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Real time PCR quantification of goat - defensin mRNA expressed by different tissues of Osmanabadi goat

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Defensins are cationic, cysteine-rich motif containing antimicrobial peptides (AMPs) produced by leucocytes and epithelial cells (Thomma *et al.* 2002, Ganz 2004). Expression levels of defensins vary in different epithelial cells, highest being in those tissues that are constantly exposed to, and colonized by, microorganisms (Meade *et al.* 2014). Defensin peptides contribute to the innate immune response against a variety of bacteria, viruses and fungi (Sørensen *et al.* 2008, Meade *et al.* 2014). The characteristics of AMPs (Xia *et al.* 2018) that contribute to their antimicrobial activity include a net positive charge, which allows them adhere to bacterial membranes via electrostatic forces and in hydrophobic microenvironments they form amphipathic structures capable of penetrating the bacterial phospholipid bilayer (Brogden 2005). In addition, antibacterial activity of AMPs could be through other mechanisms, viz. inhibition of cell wall formation, inhibition of cell respiration, bacterial protein inactivation and induction of yeast apoptosis (Xia *et al.* 2018). Other than antimicrobial functions, defensins also have been reported to have immunomodulatory effects, they serve as link between innate and adaptive immune responses, some defensin genes are also involved in hair colour, fertility, disease resistance and reproduction (Candille *et al.* 2007, Zhou *et al.* 2013, Batra *et al.* 2019, Maia *et al.* 2019).

It was recently reported that goat (*Capra hircus*) genome harbours at least 50 β-defensin genes (Zhang *et al.* 2021). Among these, three goat β-defensins (*GBD*), β 1 (*GBD-1*) and β 2 (*GBD-2*) and lingual antimicrobial peptide (*LAP*), have been studied more in goats (Zhao *et al.* 1999, Sharma *et al.* 2010). The expression of *GBD-1* and *GBD-2* AMPs in different tissues had also been reported in goats (Zhao *et al.* 1999, Bagnicka *et al.* 2005, Shao *et al.* 2012). Interestingly, Bagnicka *et al.* (2013) observed gene presence/absence (P/A) polymorphisms for defensins as *LAP* gene was reported to be absent in Polish dairy goats. Considering these facts, the present investigation was designed to study variations, if any, in *GBD* expression in different tissues of Osmanabadi goat, native dual purpose goat breed of Maharashtra state known for hardiness and resistance to diseases.

Different tissues, namely, tongue, reticulum, rumen, omasum, kidney, spleen, liver, trachea and uterus were collected from freshly slaughtered, apparently healthy Osmanabadi goats. Approximately, 100 mg of tissues was collected and total RNA was isolated using RNAiso Plus regent (Takara Bio Inc., India) following manufacturer's protocol. Reverse transcription of the isolated RNA for synthesis of cDNA was done using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio Inc., India) following manufacturer's protocol. The reverse transcription reaction was primed with oligo dT primers. The reaction mixture of 10 µL for cDNA synthesis was prepared by adding 4 µL reverse transcription 5× buffer, 1 µL 10 mM dNTP mixture, 0.5 µL RNase inhibitor, 1 µL PrimeScript RTase (200 IU/µL), 50 µM oligo dT primers 1.0 µL, 8.0 µL total RNA and 4.50 µL nuclease free water. The reaction mixtures were incubated at 42°C for 45 min, the RTase was inactivated at 95°C for 5 min and immediately the tubes were transferred on ice. All the cDNA samples were tested by amplifying the goat β-actin gene using specific goat β-actin primers designed from published sequence of goat beta actin (NCBI GenBank Acc. No. NM_001314342). The cDNAs showing optimum amplification were further used in real-time PCR to quantify the relative expression of *GBD* mRNA by different tissues.

Expression of *GBD* and β-actin (an endogenous housekeeping gene) was detected by Step-One-Plus real time PCR machine (Applied Biosystems, USA). All reactions were performed using the SYBR® Green PCR Master Mix (Applied Biosystems, USA) according to the manufacturer's instructions. Specific primers (sense primer, 5′-CATGAGGCTCCATCACCTG-3′, and antisense primer, 5′-GCGCACAGACGCCTTTATTCC-3′) for amplification of *GBD* were designed from published goat defensin sequences (NCBI GenBank Acc. No. Y17679, *GBD-1*, and NCBI GenBank Ac. No. AJ009877, *GBD*-2). The cDNA obtained from each sample was used as a template for

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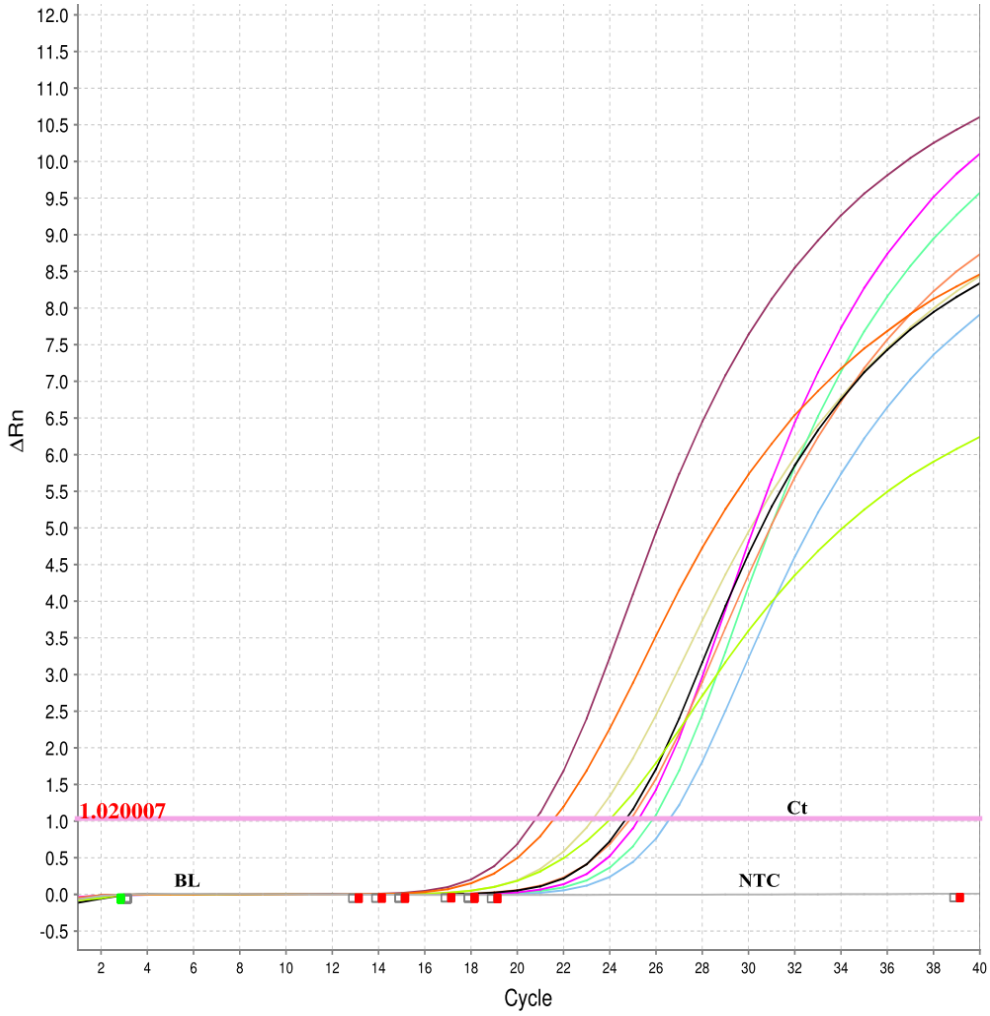

Fig. 1. Amplification plot of goat β-actin in real-time PCR (Ct, Cycle threshold; BL, Baseline; NTC, None-template control).

qPCR in optimized 25 µL reaction volume in MicroAmp optical 96-well plates. The real-time PCR mixture (25 µL) was prepared by adding 2.5 µL cDNA, 1.25 µL (10 pM) forward primer and reverse primer each, 12.5 µL SYBR green qPCR master mix, 7.5 µL nuclease free water. No template control (NTC) was included as negative control. The reaction conditions for qPCR were initial denaturation at 95°C for 10 min, and 40 cycles of 95°C for 20 sec and 62°C for 30 sec. All samples were tested in duplicate and the mean was obtained for further calculations. For each sample, a dissociation curve was generated after completion of amplification and analyzed in comparison to negative control to determine the specificity of PCR reaction. The comparative (C_T) method was employed for relative quantification of *GBD* mRNA and the values were expressed by ΔCt (dCt) value. ΔCt was calculated by subtracting mean cycle threshold (Ct) value of target gene from the value of control reference (housekeeping) gene.

Standard amplification plot for β-actin gene and *GBD* gene was generated using cDNA of different tissues, respectively (Figs 1 and 2). No signal was detectable in NTC indicating these reactions were DNA free. The mean Ct±SD values for goat β-actin in different tissues were 21.58±0.13, 23.98±0.21, 24.71±0.17, 24.87±0.12, 25.24±0.24, 25.85±0.24, 26.58±0.32, 20.79±0.11, and 23.26±0.28, respectively for reticulum, tongue, rumen, omasum, kidney, spleen, liver, trachea and uterus. While Ct values recorded for goat *GBD* in corresponding tissues were 14.67±0.14, 16.23±0.21, 18.00±0.23, 22.40±0.22, 23.81±0.26, 25.14±0.34, 26.79±0.17, 27.66±0.17, and 27.91±0.29. The ΔCt values for different tissues were obtained by subtracting the Ct value of goat *GBD* of respective tissues from Ct value of β-actin amplification. The ΔCt value for tongue epithelia was fund to be the lowest (-7.75), indicating the highest level of expression, followed by reticulum (ΔCt -6.90), rumen (ΔCt -6.70), omasum (ΔCt -2.4), kidney (ΔCt -1.4), and spleen (ΔCt -0.7). The expression of *GBD* mRNA was at moderate level in liver (ΔCt 0.20); whereas it was at minimal level in uterus (ΔCt 4.6) and trachea (ΔCt 6.8). These findings of expression of *GBD* in different goat tissues are consistent with previously published studies (Zhao *et al.* 1999, Shao *et al.* 2012, Sharma *et al.* 2020). Similar to the observations of this study, high levels of *GBD* expression in reticulum and omasum and low levels of *GBD* expression in kidney, spleen, liver had been previously reported in goat

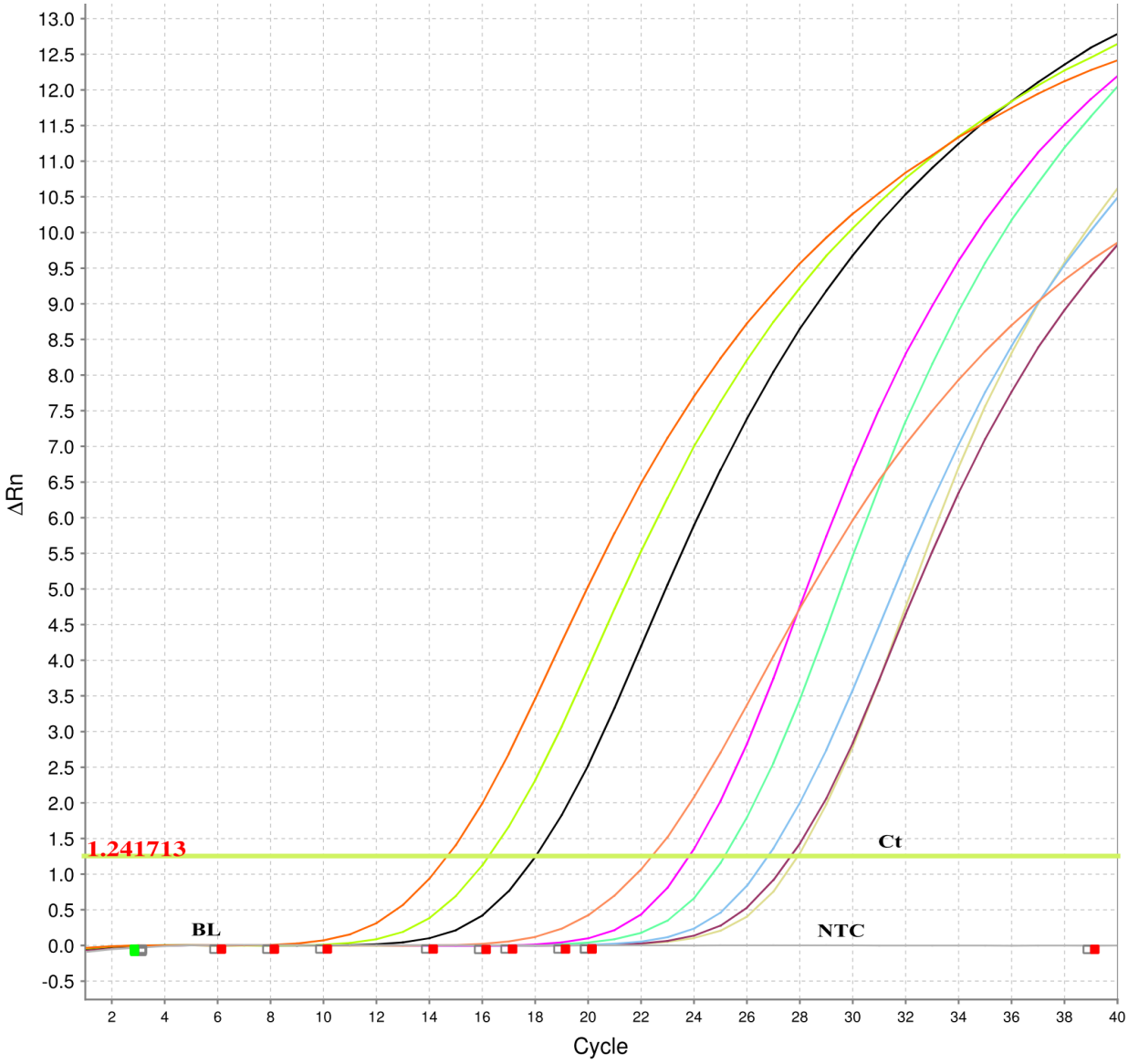

Fig. 2. Amplification plot of goat *GBD* in real-time PCR (Ct, Cycle threshold; BL, Baseline; NTC, None-template control).

(Zhao *et al.* 1999). In the present study, highest level of *GBD* expression was observed in tongue tissue This observation is divergent to previous report where moderate levels of *GBD* expression were detected in tongue tissue (Zhao *et al.* 1999). Likewise, the expression of *GBD* in trachea was previously reported to be at moderate level, however, in present study, the *GBD* expression in trachea was minimal (Zhao *et al.* 1999). The observed differences in *GBD* expression in present investigation might have occurred due to use of different goat breed (Bagnicka *et al.* 2013), single nucleotide polymorphism in *GBD* genes and presence of subclinical infection in the animals. In the present study, *GBD* expression was also seen in rumen, to our knowledge this is the first report about expression of *GBD* in this tissue. In the present study, we did not perform specific identification of AMPs at these sites. Thus, a future study to identify specific AMPs in rumen of goats is warranted.

SUMMARY

In the present investigation, goat β-defensin (*GBD)* expression in different tissues of Osmanabadi goat was studied. Goat tongue epithelia had the highest level of *GBD* expression, followed by reticulum, rumen, omasum, kidney, spleen, liver and uterus. Minimal expression of *GBD* was observed in Osmanabadi tracheal tissue. To our knowledge, this is the first study to report expression of *GBD* in goat rumen.

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