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Recent Contributions to Development of Herbal-Based Immunomodulators for Farm Animals

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Abstract

Safety and quality of food and feed today is at a high level, the challenge is to ensure a healthy daily diet but affordable for everyone. But the most important link for obtaining sanogen products for food is to use organic, eco-friendly and not polluted agricultural raw materials (vegetable and animal) and most of all, to "produce for man in harmony with nature".

Use of immunostimulants is a unique approach for farm owners as they undertake methods of controlling disease losses in their facilities. Microbial diseases are limiting factors in all forms of intensive culture and a problem is that few approved chemotherapeutic agents are available for use in animal food because of growing concerns for consumers liability and for accumulation of substances in the environment. Many medicinal plants showing immunomodulatory activity have been used instead of drugs because of their low toxicity for the host system.

This review aims at presenting recent contributions to development of herbal-based immunomodulators for farm animals, a total of 97 studies from 2000 - 2016 concerning species of economic interest (various categories of fish - tilapia, trout, carp; shrimps; cattle; pigs and chickens). In veterinary practice, innovative eco-friendly products that could improve or prevent some disorders became of great actuality and therefore the research on animals of economic interest should continue for the benefit of both animals and humans.

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Introduction

Safety and quality of food and feed in Europe today is at a high level, the challenge is to ensure a healthy daily diet but affordable for everyone. Plant breeding and development of functional food ingredients contribute to the production of high quality food and thus to ensure a healthy diet for the entire population in a sustainable manner, while a more efficient processing and distribution makes quality food to be available at an affordable price.

A pragmatic outcome of eco-development is designing and creating sanogen products[1] based on the approach centered on values which take into account man and its immediate and future requirements. But the most important link for obtaining sanogen products for food, cosmetic, pharmaceutical, etc. is to use organic, eco-friendly and not polluted agricultural raw materials (vegetable and animal) and most of all, to "produce for man in harmony with nature".

In veterinary practice, reasons for producing a poor immunity to an infection can include: the infection itself – a number of infections suppress immunity and these particularly include some viruses and mycoplasma; stress – this comes in many different forms for an animal; nutrition – an inadequate overall diet may suppress immunity; deficiency in individual feed ingredients – especially proteins, vitamin A and E, selenium, etc.; the production status of the animal – immune response is often less in the mother close to birth; age of animal –very young or old are often imunosuppressed; other diseases – especially cancer, metabolic diseases (e.g. pregnancy toxemia in sheep, fatty liver in cattle); vaccination process – insufficient vaccine given, out-

of-date, stored incorrectly, not administered by correct route, period too long from making up vaccine to its administration (Responsible use of medicine in farm animals guidelines, 2006 Responsible use of vaccines and vaccinations in farm animal production). Low immune system and responses may result in very high mortality due to specific pathogens that antibiotics are helpless against. In all forms of intensive culture, where single or multiple species are reared at high densities, infectious disease agents are easily transmitted between individuals.

Many medicinal plants showing immunomodulatory activity have been used instead of drugs because of their low toxicity for the host system, adequate absorption and capability to reach the target organ without much degradation by host enzymes[2].

The side effects of synthetic drugs such as presence of antibiotic residues leading to the problem of antibiotic resistance in humans, toxic metabolites remaining in meat and byproducts are a matter of concern in long term usage of synthetic products. Such issues have promoted use of herbal preparations which are considered to be relatively safe and affordable to rural folk. Further, absence of antibiotic or toxic residues in meat and milk products has also encouraged herb based health solutions in veterinary health care sector. Thus, traditional herbal medicines in veterinary practice have great potential as an alternate therapy[3].

Experimental research performed with extracts or active principles isolated from plants have shown that they can influence the immune response in several ways[1]: on cellular level by modulating the proliferation rate of immune cells (e.g. naftoquinones), on humoral level by influencing the antibody production (e.g. polysaccharides) or by modulating cellular functions to increase or decrease cytokine or other mediators production (e.g. phenolic compounds).

Most studies have used herbal extracts rather than the purified compounds, therefore there is still suspicion concerning the efficacy and optimum dosage of herbal plants and their derivatives as immuno stimulators. Hence, more research is required for scientific validation of herbal plants as potent animal immunostimulators[4]. Csermely et al., 2005[5] suggested that a pharmacological strategy directed toward multiple targets could result in more efficient therapeutic outcomes. It has also been emphasized that the multi-component compounds possessing broader specificity and lower affinity, as found in botanical medicines, can be more efficient than compounds with high affinity and high specificity[6]. Moreover, the use of whole plants, instead of isolated chemicals, may offer a safer clinical strategy in the treatment of many diseases[7].

There are differences in pharmaco dynamic and pharmacokinetic reactivity between animals and humans, veterinary pathology having specific anatomic and physiologic features. Although human herbal medicines are applied in veterinary field, this is not a safe way of treatment especially on food animals because of the lack of studies regarding toxicity, pharmacological effect, side effects or residues targeted on respective species.

Relationship between nutrition and the immune system has been the center of attention in scientific communities in last decade. Unfortunately, there are a small number of studies which have revealed the mechanism of action of the immuno stimulatory compounds of herbal plants. An understanding of the mechanisms through which phytochemical influences the immune system is necessary to appreciate the use of herbal plant as immunostimulators and veterinary medicinal products[4].

This review aims at presenting recent contributions to development of herbal-based immunomodulators for veterinary use. Most of the studies were performed on species of economic interest and therefore compiled data are sorted depending on animal species used in clinical trials.

Methods

Literature review

Studies were identified by conducting electronic searches of PubMed, Science Direct and Scopus from 2000 to the end of 2015. More than 100 papers related to utilization of herbs, herbal extracts or herbal products as feed or medicines in farm animals were consulted.

Study selection, inclusion and exclusion criteria

The following search terms were combined with the term immunomodulation: plants and animals, or herbs and animals, or plants and fish/ shrimp/ cattle/ pig/ chicken.

Studies were selected based on the following inclusion criteria: Farm animal studies. Tests on mice, rats and rabbits were excluded (preclinical tests). Studies presenting detailed information regarding the herbal product used (form of administration: extract, herb, single species or in combination; specifications on doses used) and the delivery route (oral, topic, associated with other drugs or vaccines; timing)

The presence of test group and control group: Studies reporting immunomodulatory effects following administration of herbal products, even if the study also reported other several effects on animal functions.

English language: Studies published between 2000 - 2016.

Results

A total of 97 studies were considered appropriate for inclusion in this review. Almost half of them (48%) were conducted in the last 6 years. The majority of animal studies explored models in which healthy animals were fed with herbal extracts, other studies investigated animals exposed to specific viruses and bacterial pathogens. The use of herbals as adjuvants for vaccines and antibiotics was also noted.

The results compiled for each animal category taken into consideration are presented as follows.

Research on poultry

Over the years in poultry industry, most of the selection emphasis has been on the improvement of growth performance and these changes have been shown to be negatively associated with immunological parameters of poultry[4]. Most of the studies focuses the therapeutic approach in three highly contagious diseases of chickens: Infectious Bursal disease caused by infectious bursal disease virus (IBDV), characterized by immuno-suppression and mortality generally at 3 to 6 weeks of age, Newcastle disease (ND) characterized by marked variations in morbidity, death rate, symptoms and lesions and coccidiosis, a parasitic

disease of the gastrointestinal tract affecting mostly young and immuno-compromised animals. The relevant studies on this topic were synthesized and presented in table 1. Although a lot of investigations were conducted, there are still many contradictory results resulted probably from different experimental models carried out.

Table 1: – Synthesis of immunomodulatory herbal extracts therapeutically or preventive applied in chicken.

Herbal preparation	Effect on the immune system	Reference
Aloe extract @15 ml/liter of drinking water	better antibody titer against IB and IBD and lower coccidia oocysts count in bedding material	Durranietal.,2008[66]; Darabighane & Nahashon,2014[67].
Momordica cochinchinensis seed (ECMS) (20, 40, and 80 micro g)	a dose of 20 microg of ECMS is capable to significantly enhance antibody levels on 14, 21, 28,and 35 days when compared with controls (inactivatedIBD vaccine alone) and to increase mitogenic stimulated lymphocyte proliferation	Rajput et al., 2010[68]
Garlic infusion	immunostimulant effect against Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB)	Shahriyar and Durrani, 2006[69]
2 and 4g/kg cinnamon and 2 and 4g/kg garlic powder added to the basal diet	none of the immune related parameters measured including antibody titers, lymphoid organs' weight, A/G and H/L ratios was neither positively nor negatively stimulated	Toghyani et al.,2011[70]
Anissed and ginger aqueous extract	Significant effect on the immune performance of broilers against IBD, IB and ND	Atiq and Durrani, 2007[34]
4, 6, 3, and 10 g of Garlic (*Allium sativum*), Ginger(*Zingiber officinale*), Neem(*Azadirachta indica*) and Berberry (*Berberis lycium*) respectively, mixed per liter drinking water.	Better immune performance against Newcastle disease, Infectious Bronchitis, Infectious bursal disease and Coccidiosis	Nidaullah and Durrani, 2010[71]
Livol (herbal product consisting in a mixture of *Andrographis paniculata, Azadirachta indica, Betafin, Magnifera indica, Terminalia chebula, Terminalia arjuna, Eclipta elba* and *Solanumigrum*) of broilers diet (@ 1 ml/7liter of water from day one to 42 of age)	potent immunostimulatory effect by potentiating humoral immunity, therefore can be helpful in ameliorating the negative and/or harmful effects of IBDV vaccination	Zahid et al., 2015[72]
Neem leaves infusion	significant effect on the immune performance against IBD	Sarang and Durani, 2005[73]
7 g neem/kg	greater antibody titers against SRBC and influenza virus compared with the control diet	Landy et al., 2011[74]
2% *Aloe vera* gel (mixed with their drinking water)	Significant increase in antibody titer against Newcastle disease virus on days 37 and 52	Valle-Paraso et al., 2005[75]
Aloe vera gel powder (at 0.5%, 0.75%, and 1% in feed)	Significant increase in antibody titer against Newcastle disease virus on days 37 and 52	Alemi et al., 2012[76]
crude extract of *Aloe secundiflora*	non-significant response against Newcastle disease.	Waihenya et al. , 2002[77]
Withania somnifera extract	does not change serum total protein, albumin and globulin and numerical decrease in HI titre against Newcastle vaccine comparing to control but combination of enrofloxacin and 1-2% W. somnifera extract is capable of increasing dose dependent all the parameters above	Arivuchelvan et al., 2013[78]
Water extracts of *Radix astragali, Radix codonopis, Herba epimedii* and *Radix glycyrrizae* individually and in different combinations were supplemented in drinking water.	Improvement of immune response and increase of antibody titers to NDV and H5-AIV after vaccination in chickens with immunosuppression induced by Reticulo endotheliosis virus (REV) infection, but did not show such immunological enhancement in clinically healthy chickens	Liu et al., 2010[79]
combination of carvacrol, cinnamaldehyde and Capsicum oleoresin	enhances coccidiosis resistance showing beneficial effects on host immune system and metabolic conditions through the regulation of gene expression in the chicken gut	Lillehoj et al., 2011[80]
T. cordifolia stem (1g/kg) which can be used extract potentially before mass vaccination	property of immunomodulation like levamisole	Bhardwaj et al.,2011[81]
Asparagus racemosus dried root powder	stimulates both humoral and cell mediated immune responses	Kumari et al., 2012[82]
Aloe vera gel powder (0,75% and 1% mixed with feed)	significant positive effects on antibody titer against SRBC	Mahadvi et al.,2012[83]

sugar cane extracts (SCE) or polyphenol-rich fraction (500 mg/kg/day) for 3 consecutive days	significantly higher antibody responses against sheep red blood cells and resulted in a significant increase in the number of IgM- and IgG-plaque forming cell responses of PBL	Hikosaka et al.,2007[84]
Sugar cane extract before or after whole body X-ray irradiation	enhanced both primary and secondary immune responses in chickens as well as cell-mediated immunity measured by delayed type hypersensitivity to human gamma-globulin	Amer et al., 2004[85]
Ocimum sanctum	during antibiotic therapy to overcome the adverse effects and enhance the immunoprotective effect high globulin level	Arivuchelvan et al., 2012[2] Mode et al., 2009[86] Singh et al., 2010[19]
	effect of O. sanctum on humoral and cell mediated immune response.	Goel et al, 2010[87]
hot aqueous extract of *O. sanctum*	enhanced the antibody level by 42.85% in comparison to control group and acted as stimulant of humoral response stimulant; stimulatory effect on both arms of immune system	Goel et al., 2008;[88] Varshney et al.,2013[89]
hot aqueous extract of *Argemone mexicana*	enhanced the antibody level by 14.28% in comparison to control group and acted as stimulant of humoral response stimulant; antibody stimulation response but suppression in cell mediated immune response	Goel et al., 2008;[88] Varshney et al.,2013[89]
rosemary powder and ethanolic extract	failed to show any significant impact on antibody titers against NDV, SRBC and influenza disease virus, but remarkably improve total serum antioxidant activity.	Soltani et al., 2016[90]
10 g anise/kg diet	Increases the antibody titer against avian influenza virus	Yazdi et al., 2014[91]
1 g *Tribulus terrestris* L./kg	higher antibody titer against avian influenza virus and sheep red blood cells at 28 and 31 days of age	Yazdi et al., 2014[92]
10 to 20 g/kg *Nigella sativa* L. seed	Improves antibody-mediated immunity	Ghasemi et al., 2014[93]
5 and 10 g/kg *Mentha pulegium* L. powder added to the basal diet.	No semnificative effects on humoral immune response	Ghalamkari et al., 2012[94]
0.2%, 0.4% and 0.6% doses of *Mentha spicata* extract in the drinking water	No stimulation of the immune system response	Nanekarani et al., 2012[95]
Urtica dioica alcoholic extract	Improves innate immune response, enhances the phagocytic capacity of leukocytes, induces higher resistance to diseases and improves post vaccination response	Sandru et al., 2016[96]
proanthocyanidin-rich extract (PAE) from Pinus radiata bark		Park et al., 2013[97]

Research on cattle

Few researches were carried out for demonstrating the specific immunomodulatory effect of different herbs/ extracts in bovines, most of the studies focusing on collateral effects of herbal administration – antioxidant, improvement of metabolic status, etc. Mastitis, a potentially fatal mammary gland infection, is the most common disease in dairy cattle. A study conducted by Bhatt et al., 2014[8] supports the use of alternative herbal therapy against bovine sub-clinical mastitis by enhancement of cytokine expression of somatic cells and reduction in total bacterial count in bovine mammary gland after topical application of 5 g of Mastilep herbal gel on each affected udder quarter including the teats, after the morning and evening milking for 5 consecutive days. Each 10g of Mastilep (Dabur Ayurvet Ltd., Ghaziabad, India) contained Eucalyptus globulus 0.20 g, Glycyrrhiza glabra 0.20 g, Curcuma longa 0.04 g, Cedrus deodara 1.00 g, Paedaria foetida 0.04 g and sulphur 1.00 g in a gel base[8].

The ability of ginseng (GS) and purified ginsenoside R(b1) to enhance the efficacy of mastitis vaccines in protection against intramammary infections was also tested and resulted that addition of R(b1) resulted both in significantly higher antibody production and lymphocyte proliferation in response to PWM (pokeweed mitogen), ConA (concanavalin A) and Staphylococcus aureus antigens than in the control group, but addition of GS induced only a significantly higher lymphocyte proliferation and had no effect on the antibody production[9]. Also, Baravalle et al., 2011[10] concluded that GS used as immunostimulant at drying off could play a role in mastitis control by enhancing intramammary defenses, either alone or in conjunction with antibiotic therapy[10].

Even if some plants, such as Ocimum sanctum, with proved immunomodulatory effect in other species are also effective in increasing both the humoral and cell mediated immune responses in cattle[2,11], in other cases the immunomodulatory effect is not confirmed -Matricaria chamomilla is a well-known immune booster in humans, but it has no stimulatory effect in cattle for rabies immunization[12].

Research on pigs

The use of immunomodulators could be a useful approach to enhance immune responses after vaccination or to overcome infectious diseases in swine[13]. Gallois and Oswald (2012)[14] emphasized that in a perspective of short or mid-term application in pig farm, a balance-sheet of the potential use of immunomodulators in pig nutrition is needed, especially during the weaning transition where they are highly sensitive to di-

gestive disorders. Plants and their bioactive components, when known, are very diverse and their potential to enhance pig health and immunity has only been scarcely evaluated *in vivo*.

The use of ginseng as a co-adjuvant provides a simple, safe and cheap alternative for improving the potency of aluminium hydroxide adjuvanted vaccines by facilitating the production not only of IgG1 antibodies but also of IgG2. It was proved that the addition of 2mg ginseng per vaccine dose, potentiate the antibody response of the commercial vaccines without altering their safety[15].

Dietary treatment of piglets with crude soap bark of Quillaja saponaria did not counteract the negative effects on feed intake and growth induced transiently by a challenge with Salmonella enteric, Serovar typhimurium[16], although saponins of this species are widely used as a vaccine adjuvant[17]. It has been suggested that these "weak immune modulations" may be due to the low purity of the extract used[18], i.e. a low content in saponins and a high content in tannins known to have anti-nutritional properties[19]. Thus, Ilsley et al. 2005[18] incorporated a purified saponin extract from Q. saponaria in the diet, alone or in combination with curcumin which has been shown to modulate lymphocyte mediated immune functions in mice[20]. Whereas piglet immune responses were not influenced by curcumin, the feed intake and serum IgA, IgG and C- reactive protein concentrations were transiently increased in saponin-fed piglets[18]. The subsequent negative impact of saponin on feed utilization could result from increased dietary requirements to mount an immune response[18]. However, the impact on health of such an increased immune response still needs to be demonstrated.

Beta-sitosterol (BSS) can be considered as immunomodulators in pigs. Pigs treated with a Spanish product (Inmunicín MAYMO®), based on food plant phytosterols (10 µg/ml (12 µM of BSS) or 100 µg/ml (123 µM of BSS), commercialized as complementary feed, prior to vaccination with porcine reproductive and respiratory syndrome virus modified life vaccine PRRSV-MLV vaccine exhibited some changes in immunological parameters at different times post-vaccination, such as the proliferation ability of Peripheral blood mononuclear cells PBMC after phytohemaglutinin stimulation and increased apolipoprotein A1 plasma concentration which may contribute to enhance PRRSV vaccine response[21].

Dietary supplementation of pig's diet with essential oils has in general beneficial effects on growth performance as showed[22] and it was also investigated by researchers focusing on immune system. Thymol used alone enhances total IgA and IgM serum levels and exhibits some local anti-inflammatory properties, as indicated by a reduction in TNF-α mRNA in the stomach of post-weaned pigs[23].

An extract of Origanum vulgare, enriched with thymol and carvacrol in similar proportions, was reported to protect low-weight growing-finishing pigs from disease[24]. This health benefit was associated with an increased proportion of CD4 +, CD8 + and double positive T cells in peripheral blood and mesenteric lymph nodes[24]. Other studies showed no beneficial effects: a plant extract containing 6% of carvacrol and 0.14% of thymol, incorporated at 0.05 to 0.15% in pig diet, had no effect on the plasma levels of acute phase proteins[25], and the inclusion of a commercial plant product composed of oregano oil mixed with anis and citrus oils did not improve health status of piglets[26].

A plant extract containing 5% of carvacrol (Origanum spp.), 3% of cinnamaldehyde (Cinnamonum spp.) and 2% of capsicum oleoresin (Capsicum annum), included in the feed at a 0.03% level, led to a decreased number of jejunal intra-epithelial lymphocytes, and an increased number of lymphocytes in the colonic lamina propria[27]. Conversely, mononuclear cell subsets from ileal peyer's patches were not affected by this plant extract combination and only the percentage of B lymphocytes was reduced in lymph nodes of piglets[28].

The immune modulations conferred by vegetal glucans (anti-inflammatory properties, increased T- lymphocyte proliferation) may be beneficial for the piglets to fight against infections, but this need to be further specifically demonstrated. Up to now, Yuan et al. (2006)[29] reported that dietary Astragalus membranaceus increases the white blood cell count, mainly through the contribution of CD4+ lymphocytes. Also, the administration of b-glucans in piglets increases the proliferation of T cells isolated from peripheral blood[30], blood concentration in IL- 2 and interferon-γ (IFN-γ), whereas IL-4 and IL-10 concentrations remained unchanged[29,30] which suggests a Th1 activation, and thus an enhancement of cellular immunity. Plant β- glucans do not seem to influence humoral immunity, as indicated by the specific antibody titres following immunization with ovalbumin[29]. Moreover, when supplied at moderate doses, glucans from A. membranaceus can counteract the increased plasma concentrations of IL-1β and prostaglandin E2 induced by a LPS challenge[14,30].

Astragalus polysaccharides (APS) extracted from the herb is recognized as an effective immune- modulating function both in humans and animals. In another study, it was showed that APS in different dosages (5,10 and 20 mg kg) rapidly increased the Foot and Mouth Disease Virus specific antibody in a dose-dependent manner in fifteen four-week-old Yorkshire pigs. APS also significantly up-regulated the mRNA expression of the production of Th1 (IFN-γ) and Th2 (IL-6) cytokines in peripheral blood lymphocytes from the immunized pigs[31].

Genistein and daidzein, two isoflavones found in soybean products, were also suggested to act as immune-modulators when given orally. Both isoflavones are efficient in promoting growth in piglets challenged with PRRS virus, which suggests that their mechanisms of action would differ. After oronasal infection of piglets with PRRS virus, genistein minimised the viraemia from day 4 to day 24 post-inoculation, as well as the serum concentration of IFN-γ[32] and increased serum α-1-acid glycoprotein concentration[33]. Accordingly, lower serum IFN-γ concentration in genistein-fed animals is in agreement with the greater virus elimination and a quicker return of IFN-γ to basal levels[33]. In the same experimental model, dietary daidzein failed to decrease serum titres of virus[33] and also serum α-1-acid glycoprotein concentration was not modulated[14,33].

Research on fish

Aquaculture is one of the farming branches with a strong ascending trend in last years. The administration of herbal extracts for nutritive and medicinal purposes in fish farms is a great challenge, due to particular features of this group of organisms. Worldwide fish and shellfish culture are subjected to many diseases that lead to great losses and decrease in fish production, but studies regarding fish immunity are still at beginning comparing to mammals. The use of immunostimulants in aquacul-

ture for prevention of diseases (especially in early stages – fish larvae) is a promising new development[34] and it can influence in a positive way both fish production and quality and also would contribute to a cleaner environment due to high biodegradability. Most of the herbs and herbal extracts can be given orally, which is the most convenient method of immunostimulation[34]. Mechanisms involved remain as yet rather obscure, although some information exists. Immunomodulators present in the diet stimulate the nonspecific immune system, while antigenic substances such as bactrian's or vaccines initiate the more prolonged process of antibody production and acquired immunity[35]. However, the effect is dose-dependent, and there is always a potential for overdosing consequently, dosage optimization is strongly recommended[34].

The use of plant extracts in practical diets for fish is a modern approach in aquaculture industry, but experimental models were carried out only on few species, as follows.

Tilapia

Specific and non-specific immune responses and disease resistance against A. hydrophila in Tilapia are influenced by Phyllanthus emblica (crude extract and water-soluble fraction); Eclipta alba (leaf aqueous extract)[34,36]. Ocimum sanctum (leaves extract) by stimulating both antibody response and neutrophil activity[34,37]. Tinospora cordifolia leaf extracts were also used as immuno-prophylactic to prevent diseases in finfish aquaculture. Both ethanol and petroleum ether extracts administered in Oreochromis mossambicus at doses of 0.8, 8 or 80 mg/kg body weight, prolonged the peak primary antibody titres up to one to three weeks and enhanced the secondary antibody response and neutrophil activity[38]. A study aimed at assessing the effects of the water- and hexane-soluble fractions of Solanum trilobatum on the nonspecific immune mechanisms and disease resistance of Tilapia found that all doses of the water soluble fraction significantly enhanced the production of reactive oxygen and decreased the percentage mortality following a challenge with A. hydrophila[34,39,40]. Another disease resistance test showed that feed supplemented with Nyctanthes arbortristis seed extract at 0.1% or 1% level significantly reduced the mortality of O. mossambicus and a 3-week feeding with 0.1% extract-supplemented diet appears to be the optimal regimen for maximal disease resistance[34,41].

On the other hand, other studies showed that administration of herbal extracts did not show an obvious immunostimulatory effects as is the case of dietary supplementation with propolis extracts and aloe (1:1) in different concentrations[42] or injectable hot-water extract of Toona sinensis at 8 microg g($^{-1}$) which had significantly increased respiratory burst, phagocytic activity and lysozyme activity towards Aeromonas hydrophila by 1 and 2 days post injection but no significant differences in total immunoglobulin levels were observed[43].

As regards single compounds, studies were carried out on azadirachtin, a triterpenoid derived from Azadirachta indica, which enhances respiratory burst activities, the leukocyte count and the primary and secondary antibody response against sheep red blood cells[34,41] and insulin which seems to have non-significant immunomodulatory effects unlike those exerted in humans[44].

Trout

Non- specific immune responses of rainbow trout is improved by diet supplementation with Origanum vulgare extract at a rate of 1%[45]; 1.0% Cotinus coggygria powder for 3 weeks[46]; 1% aqueous extract of powdered ginger roots for three weeks, mistletoe (Viscum album) or nettle (Urtica dioica) (0.1 - 1%)[47]. The experiments set to control the infection with A. hydrophila in rainbow trout (Oncorhynchus mykiss) showed that administration of dietary garlic[48] or 0.5 g ginger per 100 g of feed, conducts to reduction in mortalities to 0% compared with the controls and also to proliferation in the number of neutrophils, macrophages and lymphocytes, and enhancement of phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities[47,48].

Carp

The pathogens (especially bacteria) affect the immune system of fish and the administration of immunostimulants can increase resistance to infectious diseases by enhancing both specific and nonspecific defense mechanisms. It was showed that oral administration of Aloe vera or Aegle marmelos leaf extract can enhance some of specific and non specific immune responses by increasing lysozyme activity, serum bactericidal power and the total protein and IgM levels[49]. Also, Aloe vera supplementation (0.5%) per feed can increase the resistance to A. hydrophila and A. septicemia[34,50]. As regards bacterial challenge, Euphorbia hirta extract (50 g/kg diet) provided significant immune response (specific and nonspecific) on Pseudomonas fluorescens -infected carp enhancing the phagocytic ratio on 10th and 15th day after the infection[51] and also the administration of above mentioned extract is capable of eliminating A. hydrophila from blood and kidney[52]. Bath administration of two compounds ((1) 1, 5-Anhydro-D- glucitol and (2) 3,4,5-trimethoxy cinnamic acid) isolated from Polygala tenuifolia modulates the immune related genes in Ctenopharyngodon idella (grass carp) kidney cells and to some extent, eliminate the virus and parasitic infections[53].

Other fish species

Enhancement of both specific and non-specific immunity (higher serum antibody levels and higher serum anti-proteases) of freshwater fish Catla catla was achieved by feed supplementing with Achyranthes aspera (0.5%)[34] and also with 25 g Aegle marmelos leaf extract/kg with the highest effectiveness of the immunostimulant action for the first 5 days after challenging with pathogen[54]. KM-110 (0.5% Korean mistletoe, dietary concentration) could be also utilized as a promising immunostimulating substance for a diet in aquaculture due to its proved stimulating action on phagocytic activity in Japanese eel (Anguilla japonica)[55].

Supplementary artificial feed containing 5% Ficus benghalensis dried root powder administered to Indian freshwater murrel, Channa punctatus conducts to significantly increase of phagocytosis, phagocytotic index, nitric oxide (NO), total serum protein and immunoglobulin in the treated fish compared to control[56] and 5% Urtica dioica[57] or 10 g Mangifera indica kernel kg^{-1} dry diet[58] improve growth, biochemical, haematology, non-specific immunity and reduces mortality of Labeo victorianus after challenge with A. hydrophila.

Shrimps

Rubus coreanus ethanolic (0,5% administrated for 8 weeks) as well as Gelidium amansii extracts[59] could be used as herbal immunostimulant for shrimps to increase expression of immune genes and antioxidant enzymes activities and disease resistance against the bacterial pathogen, Vibrio alginolyticus[60]. Increased resistance on specific pathogens of shrimps is induced by the water hyacinth Eichhornia crassipes extract-containing diets at 1.0, 2.0, and 3.0 g kg($^{-1}$) that can be used as an immunostimulant for the giant river prawn, Macrobrachium rosenbergii[61], Panax ginseng root or its polysaccharides (GSP) in white shrimp, Litopenaeus vannamei[62] or injectable banana peel extract[63] to enhance immune responses and resistance against Lactococcus garvieae, a well known aquatic pathogen.

Haematological, biochemical and immunological parameters of black tiger shrimps (Penaeus monodon) are improved by feeding them for 25 days with 800 mgkg($^{-1}$) of an Indian mixture of herbal immunostimulants based on Cyanodon dactylon, Aegle marmelos, Tinospora cordifolia, Picrorhiza kurooa and Eclipta alba which enhances significantly survival rate (74%)[64]. It was showed that Gracilaria tenuistipitata, a cosmopolitan algal species, exerts protective effect against low-salinity stress and earlier recovery of immune parameters in white shrimp L. vannamei immersed in hot-water extract[65].

Discussion

With the accumulation of data on the factors that regulates immunoreactivity, we are more able to shape a pharmacological direction prophylactic or therapeutically, by reducing (immunosuppression) or increasing (immunostimulation) immune response activity. Unlike traditional therapeutic methods aimed at combating etiologic agents of various pathological entities, neglecting or suppressing the immune system's protective capacity, immunomodulatory therapy introduces a completely different perspective, to combat pathological conditions by stimulating the body's own defense mechanisms.

Only limited evidence is available considering the potential impact of plant extracts or botanicals with the general immune system of different categories of animals. Species other than ruminants, pigs and broilers were rarely considered. The difficulty in such trials is the choice of parameters and the experimental model. The current situation is characterized by a lack of well controlled studies fulfilling the requirements that would allow a comprehensive assessment of the effects on the immune system.

Most of the herbal-based products used for immunomodulation on animals are administrated orally, as feed additives but and data regarding bioavailability and the influence on immune system are scarce. Many studies emphasized the beneficial role of herbal extracts as vaccine adjuvant (for IBD, mastitis, aluminium hydroxide adjuvanted vaccines, etc.) or in enhancing the immunoprotective effect and overcoming the adverse effects during antibiotic therapy.

The absence of any breakthrough in this area has various reasons: first of all, many preparations are not chemically defined or insufficiently standardized, insufficient pharmacological characterized for species of interest making difficult to match the dose of any immunostimulants and by this to predict and optimize the efficiency in a proper way.

Moreover, in many cases, immunostimulating effects of various plant extracts or active principles by *in vitro* experiments have not been confirmed in animal experiments, while substances that have proven effective in prophylaxis or treatment of animal diseases on laboratory scale are often ineffective in clinical trial because the disease state is influenced by various internal and external factors that cannot be simulated in an Laboratory.

In veterinary practice, as part of the companion and farm animals pathology, immunomodulation is an important issue in a variety of situations; for these cases, innovative eco-friendly products that could improve or prevent some disorders became of great actuality and therefore the research on animals of economic interest should continue for the benefit of both animals and humans.

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