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

JAI SUNDER[1], T SUJATHA[2], A RAJA[3] and A KUNDU[4]

ICAR-Central Islands Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands 744 105 India

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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, T5, 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

Present address: [1]Principal Scientist, [2]Scientist, [4]Head (drakundu1@yahoo.com), Division of Animal Science. [3]Professor (raja@tanuvas.org.in), Biotechnology Department, TANUVAS, Chennai.

Table 1. Effect of feeding of *Morinda citrifolia* and *kalmegh* on growth, production and immunity in Nicobari fowl

Parameters	T1	T2	T3	T4	T5
Weight gain after treatment	253.00[a]±12.67	238.43[a] ±20.04	233.53[a] ±26.78	180.54[b] ±35.05	247.36 [a] ±21.06
Pubertal weight gain	208.90[b] ±17.06	189.53[b] ±20.15	240.41[a] ±27.18	138.20[c] ±26.34	198.64[b] ±32.67
Hen day production percent (NS)	33.11 ±2.77	31.64 ±2.45	33.75 ±2.25	31.17 ±2.26	32.38 ±3.15
Hen housed egg production percent (NS)	33.33 ± 7.77	31.74 ± 9.45	33.96 ± 9.15	31.11 ± 4.46	32.69 ± 8.25
Feed efficiency during weight gain after treatment	6.16[a]±2.67	6.47[a] ±0.04	7.24[b] ±6.78	7.66[c] ±5.05	6.51[a] ±2.06
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
Humoral immune response (HA titre)					
7th day	1.33[a]±0.07	1.50[a] ±0.04	1.53[a] ±0.07	1.09[b] ±0.05	1.36 [a] ±0.06
14th day	0.90[a] ±0.06	0.99[a] ±0.15	1.03[a] ±0.08	0.48[b] ±0.08	0.93[a] ±0.10
21st day	0.84[a] ±0.02	0.89[a] ±0.07	0.98[a] ±0.05	0.33[b]±0.03	0.86[a] ±0.09
Cell mediated immune response					
Foot index (mm)	0.84[a]±0.17	0.88[a]±0.12	1.00[a]±0.15	0.39[b]±0.08	0.96[a]±0.14

Table 2. Fold difference in expression of different TLRs

Treatments	TLR2	TLR3	TLR4	TLR5	TLR7	TLR15	TLR21
10 ml *Morinda citrifolia* juice + 200 mg *kalmegh* powder / bird / day	0.231	0.016	1.310	0.387	0.678	2.188	1.580
15 ml *Morinda citrifolia*+ 400 mg *kalmegh* powder / bird / day	0.252	0.057	0.268	4.469	0.953	1.547	2.657
GT 4 ml/bird/day	1.165	0.0173	same	1.072	1.319	0.926	0.877
Alternate days: 10 ml *Morinda citrifolia* + 200 mg *kalmegh* powder / bird / day	0.113	0.089	0.567	0.611	0.858	0.476	1.972

Values are in reference to control.

of 10 ml *Morinda citrifolia* juice + 200 mg *kalmegh*, 15 ml *Morinda citrifolia* + 400 mg *kalmegh* and alternate days treatment were lower as compared to the commercial tonic. Expression of TLR-7 gene was low with all treated groups as compared to commercial tonic. The increase in TLR-3, TLR-4 and TLR-5 gene expression and decrease in TLR-7 gene expression in gut associated caecal tonsil in chickens fed dietary noni and *kalmegh* indicated that combination of herbal extracts have better immunomodulatory properties than commercial tonic. Phytochemicals and polysaccharides present in noni fruit showed immunomodulatory activity by modulating NF-kB 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 this present study may be due to effects of phytochemicals on these TLR signal transduction pathways. This is supported by the fact that some phytochemicals like quercetin, which is present in the noni fruit, stimulate the production of interferon-γ (IFN-γ), an antiviral product (Park *et al.* 2009). IFN-γ then acts in an autocrine manner and provides positive feedback for expression of TLR-3, TLR-4 and TLR-5 (Tanabe *et al.* 2003, Tohyama *et al*. 2005). Also, Han *et al*. (2002, 2003) showed that polysaccharides bind with TLR-2, -3, -4 and -5 on the surface of immune cells and stimulate production of various cytokines and chemokines by the NF-kB pathway. Also, the location of TLR-7 on internal cellular endosomes might limit its stimulation by noni polysaccharides, as they cannot enter through the cell. In contrast, several phytochemicals 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, 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.

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 expression of TLR-7 indicated that supplementing noni fruit and kalmegh (@ 10 ml + 200 mg / day / bird) induces antiviral and antibacterial responses in chicken. In conclusion, noni and kalmegh might be promising alternatives for antibiotic growth promoters and commercial immune boosters to improve the production of

safety poultry produce.

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