immunomodulatory effect of Morinda citrifolia and Andrographis paniculata on expression of toll-like receptors in Nicobari fowl

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Noni (Morinda citrifolia), a popular medicinal plant prevalent in Andaman & Nicobar Islands, is rich in various phytochemicals and polysaccharides, and its fruits could be used as a feed supplement for poultry (Sunder et al. 2011) to boost the growth performance and immunity. Kalmegh (Andrographis paniculata), a promising medicinal plant, found to inhibit lipid peroxidation and free radical activities. Herbal feed additives as an alternative to antibiotic growth promoters were indicated to exert immunomodulatory action (Mishra et al. 2008); which confer birds with greater general immunity from various diseases. Toll like receptors (TLRs) are innate immune receptors and induce fast and appropriate host defence reaction against pathogens. TLRs recognise the conserved microbial patterns such as flagellin, LPS, peptidoglycan in an efficient and non self reactive manner to initiate pro inflammatory and cytokine. However, the basic information on changes at expression of toll like receptors by Morinda citrifolia, kalmegh and their synergistic effect is not very well known. Hence, the objective of this study was to observe the effects of dietary noni and kalmegh supplementation on production performance, immunity and gene expression of toll-like receptors in Nicobari fowl.

Nicobari chicks were randomly assigned to 5 groups of 5 dietary supplements namely, T1–10 ml Morinda citrifolia juice + 200 mg kalmegh powder / bird / day; T2–15 ml Morinda citrifolia + 400 mg kalmegh powder / bird / day; T3– commercial tonic (GT) 4 ml/bird/day; T4–alternate days: 10 ml Morinda citrifolia + 200 mg kalmegh powder / bird / day; and T5 – control (no tonic). Growth performance and immune response was evaluated.

Total RNA was extracted from caecal tonsil samples (6) of each group by using RNA isoplus and quantification of total RNA was done by biospectrophotometer. cDNA was synthesized from 2 µg of total RNA by using high capacity cDNA synthesis kit. Primers were synthesized from the sequences mentioned by Michailidis et al. (2010) and used in PCR and real-time PCR. Real-time PCR was done in Realplex 4S machine with SYBR green master mix. Real-time PCR was carried out with 1 µl of cDNA, 5 pmoles of each forward and reverse primers, 5 µl of SYBR green master mix and nuclease free water to make up the volume to 10 µl. Cycling parameters were as follows: an initial denaturation at 95°C for 10 min; 40 cycles of 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec.

Growth performance (Table 1) revealed that the T1 showed best body weight gain after the feeding of morinda and kalmegh. Body weight gain at pubertal age was better in T3 followed by T1, T2 and T5 respectively. No significant difference was obtained in terms of egg production both for hen day egg production and hen housed egg production. Feed efficiency with respect to the body weight gain was significantly better (P<0.05) in T1 followed by T2, T3 and T4 respectively. Sapcota et al. (2005) and Mathivanan et al. (2006) also recorded better feed efficiency with Andrographis paniculata and similarly feeding of noni juice and morinda based products as growth promoter and enhancer of egg production was reported earlier (Sunder et al. 2008, 2011, 2012). In all the groups, the immune response (Table 1) was higher compared to control. Sunder et al. (2008, 2011) reported the high B cell and T cell response in Nicobari fowl, broiler and Japanese quail by feeding of M. citrifolia. Neutraceutical compounds, amino acids, vitamins, minerals, coenzyme carbohydrates and alkaloids might have helped in overall growth and elicited immune response (Singh et al. 2008). Supplementation of noni and kalmegh in the present study influenced the expression levels of TLR2, TLR3, TLR4, TLR5, TLR15 and TLR21 significantly (P<0.05) (Table 2) as compared to control. Analysis by real - time PCR revealed that 15 ml Morinda citrifolia + 400 mg kalmegh treatment increased (4.4 fold) TLR-5 gene expression and did not show any significant effect on the expression of other genes; whereas 10 ml Morinda citrifolia juice + 200 mg kalmegh significantly (P<0.05) increased (1.3 fold) TLR-4 gene expression as compared to control and commercial tonic groups and other treatment groups. There was no significant increase in the expression of TLR4 with commercial tonic as compared to control and TLR–2 gene expression levels.
Feed efficiency during pubertal weight gain 7.29 b ±7.06 6.98 b ±2.15 7.46 a ±7.18 7.87 c ±6.34 7.43 b ±3.67

production of interferon- \( \gamma \) (IFN- \( \gamma \)), which is present in the noni fruit, stimulate the TLR signal transduction pathways. This is supported by the fact that some phytochemicals like quercetin, which is present in the noni fruit, showed immunomodulatory activity by modulating NF-κB signal transduction pathway in a dose dependent manner (Desai et al. 2009, Murakami et al. 2007). The increased expression of TLR-3, TLR-4 and TLR-5 in noni can easily enter the cell. These might inhibit the basal expression level of TLR-7. However, TLR-7 in chicken is less important in viral infection (Philbin et al. 2005). The present study has given the base justification for how the immunity improves with the supplementation of medicinal plants. In conclusion, noni and kalmegh might be promising alternative medicinal plant combination for antibiotic growth promoters and commercial immune boosters in the platform of production of antibiotic residue free poultry produce.

**SUMMARY**

This study was conducted to observe the effects of dietary noni and kalmegh supplementation on production performance, immunity and gene expression of toll-like receptors in Nicobari fowl. Supplementation of noni and kalmegh significantly influenced the gene expression levels of TLR-2, TLR-3, TLR-4, TLR-5, TLR-15 and TLR-21 as compared to control. The selectively increased TLR-3, TLR-4 and TLR-5 and decreased TLR7 gene expression indicated that supplementing noni fruit and kalmegh (@ 10 ml + 200 mg / day / bird) induces antiviral and antibacterial responses in chicken and noni and kalmegh might be promising alternative medicinal plant combination for antibiotic growth promoters and commercial immune boosters in the platform of production of antibiotic residue free poultry produce.
safety poultry produce.

REFERENCES


