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Review Article

PROMISING PHYSIOLOGICAL EFFECT OF VARIOUS BIOLOGICAL AND INORGANIC AGENTS AS FEED SUPPLEMENTS FOR LIVESTOCK AND POULTRY WITH DISCUSSION ON RESEARCH PROVEN FACTS AND ESTABLISHMENT OF CONCEPT: AN ELABORATE AND SPECIALIZED REVIEW

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Abstract

This review is constructed with the aim of highlighting the pharmaceutical and physiological effect of purified β-glucan from an edible mushroom (*Pleuratus florida*) as an immunomodulator on the innate immune responses in broiler Also, mushroom glucan as a feed supplement significantly provides protection against disease. This article portrays the potentiality of β-glucan (mushroom origin) as an immunostimulant in poultry. Plant derived and herbal feed additives (often also called phytobiotics or botanicals) are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the individual production performance, and improving the quality of food derived from those animals, such as herbs (flowering, non woody, and non persistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by non aqueous solvents). Cow urine therapy and all traditional practices from Indian systems of medicine have a strong scientific base. Traditional systems in medicines, whether from Ayurveda or Siddha or the use of cow urine distillate as immunomodulator are based on classical texts and systems, practices and products handed down over generations going back to Charak, Sushrutha, Vagabhatta, the Ashtangahridaya and the Samhitas. Cow urine has been described in 'Sushrita Samhita' and 'Ashtanga Sangraha' to be the most effective substance/secretion of animal origin with innumerable therapeutic values. In Ayurveda cow urine is suggested for improving general health. The present article highlights and portrays the immunopotential effect of CUD and CUD can be recommended in broiler ration at optimum dose level against NDV.

Keywords: Fungus, Chicken, Cow urine distillate, Herbs, Immunomodulator, Yeast

INTRODUCTION

Immunomodulator stimulates leucocytes, particularly cells of the macrophage system and modulates and potentiates the immune system of the body[1]. It has been recommended earlier that the constant addition of immunomodulators to feed is beneficial for prevention of diseases[2]. One of such immunostimulant compound is β-Glucan, polymers of glucose which consists of a linear backbone of β-1, 3 linked D- glucopyranosyl residues having varying degree of branching from the C_6 position[3]. β- Glucans are major structural components of yeast, mushrooms and fungal mycelia. Supplementation of β- glucan in diets increase the macrophage phagocytic activity, PHA-P- mediated lymphoproliferative response and also humoral response[4]. β-Glucan provides significant protection against pathogen as a feed additive by up regulating phagocytosis, bacterial killing, and oxidative burst in chicken[5]. In the mammalian system, action of β- glucan is mediated through toll-like receptors (TLR) and dectin-1[5]. In the present work evaluation was carried out for short term dietary influence of a purified β-glucan, prepared from an edible mushroom, on the innate immunity and disease resistance of broiler birds. Immunomodulator is a substance that stimulates leucocytes-particularly cells of the monocyte/ macrophage system and thereby modulates, and most often potentiates, the immune system of the body[1]. The term immunomodulator was often used inter changeable with immunostimulants, adjuvants and biological response modifiers. Glucan and mannan are the main components of yeast cell wall (YCW) that are gained from pure culture of yeast, *Saccharomyces cevisiae*. β-D-glucan is major component of yeast cell wall and has been shown to stimulate non-specific immune response. Glucans with β 1-3, β 1-4 and β 1-6 glucosidic linkages are major structural components of YCW[6], mice[7], rats[8], rabbits[9], sheep and pigs[10]. The phytogenic growth promoters supplemented in the diet or added in the drinking water in the broiler birds have a promising biological effect on their growth performance, to reduce the pathogenic bacteriological load in different parts of digestive tract and to increase villus height in different segments of small intestine mainly in duodenum. Within phytogenic feed additives, the content of active substances in products may vary widely, depending on the plant part used (e.g. seeds, leaf, root or bark), harvesting season, and geographical origin. The technique for processing

(e.g. cold expression, steam distillation, extraction with non aqueous solvents etc.) modifies the active substances and associated compounds within the final product. Experimentally, it has also been proved that among urine from various species the urine of the Indian cows is most effective[11] for its medicinal properties. Immunomodulation is gaining importance for immunopotentiation in hosts against various infections[12]. The cow urine distillate (CUD) is found to have immunomodulatory effect in mice as it enhances both T-and B-cell proliferation and also increases the level of IgG[13]. Recently, the cow urine has also been granted U.S. patents (No. 6896907 and 6410059) for its synergistic properties with antibiotics, antifungal and anti-cancer drugs as bio-enhancer. It has provided the base for further research on immunomodulatory properties of indigenous cow urine. It has also been reported that CUD enhances B and T lymphocyte blastogenesis, increases IgG antibody titer in avian species[14,15]. Keeping in view all the above facts, the present investigation was planned to study the immunomodulatory effect of cow urine distillate on humoral and cell mediated immune response against NDV vaccination in broiler chicks when administered orally.

Importance as Dietary Supplement

Yeast β-glucan has been reported to enhance the immune responses in fish[16-19], cattle[20] and humans[21]. However, information regarding the effect of dietary administration of yeast cell wall preparation on immune responses in birds is limited. In the present study we evaluate the augmentation of the non-specific immune responses, viz., production of oxygen and nitrogen species, lympho proliferation and IL-2 (cytokine) production in broiler birds following YCW treatment. Previous studies showed that infections caused by *Staphylococcus aureus* and *Eimeria vermiformis* in mice can be prevented by β-glucan administration[22]. Experimental respiratory challenge with *Escherichia coli* in broiler chicks can also be prevented by β-1, 3 / 1, 6 glucan derived from *Saccharomyces cerevisiae*[23]. Rice *et al.*[24] showed that dietary administration of glucan to rat enhanced survivability against *Staphylococcus aureus* infections. Orally administered yeast β-glucan to mice could reduce the mortality in anthrax infections[25]. The phytogenic growth promoter remains active throughout the gastrointestinal tract and as a consequence, it will exert broad spectrum antimicrobial action, will enhance nutrient utilization by exhibiting improvement in overall growth performance of broilers and by augmenting the gastrointestinal histomorphology thereby enhancing the host immunity[26]. Immunomodulatory effect of cow urine or its distillate has been reported by many workers[27-29] and therefore this has made the base for present research. The dose of CUD selected in the present study is according to the recommendation by Kumar *et al.*[30]. Jojo *et al*.[31] documented that the levamisole treated group of chicks also showed significant effect on MHI antibody titer in comparison to CUD suggesting its superior immunopotentiating effect over CUD on humoral immune response upon vaccination. Awadhiya *et al.*[32], Srikumar *et al.*[33], Kumari[34] and Rakhi[35] showed increased cell mediated immune (CMI) response correlated with the findings. The findings were also in accordance with those of Chauhan *et al.*[13,36], Ambwani[28] and Garg *et al.*[15] who worked on lymphocytes blastogenic activity with respective mitogens using lymphocyte proliferation assay.

Implications in Immunomodulation and Body Growth Promotion with Influence on Hematological and Biochemical Parameters

In vertebrates, the immunomodulating abilities of β-glucans are thought to stem from their ability to activate leukocytes, but there is some confusion about their precise biological effects[37]. Paul *et al*.[38] assessed the immunostimulatory role of glucan extracted from yeast (*Saccharomyces cevisiae*) cell wall was assessed in two different doses in terms of cellular immune effecter activity. The production of oxygen radicals by YCW (both dose group) fed broiler birds was higher up to 20th day post treatment than control values. The O.D. value was in peak level at 10th day post treatment and significantly higher than control group ($P < 0.05$) and then the O.D. values on 20th day decreased. The oxygen radical production in 0.8 g/kg treatment group was higher than 0.4 g treatment group on 10th day post treatment. Nitrite production was increased in both YCW fed groups than control group at 0 day[39]. From 10th day onward the nitrite production level was decreased in 0.8 g treatment group but in 0.4 g treatment group nitrite production was peak level at 10th day post treatment. In 0.4 g treatment group *in vitro* non-specific lymphocyte proliferation and IL-2 production was first increased and then decreased abruptly. But in 0.8 g treatment group *in vitro* non-specific lymphocyte proliferation and IL-2 production was increased and then decreased gradually and IL-2 production was in peak level at 10th day post treatment[39]. The previous workers showed that the use of yeast glucan was enhanced oxidative respiratory burst in human and chicken[40], monocyte activity and nitrite production also enhanced in sheep and chicken[41]. Guo *et al.*[4] and Waller *et al.*[41] observed glucan enhanced the lymphocyte proliferation in cattle. Oral administration of yeast glucan enhanced the cytokine production in mice[42]. The enhancement of oxygen radicals, nitrite, cytokine (IL-2) production and lympho proliferation of broiler birds might be related to the oral administration of yeast cell wall preparation (Nutriferm^{TM}) from *Saccharomyces cevisiae*[26]. Burt[43] stated microbial analysis of minimum inhibitory concentration (MIC) of plant extracts from spices and herbs, as well as of pure active substances revealed levels that considerably exceeded the dietary doses when used as phytogenic feed additive. Aksit *et al.*[44] reported antimicrobial action of phytogenic feed additive may be in improving the microbial hygiene of carcass. Batal and Parsons[45] indicated that micronutrients also influenced the morphology of intestines. They observed an increased height of villi of jejunum in broilers at 28th day of age when fed with 5 g BioMos/kg from 7 to 28 day. Jamroz *et al.*[46] have conducted a study that phytogenic formulations contained pungent principles (e.g. capsaicin) significantly increased intestinal mucus production. Jamroz and Kamel[47] observed on the improvements in daily weight gain (8.1 %) and in feed conversion ratio (7.7 %) of chickens when feed with diets supplemented (300 mg/kg) with a plant extract containing capsaicin, cinnamaldehyde and carvacrol. Biavatti *et al.*[48] reported *Alternanthera brasiliana* extracts (180 ml/200 kg feed) improved broiler performance from 14 to 21 days. Hernandez *et al*.[49] studied that blend of essential oils of cinnamon, pepper and oregano compounds improved digestibility of nutrients in chicken. Jang *et al*.[50] in chicken is the benefit of some natural substances on gastro intestinal enzymatic activity, most likely improving nutrient digestibility. An experiment was conducted for evaluating the

efficiency or effect of the phytogenic growth promoter. The phytogenic growth promoter was active throughout the gastrointestinal tract and as a consequence, it will exert broad spectrum antimicrobial action, will enhance nutrient utilization by improving gastrointestinal absorptive properties and will augment the host immunity. In the experiment, two proven and approved phytogenic growth promoters, Digestarom 1317 (dosage 150 ppm) and Digestarom 1440 (dosage 800 ppm) AC were fed to the broiler chickens against an antibiotic growth promoter, Bacitracin Methylene Disalicylate (BMD)[51]. Digestarom AC is a combination of phytogenic components with glycerides of short chain fatty acids. Basically, Digestarom AC is a complex of plant extracts and plant essential oils along with monoglycerides, lactic acids and multiglyceride complexes. Being a complex of plant extracts and essential oils, Digestarom AC is hypothesized to stimulate feed intake, intestinal secretion of enzymes and enhance digestibility of nutrients. Additionally, Digestarom AC is anticipated to act as a broad spectrum antimicrobial substances throughout the gastrointestinal tract and promote development of the villus structure of the gut[51]. Combining cow urine distillate (the term 'distillate' itself is a misnomer, since the material used is the residue, not the distillate) with antibiotics is not recommended at all and its combination in liquid or lyophilized powder form with modern drugs is irrational, since the relative bioavailability and pharmacokinetics of the components remain unknown. *In vitro* experiments with cow urine distillate have little relevance, since activity *in vivo* largely depends on plasma levels, which in turn are related to serum binding properties and absorption[27,52]. Mammalian urine contains useful constituents like adrenocorticotropic hormaone (ACTH) isolated from pregnant female urine. Other constituents include various enzymes, amino acids and Erythropoetin. The reported results of experiments which have been carried out on cow urine distillate in India and the grant of the U.S. patent vindicates the use of cow urine as a bio-enhancer[53]. According to a recent online report of 'Love4Cow Trust', researchers at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India have identified a fraction of cow urine distillate as bio-enhancer of commonly used antibiotics and anti-cancer drugs. Bio-enhancers do not possess drug activity of their own but promote and augment the bioactivity or bioavailability or the uptake of drugs in combination therapy. Such bio-enhancers have been earlier isolated only from plant sources. In the study at CIMAP, Lucknow, India researchers found that 'cow urine distillate fraction' enhances the activity of antibiotics such as rifampicin by about 5-7 folds against *E. coli* and 3-11 folds against Gram-positive bacteria. Rifampicin is a front-line anti-tubercular drug used against tuberculosis. Interestingly, it was also found that 'cow urine distillate fraction' enhanced the potency of 'Taxol' (paclitaxel) against MCF-7 a human breast cancer cell line in *in-vitro* assays (US Patent No.6, 410, 059).

CONCLUSION

It can be concluded that dietary β-glucan may provide immunostimulatory properties necessary to reduce the incidence of any infection in poultry. Cow urine distillate (CUD) possesses immunomodulatory effect as judged by increase in HI antibody titer against viral infection. The immunopotentiating effect of CUD has been analyzed on humoral and cell mediated immune response with virulent virus vaccination, its use as an immunomodulating agent at proper dose level may be advocated. The phytogenic growth promoter enhance productive performance of the broiler in terms of body weight gain with minimum alteration of gut morphology and the possibility of bacterial invasion is much less. Phytogenic growth promoter can be used as a potent replacer of antibiotic growth promoter if used at optimum level.

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