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Immunomodulation ability of curcumin based 'TOC' against infectious diseases in cattle including mastitis

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Abstract
The study was conducted to assess the effectiveness and immunomodulation ability of 'TOC' product of M/S Arjuna Natural Pvt Ltd as part of validation against infectious diseases including mastitis in cattle. Each animal in the study group (Milch and dry) was given one gram of product orally for a period of 90 days. Research findings indicate that Arjuna Natural's product TOC offers immunity in dairy cattle against infectious diseases including mastitis due to positive modulation of complement protein (C3), Interferon, IgM, lymphocytes and monocytes. Increase in Calcium- Phosphorus and Albumin-Globulin ratio substantiates the 10 per cent increase in milk production. Moreover, there was no incidence of mastitis during the study period. The observations indicate that Arjuna Naturals product TOC offers potential modulatory effect for the test compound with respect to some of the key immunology related factors in dairy cattle against infections. This observation can be correlated with other physiological observations made from the study. At a time when economic loss due to mastitis in India exceeds Rs 13000 Crores annually, there are immense opportunities for transferring the research findings among dairy farmers through industry interface.

Keywords: validation, antibiotic resistance, ethno-veterinary practice, infectious diseases, mastitis, immunomodulation

Introduction
The increasing incidences of infectious diseases in food producing animals are causing huge economic burden for farmers. Uncontrolled use of antibiotics results in increased antibiotic resistant microbes and that eventually make the antibiotic treatment of infections ineffective. The irrational use of antibiotics for treating mastitis resulted in inter-species and inter-genus transfer of antibiotic resistance and the creation of various multi-drug resistance microorganisms in the environment which affects livestock and human health as well.
This situation prompted the biologists to look for alternate strategies to control various infections in food producing animals like cows. One of the strategies in India is the use of Ethno-veterinary practice based on the *Mrigayurveda* concepts to manage infections thereby facilitating reduction of antibiotic residues in animal products like milk, meat, eggs as well as the environment (Datta *et al*., 2020, 2022, Hegde *et al*. 2021, Katrien van't Hooft *et al*. 2021, Nair, 2019, Nair and Punniamurthy 2021, Nair *et al*. 2017, 2021a, b, 2022, Ngwisha *et al*. 2021, Punniamurthy *et al*., 2017 a b, Rana *et al*., 2021) [1-13] The ethno-veterinary practices are found to be effective at two levels which include curative and preventive actions.
It is therefore considered that the ability to increase the resilience, through immunomodulatory effects, as a better means of disease management. The goal of this approach is to positively regulate the immunity for the benefit of the animal and production efficiency. Traditional medicinal plant-based formulations can act as effective immuno modulators (Bhatt *et al*. 2014) [14]. Discovery of new immune regulators and increased understanding of immunity in food-producing animals will ensure new opportunities for the use of plant-based formulations to prevent and /or reduce infectious diseases in in food-producing animals. The proposed study is based on assessing the effectiveness and immunomodulation of a product developed with curcumin as the active ingredient by M/s Arjuna Natural Pvt limited, Kerala.

Materials and Methodology
Sampling & research design
Purposive sampling techniques were used for sampling. Twenty-one cows in the age group of 4-6 years were identified and included in the study. A baseline survey using a structured questionnaire/interview schedule was conducted to understand prevalence of disease conditions and the management practices.

Animals were divided in to three groups of seven animals each which include lactating, pregnant (Dry) and control groups. Each animal in the study group were orally fed with one gram of TOC product for a period of 90 days.

Haematology methods and parameters

CBC parameters were done using a 3-part machine by Mythic 18 vet auto haematology analyser which analyses WBC parameters in 3 parts viz. granulocytes, lymphocytes and monocytes, RBC parameters and platelets. Serum biochemistry was done using Master T semi-auto serum analyser for analysing serum parameters like calcium, phosphorus, total protein, albumin and globulin. Screening of faecal samples and blood smear examination were conducted for detection of parasitic ova for detecting parasitic diseases (Michael Laposata and Peter McCaffrey 2022) [15].

The cytokines, Interferon gamma (IFN-γ), Transforming Growth Factor – beta (TFG-β), Interleukin – 6 (IL-6) and antibodies IgM and IgG as well as complement 3 (C-3) were analysed using ELISA method following standard ELISA kits instruction. Briefly, the assay was carried out in 96 well plates. To the antibody coated plates 50µL of serum samples was added and incubated for the specific period of time mentioned in the kit protocol. After the reaction, colour was developed by adding horse radish peroxidase reagent and the intensity of the colour was measured using ELISA plate reader. The amount of each protein was calculated using standard graph and the fold difference was considered

Results and Discussion

From the study carried out in seven cows each belonging to control and two test groups which includes milking and dry respectively the following conclusions were arrived at.

Screening of faecal samples and blood smear examination revealed that mild infections of strongylosis and mild infections of theileriosis in all the groups studied. Incidence of coccidiosis and strongylosis were reduced among the dry and milch cow groups during the study period.

There was not much variation in calcium, phosphorus, total protein, albumin and globulin content among test groups (milch, dry) and control group (Table 1). But Albumin-Globulin and Calcium-Phosphorus ratio increased in milch animals when compared to control group. (Table 2) WBC, Lymphocytes, Monocytes and Granulocytes (Neutrophils, Eosinophils, and basophils) were within the normal limits in all the groups (Table 3). They were slightly higher in the milch cows when compared to dry test cows and controls. The percentage of Lymphocytes and Monocytes was higher in the test cows (Milch and dry) than the controls. Percentage of Granulocytes was low in Milch cows compared to the dry and control groups.

Table 1: Serum Calcium, Phosphorus, Total Protein, Albumin, and Globulin content

Items	Units	Normal limits	Milch cows	Dry cows	Controls
Calcium	mg/dl	8.9-10.9	9.36	9.8	9.5
Protein	mg/dl	5.6-8.0	5.19	6.76	6.09
Total Protein	g/dl	6.6-8.4	7.21	7.21	7.44
Albumin	g/dl	3.2- 4.3	3.45	3.27	3.2
Globulin	g/dl	2.92-4.7	3.74	3.99	3.88

Table 2: Calcium: Phosphorus ratio & Albumin: Globulin ratio

Particulars	Normal limits	Milch cows	Dry cows	Control
Albumin-Globulin ratio	0.91	0.92	0.82	0.82
Calcium-Phosphorus ratio	1.4	1.8	1.45	1.56

Table 3: WBC, Lymphocytes, Monocytes and Granulocytes (Neutrophils, Eosinophils, and basophils) in all the groups

Items	Units	Normal limits	Milch cows	Dry cows	Controls
WBC	$10^3/\mu$	4.0 - 12.0	5.66	5.1	5.0
LYM	$10^3/\mu$	1.8 - 9.0	3.2	2.9	2.9
MON	$10^3/\mu$	0.1 0.8	0.31	0.26	0.26
GRA	$10^3/\mu$	1.5 – 4.6	3.68	1.58	1.88
LYM	%	45 – 75	58.37	60.25	51.23
MON	%	2 - 7	7.0	6.0	5.04
GRA	%	30 - 38	37.5	60.28	57.7
SNF	%	8.5 - 9	8.27	-	8.16
FAT	%	3 - 4	3.42	-	3.56

There was only marginal increase in SNF (solid non-fat) and fat contents in the test cows compared to the control group. However, 10 per cent increase in milk production was noticed in the milking group compared to the control group.

Feed analysis and digestibility

The chemical composition of the sample mixed feed showed 21 per cent Nitrogen which is adequate for high yielding cows. Higher production of milk and protein levels in milk substantiates this finding. The NDF (Neutral detergent fibre) value of the ration showed higher which was found to be digestible with lower levels of ADF (Acid detergent fibre). Higher total gas values obtained on 24 hr *invitro* production showed better energy availability thus substantiating the increased digestibility and milk production.

Markers for immunomodulation

Compliment 3 (C3) proteins- The study observed an increase of 1.42-fold increase for the dry test cows between 0^{th} day and 90^{th} day (Fig-1), whereas C3 found to remain unchanged in control and milking group and control group for all time points tested.

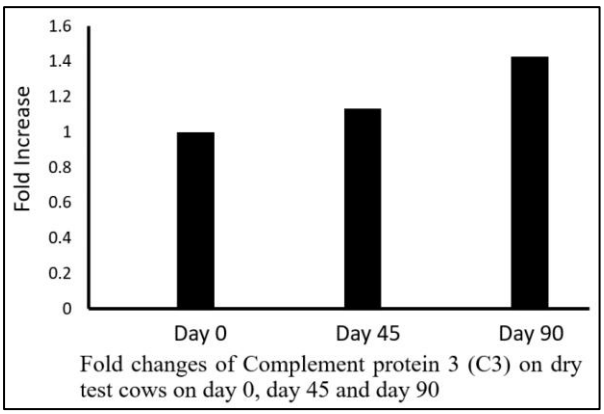

Fig 1: 1.42 folds increase of complement protein 3 in the dry test cows between 0^{th} day and 90^{th} day

Compliment 3 (C3) proteins is one of the proteins which fight the infection. This is an important part of immune system.

It helps to kill bacteria and viruses that cause disease. High or low values of this protein may indicate medical problems ranging from autoimmune diseases and immune disorders to infections. Other conditions linked to low C3 levels include C3 deficiency, a condition that causes recurrent bacterial infections. (Daniel *et al*. 2017) [16]

Interferon gamma was found to increase in milking group on 45th day with an increase of 1.48 folds and in dry test group it was increased 1.58 folds (fig – 2). It is reduced to 1.1 times by 90 days in milking cows. However, in the dry cows it is decreased below the zeroth day level. In control IFN-gamma remained unchanged.

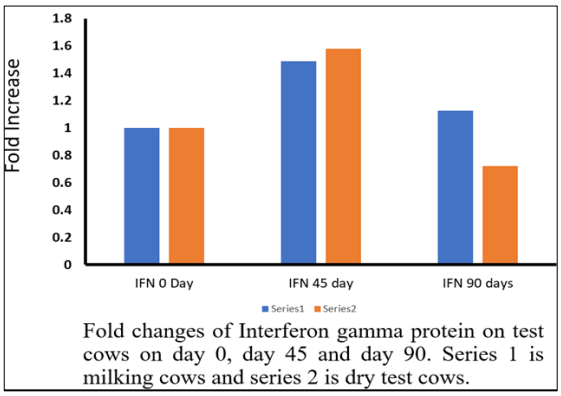

Fig 2: The change in the Interferon gamma in milking group and in dry test group on 45th and 90 days

Interferon- gamma (IFN- γ) is a cytokine that plays an important role in inducing and modulating an array of immune responses. Interferon was first described as an antiviral factor that interferes with viral replication in mammalian cells. They are secreted from infected cells and activate innate immune response that promotes not only cytokine production but also natural killer cell functions and antigen presentation. Interferon are antiviral agents produced by virus-infected cells and can fight tumours. Thus, interferon protects non-infected cells from further viral infections by creating cytokine barriers. (Kate Schroder *et al*, 2003, Zane Vitenberga-Verza *et al*. 2022) [17, 18].

Immunoglobulin G (IgG) was found to increase only in dry test cows up to 1.8-folds at 45th day and was then reduced to 1.6-folds on 90th day (Fig-3). No change in milking test group and controls were noticed (Gestur *et al*. 2014) [19]. IgG in blood and other body fluids and protects against bacterial and viral infections. It can take time to form after an infection or immunization. High levels of IgG may mean infection or an inflammatory or autoimmune disease.

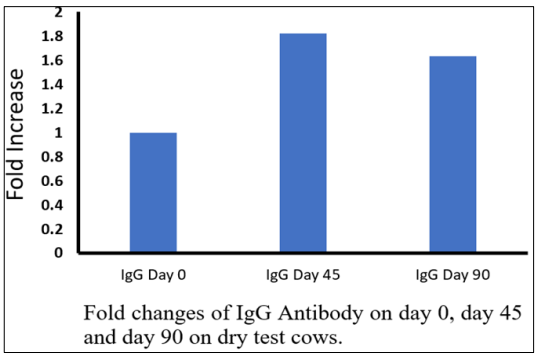

Fig 3: Immunoglobulin G (IgG) increase in dry cow group

Immunoglobulin M (IgM) antibody found to increase up to 1.89-folds on 45th day for milking cows and 2.2-folds in dry test group. They were reduced below 0th day level by 90 days (Fig – 4). There was no changed for control group for all the time points 0 to 90th day).

IgM is specialized to activate complement efficiently upon binding antigen. IgM antibodies are usually of higher affinity and are found in blood and in extracellular fluid. They can neutralize toxins, viruses, and bacteria, opsonize them for phagocytosis, and activate the complement system. IgM not only serves as the first line of host defence against infections but also plays an important role in immune regulation and immunological tolerance. Because this antibody helps provide protection against bacteria and viruses, having low IgM levels has been associated with a higher risk of recurring infections

The other parameters tested like IL-6. TGF-beta and Vitamin-D3 were found to be not changing between the test and control groups at any of the tested time points. Interleukin 6 (IL-6) is essential for the production of antibodies by B cells and general markers for Inflammations and infection. Interleukin 6 (IL-6) was identified as a soluble factor secreted by T cells, which is essential for the production of antibodies by B cells (Kunal *et al*. 2018) [20].

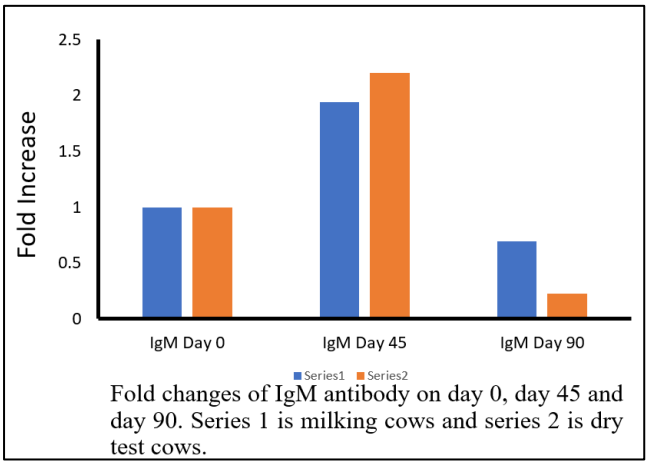

Fig 4: Immunoglobulin M (IgM) antibody found to increase on 45th day for milking cows and 2.2 folds in dry test cows and subsequently reduced by 90 days.

Conclusion

The observations indicate that Arjuna Naturals product with active ingredient as curcumin offers potential modulatory effect for the test compound with respect to some of the key immunology related factors in dairy cattle against infectious diseases including mastitis. This observation can be correlated with other physiological observations made from the study. So, there are immense opportunities for transferring this technology among dairy farmers through industry interface.

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