Biological Forum – An International Journal **15(5): 1424-1427(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

An Experimental Study on *AQP1* expression in Skin Fibroblast Cells of Native Goat Breeds in Water Stress conditions

Femi Francis[1]*, Ashutosh[2], Ditty Maria Dominic[3] and Namratha Valsalan[4]

[1]Ph.D. Scholar, Department of Veterinary Physiology,
College of Veterinary and Animal Sciences, Pookode (Kerala), India.
[2]Principal Scientist, Division of Animal Physiology,
ICAR-NDRI, Karnal (Haryana), India.
[3]Research Fellow, Centre for Research on Innovation and Science Policy,
Hyderabad (Telangana), India.
[4]Ph.D. Scholar, Department of Livestock Products and Technology,
College of Veterinary and Animal Sciences, Pookode (Kerala), India.

(Corresponding author: Femi Francis)*
(Received: 14 March 2023; Revised: 16 April 2023; Accepted: 26 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Coming decades are to be affected by erratic climate changes and water scarcity. The goat breeds are efficient water utilisers especially of arid adapted regions such as Sirohi. Inherent genetic traits together with physiological and morphological mechanisms helps these animals to sustain these hurdles. Genetic adaptations being the most important factor deciding the adaptation ability of the animal, these needed to be focussed. Various genes act together for the survival of the breeds in water deficit areas. The study of the genetic mechanisms used by desert breeds that have co-evolved and adapted to the arid environments where water shortage is frequently experienced is necessary due to the current water deficit and irregular precipitation pattern. So, the present study was focussed on the variation in the expression pattern of the *AQP1* gene in the skin fibroblast cells of the animal at different temperature and different temperature- hypoosmotic conditions. The overall expression was found to be lower in both the breeds with much lower expression in the Barbari breed. There was a significant ($p<0.05$) variation in the expression of the *AQP1*gene between the breeds at higher temperature, normal temperature with hypoosmotic condition, and high temperature with hypoosmotic conditions. The comparatively lower expression in Barbari breed and higher expression in Sirohi breed at higher temperature with hypoosmotic condition depicts the less water stress affected by the Sirohi breed, indicating its drought adaptability. The study indicates that the Sirohi breed are well adapted to thrive in water deficit areas as compared to the Barbari breed utilising their inherent genetic adaptation on water balance. The studies may help the policy makers in deciding cross breeding policies for future era of droughts and climatic shifts.

Keywords: AQP1, Barbari, Sirohi, water stress, hypoosmotic.

INTRODUCTION

Genetic makeup affects fitness and adaptation and defines an animal's tolerance for harsh environments including high temperatures, water deficit, and drought (Naskar *et al*., 2012). The survival of a population in unfavourable climatic conditions is facilitated by genetic adaptability and other heritable animal traits. Native livestock breeds have special adaptation traits that have evolved in demanding tropical habitats that allow them to thrive and to be productive in challenging environments (Niyas *et al*., 2015). P henotypic variation is produced when innate genetic variation interacts with environment and affects adaptation ability of the animal which is evaluated through survival and reproduction (Naskar *et al*., 2012). Adaptive evolution of genes including *SLC14A2*, *ACE*, *AGT*, and *AQP* has improved the ability to transport water and urea, which has increased the concentration of urine, an effective method for keeping the water balance in animals (Xu *et al*., 2013).

AQUAPORINS are a family of membrane-bound proteins that are found in a wide variety of organisms, including plants, animals, and microbes (Calamita, 2000; Agre *et al*., 2006; Agre and Kozono 2003; King *et al*., 2004). The movement of water and other tiny solutes throughout the cell is the primary role that aquaporins are known to play in. The protein aquaporin, a molecular water channel protein found in the cells of several tissues, is encoded by this gene. Many scientists have discovered 13 isoforms of aquaporins (labelled AQP0 to AQP12) in the various tissues of mammals. AQP 1 plays a role of selective water channel.

Numerous research has been conducted on the tissue distribution, cellular localization, regulation, structure, and function of mammalian AQPs. Even though many

studies have been done on the various ways that AQPs act in various tissues, only a small number of studies, if any, have been done on the AQP expression and its osmoregulatory function in skin. It has been discovered that AQP-1 plays a crucial role in the osmotic water transport through epithelial and endothelial barriers' cell membranes. Skin hydration and water transport may be impacted by the AQP's fluctuating expression and cellular location. Higher water loss is triggered by the increased expression of *AQP1* and *AQP3* in the developing skin. However, there is still much to learn about AQP-1's distribution and role in various human and animal tissues (Mobasheri and Marples 2004). The *AQP3* gene, a different member of the AQP family involved in preserving the water balance in the Sirohi breed, was altered, according to Francis *et al.* (2020). Several studies have been conducted on the role of *AQP1*expression in wound healing process (Hara-Chikuma and Verkman 2008), upon repeated UV irradiation (Kim *et al.,* 2020) etc, but few studies have been carried out in relation with the *AQP 1* expression and water stress in animals.

Goat being one of the most water efficient animal and known for its survival in water deficit areas especially arid zones. The native breeds such as Sirohi thrives well in water scarce conditions. Therefore, the present study was undertaken to study the changes in the *AQP1*expression in the skin fibroblast cells of native goat breeds, Barbari and Sirohi when exposed to different temperature and osmotic conditions.

MATERIALS AND METHODS

The present study was carried out in the Division of Animal Physiology, ICAR-National Dairy Research Institute, Karnal, India, as part of the MVSc research work.

A. Skin fibroblast cell culture

Three each adult Barbari and Sirohi goats were selected for the study. Skin explants were collected from the ear of each animal and the primary fibroblast cell culture was established in the lab. The skin fibroblasts were cultured in the cell culture medium (DMEM/F12 with 2-mM l-glutamine, 20% fetal bovine serum, 100 IU/mL of penicillin and 100 µg/mL of streptomycin) under humidified atmosphere containing 5% CO_2 at 37 °C. When the cultured flasks reached about 80% confluency, the fibroblast cell were passaged using 0.25% Trypsin- EDTA. The subcultured cells were passaged four times and the cells obtained after the fourth passage were used for the specific treatments.

B. Treatments on Skin fibroblast cell culture

The skin fibroblast cells of Barbari and Sirohi goats were subjected to five different treatment conditions for 3 hours. The fibroblast cells were exposed to different temperatures and different temperature- hypo-osmotic conditions. The skin fibroblast cells were maintained at 25°C, 37°C, and 42°C for 3hrs in treatment 1, treatment 2, and treatment 3 respectively. The fibroblast cells were subjected to the hypoosmotic conditions at 37°C and 42°C in treatment 4 and treatment 5 respectively. The treatment media was added with 100mM NaCl to mimic the dehydration (hypo-osmotic) condition in the cells. Three samples were taken from the two breeds each and these samples were subjected to different treatments in triplicates. The cells grown in the culture medium exposed to the normal temperature *i.e.* 37°C is considered as the control sample.

C. Isolation of RNA and Real time PCR

Total RNA was extracted from treated skin fibroblast cells using the miRNeasy kit (Qiagen, USA). RNA quality and quantity was assessed using the Nanoquant and only those RNA samples isolated from fibroblast cells with purity between 1.9 and 2.0 were used for cDNA synthesis. By using 1.5% agarose gel electrophoresis, the acquired RNA's purity and integrity were evaluated. Using Thermoscientific'sRevert Aid First Strand cDNA Synthesis kit, first strand cDNA was created.

A quantitative real time-PCR (qPCR) was carried out to study the relative expression of *AQP1* mRNA. Gradient PCR was carried out to determine the annealing temperature of the respective gene and the housekeeping gene (*GAPDH*) (Fig. 1 and 2). A master mix was prepared using the Maxima SYBR Green qPCR Master Mix(2X) as follows: 5 µl master mix(2X), 0.5 µl forward primer, 0.5 µl reverse primer, 2 µl cDNA sample and 2 µl nuclease free water. The sequence information for the gene was obtained from the NCBI database, and appropriate primers were built using the primer 3 web services. Table 1 shows the primer sequences and annealing temperatures for the genes.

Table 1: Primer sequences of the genes under study.

Sr. No.	Gene	F/R	Sequence (5'-3')	Tm (°C)	Product size (bp)
1.	*GAPDH*	F	CCAACGTGTCTGTTGTGGATCTGA	58	218
		R	GAGCTTGACAAAGTGGTCGTTGAG		
2.	*AQP1*	F	TCTGTGGCCCTGGGACATCTGCTGGCG	58	111
		R	AGAAGATCCAGTGGTCCTGGAAATTGTGC		

RT- PCR was carried out as follows:(50°C X 2 min; 95°C X 10 min; (95°C X 30 s; Tm of gene X 30 s; 72°C X 30s) X 35 cycles; 95°C X 1 min; 55°C X 30 s; 95°C X 30 s). GAPDH gene was taken as the internal control gene and the sample cultured at 37°C in normal cultured medium was considered as the control sample. The relative gene expression of *AQP1* was then analysed using $^{\Delta\Delta}$Ct method.

D. Data analysis

Significance in the change in *AQP1* expression was analysed by using one way and two-way ANOVA using

SPSS software version 24. A difference with a P<0.05 value was judged statistically significant.

RESULTS AND DISCUSSION

The variation in the *AQP1* expression in the skin fibroblast cells of Barbari and Sirohi got breeds when subjected to different temperatures and temperature-hypo-osmotic is presented in Table 2. As a calibration factor, the relative *AQP1* gene mRNA expression in treatment 2 was used.

Table 2: Relative gene expression of *AQP1* mRNA in Barbari and Sirohi breeds.

AQP1					
Breed	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Barbari	0.55±0.26	1.0±0	0.26^{A}±0.09	0.45^{A}±0.30	0.26^{A}±0.12
Sirohi	0.56±0.05	1.0±0	0.44^{B}±0.01	0.50^{B}±0.10	0.57^{B}±0.14

^{ABC} Bars with different superscripts are significantly different (p<0.05) between breeds

The relative expression of *AQP1* mRNA was found to be higher in the Sirohi breed when compared to the Barbari breed though the general expression was lower when compared with the control. Variations were observed between the treatments, in both the breeds, but were not statistically significant. *AQP1* expression was found to be decreasing with increasing temperatures in both the breeds with a major decrease being observed in the Barbari breed. During hypo-osmotic conditions at normal temperature, the Sirohi breed showed a significant (p<0.05) increase when compared to the Barbari breed. But with an increase in temperature in hypo-osmotic conditions, Sirohi breeds showed an increase in the *AQP1* gene expression meanwhile the Barbari breed showed a decrease in the respective gene expression when compared to treatment with a hypo-osmotic medium at normal temperature. There was a significant (p<0.05) increase in the *AQP1* expression in the high temperature-hypo-osmotic condition in the Sirohi breed as compared to the Barbari breed.

The majority of AQP1 research is conducted in vascular endothelial cells, where it is crucial for the exchange of water between the blood and dermis to maintain hydration. Their physiological significance in fibroblasts and melanocytes is less well understood. AQP1 expression has been observed in the cells during osmotic stress conditions (Boury-Jamout *et al*., 2006) and found to be upregulated during periods of hypertonic stress (Leitch *et al*., 2001). The present study has detected a lowered expression of *AQP1* in the hypo-osmotic conditions at normal temperature with less variations in the expression when compared between the Barbari and the Sirohi breed. At higher temperatures with hypo-osmotic stress the Barbari breed may be experiencing severe water stress such that the expression of *AQP1* is too low when compared with the Sirohi breed. Though not statistically significant, the difference in the expression of *AQP1* in hypo-osmotic conditions at normal temperature and higher temperatures is much more in the Barbari breed as compared to that of the Sirohi breed. The Sirohi breed showed a higher expression in the *AQP1* at hypo-osmotic conditions. This may be due to the fact that the Sirohi breed may have developed several innate mechanisms over generations to adapt to water stress and to thrive well in higher temperatures. The reduced expression in the Barbari breed during hypo-osmotic condition and higher temperature can be a mechanism to reduce the water loss from their body. Though with reduced water intake and increased expression of the *AQP1* gene during hypo-osmotic stress, clearly indicates Sirohi as an arid adapted animal that can survive well in future decades of water scarcity. Though other genes such as *AQP3* and *AQP5* showed significant variations during the similar treatments (Francis *et al*., 2020; 2022), *AQP1* showed much lesser expression and variations. The role played by the *AQP1* gene may be lesser as compared to other *AQP* genes in the skin fibroblast cells or detailed studies are further required. An experimental study conducted by Debbarma *et al.* (2020) also observed altered variations in the expression patterns of *AQP1* in the skin fibroblasts of Buffaloes during different seasons suggestive of its role in thermotolerance and water balance. He also observed down regulated expression of *AQP1* in tracheal mucosa in higher temperatures suggestive of the lesser role played by the *AQP1* in water balance in thermoregulation (Debbarma *et al*., 2021).

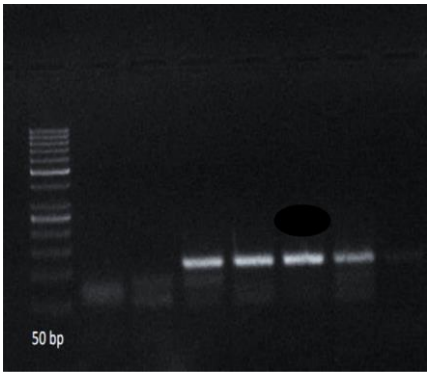

Fig. 1. Gradient PCR of *AQP1*.

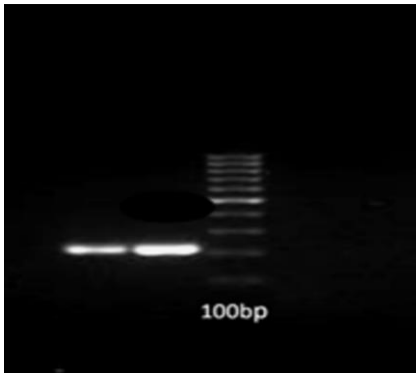

Fig. 2. Gradient PCR of *APDH*.

CONCLUSIONS

The present study concluded that *AQP1* genes are expressed in the skin fibroblast cells of goat. The expression of *AQP1* is generally lower in both the Barbari and Sirohi breed with lowest expression in the Barbari breed. The Barbari breed showed lowest expression during hypo-osmotic stress at higher temperature as the concerned gene specifically transports water, which may be a mechanism for water conservation The Sirohi breed despite of the hypo-osmotic condition increased the expression of the *AQP1*, shows its thermotolerance and water scarce adaptability suggesting that the Sirohi breed can be bred easily in future era of erratic climate change.

FUTURE SCOPE

More studies have to be conducted on the role of *AQP1* expression in the skin fibroblast cells along with other AQP genes. Since this experiment is carried out *invitro*, *invivo* studies are to be carried out. Molecular mechanisms pointing towards the osmotic-stress induced *AQP* expression have to unravelled in detail.

Acknowledgements. I would like to thank the Director, ICAR-NDRI for providing with the facilities for carrying out the smooth conduct of the research.
Conflict of Interest. None.

REFERENCES

Agre, P. and Kozono, D. (2003). Aquaporin water channels: molecular mechanisms for human diseases. *FEBS Let*, *555*, 72–78.

Agre, P., King, L. S., Yasui, M., Guggino, W. B., Ottersen, O. P. and Fujiyoshi, Y. (2002). Aquaporin water channels –from atomic structure to clinical medicine. *J Physiol*, *542*, 3–16.

Boury-Jamot,M., Sougrat, R., Tailhardat, M. and Le Varlet B. (2006). Expression and function of aquaporins in human skin: Is aquaporin-3 just a glycerol transporter? *Biochim. Biophys. Acta*, *1758*, 1034-1042.

Calamita, G. (2000). The Escherichia coli aquaporin-Z water channel. *Mol. Microbiol*, *37*, 254-262.

Debbarma, S., Ashutosh, Saini, S. and Gowda, S. B. (2020). Seasonal effect in expression of AQP1, AQP3 and AQP5 in skin of Murrah buffaloes. *Journal of Thermal Biology*, *93*,102727.

Debbarma, S., Ludri, A., Saini, S., Devi, P. and Kumar, G. (2021). Expression of AQP1, AQP3, AQP4 and AQP5 in upper respiratory tract of buffaloes during different seasons Expression of AQP1, AQP3, AQP4 and AQP5 in upper respiratory tract of buffaloes during different seasons. *Biological Rhythm Research*, *53*, 10.

Francis, F., Ashutosh and Parkunan, T. (2020). A comparative study on the expression profile of aquaporin 3(AQP3) gene in the skin fibroblast cells of Barbari and Sirohi breeds of goat. *Ind. J. Dairy Sci*, *73*(2), 140-144.

Francis, F., Ludri, A. and Parkunan, T. (2022). Comparative study on the expression profile of Aquaporin5 (AQP5) gene in skin fibroblast cells of native goats enabling their water utilization efficiency. *Ind.J. Dairy Sci*, *75*(2), 151-155.

Hara-Chikuma, M. and Verkman, A.S. (2008). Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *J. Mol. Med.* (Berl), *86*, 221–231.

Kim, W. O., Kim, S. A., Jung, Y. A., Suh, S. I. and Ryoo, Y. W. (2020). Ultraviolet B Downregulated Aquaporin 1 Expression via the MEK/ERK pathway in the Dermal Fibroblasts. *Annals of dermatology*, *32*(3), 213–222.

King, L.S., Kozono, D. and Agre, P. (2004). From structure to disease: the evolving tale of aquaporin biology. *Nat Rev Mol Cell Biol*, 5, 687–698.

Leitch, V., Agre, P. and King, L. S. (2001). Altered ubiquitination and stability of aquaporin-1 in hypertonic stress. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 5, 2894–2898.

Mobasheri, A. and Marples, D. (2004). Expression of the AQP-1 water channel in normal human tissues: a semiquantitative study using tissue microarray technology. *Am. J. Physiol. Cell Physiol*, *286*, C529–C5237.

Naskar, S., Gowane, G. R., Chopra, A., Paswan, C. and Leo, L. P. (2012). Genetic adaptability of livestock to environmental stresses In: Sejian V, Naqvi SMK, Ezeji T, Lakritz J Lal R (eds) environmental stress and amelioration in livestock production. Springer Verlag publisher, New York, 319-374.

Niyas, A., Chaidanya, K., Shaji, S., Sejian, V., Bhatta, R., Bagath, M., Rao, G. S., Kurien, E. K. and Girish, V. (2015). Adaptation of Livestock to Environmental Challenges. *J Vet* Sci *Med Diagn*, *4*, 3.

Xu, S., Yang, Y., Zhou, X., Xu, J., Zhou, K. and Yang, G. (2013). Adaptive evolution of the osmoregulation-related genes in cetaceans during secondary aquatic adaptation. *Evol Biol*, *13*, 189.

How to cite this article: Femi Francis, Ashutosh, Ditty Maria Dominic and Namratha Valsalan (2023). An Experimental Study on *AQP1* expression in Skin Fibroblast Cells of Native Goat Breeds in Water Stress conditions. *Biological Forum – An International Journal*, *15*(5): 1424-1427.