ABUNDANCE OF SERUM BETA-miR-2285u MICRORNAs SUGGESTED ENHANCED RUMEN HEALTH OF GOATS FED FORAGE AND HIGH-GRAIN BASED DIETS.

Agbana, J.A., Fanwo, R.R. and Aina, E.S.

Department of Animal Health and Production Technology, Faculty of Agricultural Engineering, Kogi State Polytechnic, Lokoja.
Corresponding author: jagbanah@gmail.com; +234 (0) 706 820 0397

ABSTRACT

The knowledge on how diets influences metabolic disorders in farm animals through modification in the activity and functions of microRNAs is essential. microRNAs are small non-coding RNAs that regulate post-transcriptional gene expression, which could serve as biomarkers for several metabolic conditions. Sub-acute ruminal acidosis (SARA), a metabolic disorder characterized by rumen inflammations as a result of low ruminal pH and the accumulation of volatile fatty acids, is linked to feeding of grains or starch-rich diets. This study evaluated the suitability of miRNAs as potential biomarkers in goat for the diagnosis of SARA, employing next generation sequencing technology for comprehensive identification of miRNAs in plasma and leucocytes. Six bucks were fed a control diet of 100% forage and subsequently, modulated to a 70% high-grain diet. 421 and 523 miRNAs were recorded for plasma and leucocytes, respectively. Bta-miR-2285u, bta-miR-30b-3p and bta-miR-1271 were differentially expressed between diets. Thus, they were chosen as potential SARA biomarker candidates and further analyzed by RT- qPCR in more bucks. Bta-miR-30-3p, a rumen specific miRNA that can detect short term ruminal imbalances was highly expressed in plasma of goats. Hence, abundance of serum bta-miR-30-3p could serve as effective biomarker for rumen health. Further analysis on mechanism involved may be explored.

Keywords: Acidity, Acidiosis, miRNA, Potential, Ruminal, Starch-rich diets.

INTRODUCTION

Information on how choices of diets, feed intake and dietary additives influence molecular processes in farm animal to maintains gastrointestinal health, is essential for study. Animal nutritionists have the understanding of the nutritional value of feed as both nutrients present and availabile to farm animals, with considerations of consumption rates, flavour, and the impact of the meal on the health status of animals and standard of animal products (Rome, 2015). However, the knowledge of mechanism through which nutritional value of feeds transcend to variations in phenotypic expressions remains unclear. Understanding farm animal responses to dietary components and other environmental factors elucidates the importance of the environment-gene expression interaction, and post transcriptional regulation through microRNAs (Ojo and Redmer, 2023). A better understanding of this interactions presents opportunity to modulate metabolism, health and diseases based on nutritional strategies.

Livestock diseases cause significant financial losses worldwide by increasing mortality and decreasing productivity in herds (Correia *et al*, 2017). Though, external and internal parasites, mastitis, and sub-acute ruminal acidosis (SARA) may not directly kill animals, but they indirectly limit the production efficiency of animals by lowering milk production, reducing feed conversion and may delay puberty in young herds (Ojo and Redmer, 2023). Some health risks associated with these animal diseases have been reported to spread to human as a result of consuming their products (Zhang *et al,* 2020). The vulnerability of human to these numerous health conditions is a concern for the Animal nutritionist.

Numerous studies have demonstrated a connection between circulating microRNAs (miRNA) and most ruminant diseases such as mastitis, tuberculosis, foot and mouth diseases and other metabolic disorders (Ngoc Do *et al*, 2022 & Ojo and Redmer, 2023). Hou *et al*. (2018) and Bourdon *et al*. (2021) also showed that serum circulating bta-miR-223 and bta-miR-142-5p miRNA levels could accurately (100 % sensitivity) identify inflammation in tissues of cows as evidences of early diagnostic indicators of mastitis. Thus, circulating miRNAs in bio fluids of animals may be employed as helpful marker for therapeutic, prognostic and diagnostic for better health, welfare and production outcomes in ruminants (Rome, 2015).

MicroRNAs (miRNA) are an inexhaustible class of small endogenous non-protein coding RNAs of 18 to 25 nucleotides that target either the 3' untranslated (UTR) or coding region of genes, crucial as regulators of gene expression (Zhang *et al,* 2020). These short transcripts may restrict protein translation through gene splicing process, where miRNA binds to mRNA molecules and either rapidly blocks translation of proteins, or degrades the bound mRNA (Bourdon *et al*, 2021). MicroRNA in bovine are suggested to play important role in vast molecular functions and biological processes chiefly, rumen development, adipogenesis, ovary development, immunity and infection (Ma *et al*, 2020).

MATERIALS AND METHODS

Experimental site

The animals were kept at the Research and Training farm of the Department of Animal Health and Production Technology, Kogi State Polytechnic, Itakpe Campus on Latitude 6^0 5 N and Longitude 6^0 4 E.

Animals' management

Bucks were fed a diet of 100% forage and were transitioned after one week of adaptation to a 70 % high-grain based diet compounded from 15% maize, 13% maize bran, 5% dried brewer waste, 12 % Bambara nut waste, 0.5% limestone, 3% bone meal and 0.5 % vitamin premix; which was fed for a total of 5 weeks.

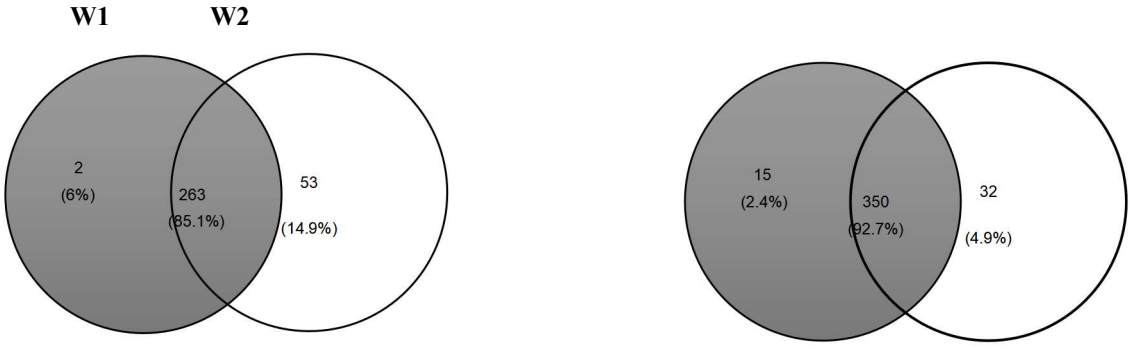
Sample collection and Analysis

At the end of the 5 week trial, blood samples were collected in EDTA coated tubes from the jugular vein of six ruminal-cannulated West African Dwarf bucks, average age of 7 ± 5.5 months, and weight of 35± 7.3 kg. The animals were cared for and experimental protocols were followed as approved by the ethical Committee of the Institutional Animal Care and Use (KSPACUC-2022). Cell-free plasma and leucocytes were obtained and analyzed from serum using a two-step centrifugation protocol at African Bioscience Laboratory, Ibadan. Total RNA was isolated using the NucleoSpin miRNA kit following the manufacturer's protocol. Small libraries were prepared from blood plasma and leucocytes using the NEXTflex small RNA-seq kit (Bio Scientific). Read processing and pre-filtering was conducted in sRNAbench and R software 4.0.2. Normalized reads counts (per million) obtained from sRNA bench were filtered in R using the *tidyr* (version 2.1.4) package. Reads with an average Phil`s Read Editor (PHRED) score below 20 were discarded and all miRNAs less than 10 total read counts across the studied samples were filtered out.

Differential expression analysis of miRNAs was carried out according to the procedures of Ojo and Redmer (2023) using DESeq2 (version 4.2). TargetScan and miRBase were used to predict DE miRNA target genes according to the procedures of Ojo and Redmer (2023). RT- qPCR assays were performed using miRNA QPCR Master Mix (Agilent). MiR-107 and MiR-103 were used as reference miRNAs to normalize for RNA content. Relative expression was calculated using ddCT-method with values as mean expression of forage-based diet.

RESULTS AND DISCUSSION

Four hundred and twenty one (421) miRNAs were discovered in plasma of bucks, with 263 miRNAs (figure 1a) shared between forage (week 1) and high grain (week 2-5) diets, while 523 miRNAs were identified in leucocytes, with 350 miRNAs (figure 1b) shared between diets fed for week 1 and 2 to 5 in all bucks. Similarly, the bucks fed high-grain based diet had 53 miRNAs that were highly expressed in the plasma suggesting higher circulating miRNAs. A total of 421 miRNAs were found in leucocytes while, 350 miRNAs shared between Week 1 and 2. Fifteen (15) miRNAs were expressed when the forage-based diet (week 1) was fed, while 32 miRNAs were only expressed during high grain feeding (Week 2-5) suggesting influence of circulating dietary miRNAs.



(A) **(B)**

Figure 1: A schematic diagram of shared miRNAs in A) plasma and B) Leucocytes of animals on forage and high-grain based diet.

Fifteen (15) miRNAs were differently expressed in plasma of forage fed and animals on high-grain based diets, from which 9 miRNAs were up-regulated and 6 miRNAs were down-regulated. A total of 28 miRNAs were also differentially expressed in leucocytes of animals on forage and high grain based diet, from which 7 miRNAs were up-regulated and 21 mRNAs were down-regulated. This differential expressions of microRNAs in plasma of West African Dwarf bucks fed either forage alone and high grain diet implies the affinity ability of circulating sera miRNAs for targeted gastrointestinal tissues (Ioannidis *et al*, 2018); as each miRNA is considered to have specific target, even though multiple miRNAs may converge on a single mRNA port (Zhang *et al,* 2020). Three

miRNAs (bta-miR-30b-3p, miR-17-5p, and miR-2285-u) were however, similar in expression for high grain diet. Therefore, the miRNAs were further sampled and analyzed by RT- qPCR.

The candidate miRNAs sorted from plasma in goats on high grain diets (n=15) were differentially expressed in terms of their read count in increasing order and log2 fold change. High grain diets significantly ($P<0.05$) influenced the expression of miR-2285u, which is rumen specific (Ma *et al*, 2020). Thus, it abundance in rumen could be a promising circulating candidate biomarker for rumen heath. Furthermore, there was higher expression of miR-30b-3p in Week 2 ($P=0.05$), compared to the forage feeding suggesting that miR-30-3p has potential to be a good candidate to detect short term rumen imbalances from high grain diets (Correia *et al*, 2017). On week 5, after 4 weeks of high grain feeding, the expression level was again at the level of forage feeding. The results agreed with the findings of Ojo and Redmer (2023) who identified bta-miR-2285u to be highly expressed in plasma of cattle fed a high grain diet compared to grazing cattle. Thus, Beta-miR-2285u also act as potential miRNA biomarker involved in ruminal feed efficiency and major diseases in ruminants (Rome, 2015). This results also infer that contrary, to the assertion of Rome (2015) that transition from forage-based diets to concentrate based diets or abrupt consumption of diets containing greater levels of fermentable carbohydrates could lead to accumulation of acids that predisposes to ruminal acidosis. it can be established that in bovine stock, transition in diets may not damage ruminal epithelium and impair tissue functions.

Table 1: Relative miRNA of selected Caprine miRNAs by RT-qPCR

		miRNA	
Weeks	**miR-30b-3p**	**miR-2285u**	**miR-17-5**
Week 1(forage diet)	1.25^{a}	1.21^{b}	1.00
Week 2(grain based diet)	0.60^{b}	2.50^{a}	1.51
Week 5(grain based diet)	0.40^{c}	0.80^{c}	1.52
p-Value	*0.035*	*0.035*	*0.09*

CONCLUSION AND APPLICATION

The findings of this work is in consonant with the fact that dietary feed components plays crucial role in regulation of miRNA and circulating miRNAs. Variety of bioactive feed ingredients affect how miRNAs are made and elements in feeds may also affect how diseases are susceptible to developing, intensifying and progressing (Ngoc Do *et al*, 2022). In a recent study, it was discovered that different diets could affect miRNA expression of bovine serum, and influence rumen immunity. Therefore, one could use effective dietary strategies to alter the physiological state of animals.

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