using the Chi-square test, and were the details of this analysis provided? 26. Were mean differences tested for statistical significance with a threshold of $p \leq 0.05$, and was this criterion explicitly stated? 27. Was the statistical method for assessing repeatability and agreement clearly described, and how were p-values calculated? 28. Was the statistical method for assessing repeatability and agreement clearly described, and how were p-values calculated? 29. What factors contributed to the lower but substantial agreements observed in some categories, and how were these factors addressed or accounted for? 30. Was Table 3 clearly referenced and did it include all necessary details for interpreting the degree of agreement between observations? 31. Ensure that the specific statistical methods used for analyzing the association between hygiene scores and SCM are clearly detailed, including how the chi-square tests were applied. 32.	

	Provide a more detailed interpretation of the hygiene scores ('clean,' 'moderately clean,' 'dirty,' 'extremely dirty') and how they relate to the prevalence of SCM. 33. Confirm that the p-values for all statistical associations (e.g., hygiene scores and SCM, bacterial counts) are appropriately reported and that the results are clearly linked to their significance levels. 34. Ensure that Figure 1a, 1b, and 1c clearly present the trends in bacterial counts and are referenced correctly in the text to support the findings. 35. Ensure that Figure 1a, 1b, and 1c clearly present the trends in bacterial counts and are referenced correctly in the text to support the findings. 36. Elaborate on the relationship between absolute and log10 SCC values with SCM and hygiene scores, providing specifics on how these associations were measured. In discussion: The discussion presented provides a detailed interpretation of the study's findings regarding the relationship between hygiene scores, somatic cell count (SCC), and subclinical mastitis (SCM) in dairy cows. However, to improve clarity and scientific rigor, consider the following recommendations: • Clearly define and interpret the impact of each hygiene score category (clean, moderately clean, dirty, extremely dirty) on SCC and SCM. • Provide more context on why the prevalence of SCM in this study is higher compared to other studies, and discuss potential differences in methodology or herd management practices. • Elaborate on how the cleanliness of specific body parts (udder, flank, lower legs) correlates with the presence of different types of bacteria and their impact on SCC. • Clarify the factors contributing to the observed consistency and repeatability of hygiene scoring, and address any potential sources of variability. • Discuss the implications of increasing bacterial loads with poor hygiene scores, particularly in relation to specific pathogens like Staphylococcus aureus and Escherichia coli. • Provide a more detailed analysis of how barn management practices, such as cleaning routines and bedding quality, influence the hygiene scores and overall udder health. • Explain why differences between SCC scores (e.g., score 1 and 2) were non-significant, and discuss the biological or practical significance of this finding. • Summarize the key findings of the study and their implications for dairy herd management and mastitis control practices.	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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Reviewer Details:

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