Addition of Mycobacterium Cell Wall Fraction as Immunomodulator to Improve the Efficacy of Oil Emulsion-Inactivated Avian Influenza Vaccine in Broiler Chickens

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Abstract: Mycobacterial lipids have strong immunomodulatory effects which can be exploited for vaccine development. In this Study, we associated the injection of Mycobacterium Cell Wall Fraction (MCWF) with vaccination against Avian Influenza H9N2 by using a commercial inactivated oil emulsion vaccine in Broiler chicken and assessed the immune response. High levels of mucosal hemagglutinin inhibitory antibodies were detected in groups immunized with MCWF and AI vaccine, compared to group vaccinated with AI vaccine alone. MCWF induced a longer and an increased local protection in both respiratory and intestinal tracts. Serum levels of antibodies were not significantly different among groups. Cytokines of humoral and cell mediated immunity were also assessed. MCWF influences the balance Th1/Th2, by increasing Th2 cytokines that downregulate the cellular immune response and promote innate immune response, and inducing the Th1/Th2 network. Our results show that MCWF has a potential to be used as adjuvant in AI vaccines by inducing a robust, broad and long lasting protective immune responses in broiler chickens.

Keywords: Immunomodulation, Avian Influenza, vaccination, Mycobacterium Cell Wall fraction, Adjuvant.

INTRODUCTION

Avian Influenza (AI) is a disease caused by virus belonging to the type A group of Orthomyxoviridae family, that affects domestic and wild avian species [1]. This disease is highly contagious between birds, and occasionally transmissible to mammals, including humans.The World Organisation for Animal Health (WOAH) has listed High Pathogenic Avian Influenza with the OIE-Listed diseases, infections and infestations in force in 2019, list that regroups the rapidly spreading diseases with serious socio-economic and public health consequences [2]. Globaly, AI is a major threat to the poultry industry and to public health. In Morocco, low pathogenic avian influenza subtype H9N2 virus was first detected in poultry flocks in January 2016, and it was reported to generate large economical losses in different types of poultry production [3].

Viruses of AI undergo several mutations [4], and the epidemiology and control of AI are complex [5]. Although the wide use of commercial vaccines in the poultry industry, disease outbreaks are still common [2]. One of the reasons is the presence of a variety of stress factors in the intensive production systems (i.e. high density, management and nutritional issues, etc.) that adversely affect the immune status of the birds [6]. In addition, AI spreads easily through people, fomites and contaminated equipment.

Currently, most of the used AI vaccines are inactivated whole AI virus, that are administered either subcutaneously or intramuscularly [5]. Inactivated vaccines are preferred to live attenuated vaccines because of its safety profile [5]. However, to induce a good protection threshold with inactivated vaccines, high antigen quantities are needed and the inclusion of adequate adjuvants to enhance immunogenicity [7]. Most of adjuvants used in commercial vaccines (Alum-containing adjuvants, oil-based emulsions) act by boosting the immunoavailability of the antigen [8]. Whereas, other adjuvents (such as bacterial cell) work by providing the correct co-stimulation signals for the antigen-specific adaptive immune cells during the antigen recognition [8].

For many decades, the immunomodulatory effects of mycobacteria and components associated with the mycobacterial cell wall have been investigated and have been used largerly as a source of adjuvant preparations [9]. Mycobacterium Cell Wall Fraction (MCWF) derived from *Mycobacterium phlei* activates and stimulates the innate and cell-mediated immune

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responses [10,11]. In the last years, a commercial MCWF has shown the potential to increase the immune response in different species; Equine [12,13]; Bovine [14, 15]. To our knowledge, the use of MCWF as an adjuvant in avian influenza vaccines has not been investigated.

In the present study, we aimed to investigate the immunomodulating effect of Mycobacterium Cell Wall Fraction (MCWF) injected with a commercial oil emulsion-inactivated AI H9 Vaccine, to enhance the response of broiler chickens, by monitoring the local immunity, and evaluating innate and adaptative immune response.

MATERIALS AND METHODS

Ethics Statement

All animal studies were carried out on the experimental station of Avian Pathology Unit at Hassan II Agronomy and Veterinary Institute in Rabat, Morocco. in strict accordance with the recommendations of the animal welfare agency guidance. The blood was collected from wing vein in humanely way, and euthanasia was performed under gas anesthesia to minimize suffering.

Chickens and Experimental Design

Two hundred sixty (260) one-day-old Ross broiler chicks, were weighed and randomly divided into 6 groups (n = 40/group). Twenty individuals were randomly euthanised, in strict accordance with the recommendations of the animal welfare agency guidance, for the quality control and detection of H9 Maternal Derivated Antibodies (H9 MDA)

The group 1 (G1) was identified as a negative control group; all birds did not receive the adjuvant (MCWF) and were not vaccinated.

All the other groups were vaccinated using a commercial H9 inactiveted AI vaccine; group 2 (G2) was considered as a positive control of vaccination to assess immunity and safety, and birds were not given MCWF.

Chicks from groups (G3), (G4), (G5) and (G6) have received the commercial H9 vaccine and MCWF ajduvant in a single administration, containing one of the 4 concentrations of MCWF: 2, 5, 10 and 20 µg, respectively.

According to each group, the vaccine and/or MCWF were administered by the sub-cutaneous route at day 1 of the trial (Table **1**).

Description of the Adjuvant

Mycobacterium Cell Wall Fraction adjuvant derived from *Mycobacterium phlei* was provided by NovaVive Inc (Napanee, ON, Canada).

Zootechnical Performances

Chickens were monitored daily for clinical signs of disease and occurrence of mortality, all the animals were weigntened weekly, and the food intake was daily recorded in order to calculate the feed conversion ratio (FCR).

Muccosal Immunity Monitoring

At day 7, 14, 21 and 35, five chickens per group were humanely sacrificed to collect brancoalveolaire liquids (BAL) and intestinal fluids (IF).

To collect the BAL, 5 mL of phosphate buffer solution was injected in the trachea, bronchus and suctioned back with a syringe. The intestinal tract was gently scalped using a glass slide and transferred into 1 mL of PBS. All the samples were centrifuged 10 min at 3000 rpm then the supernatants were collected and stored in Eppendorf tubes at -20°C for further analysis.

Table 1: The Distribution of the Groups During the Trial

Group	Number of birds	Purpose of the group
G1	40	Negative Control – Not vaccinated not treated
G2	40	Positive control vaccinated with AI H9
G3	40	Group vaccinated with AI H9 and adjuvanted with a dose of 2ug of MWCF
G4	40	Group vaccinated with AI H9 and adjuvanted with a dose of 5ug of MWCF
G5	40	Group vaccinated with AI H9 and adjuvanted with a dose of 10ug of MWCF
G6	40	Group vaccinated with AI H9 and adjuvanted with a dose of 20ug of MWCF

To evaluate the mucosal level of hemagglutinin inhibitor (HI) antibodies titers, Hemagglutination Inhibition assay using H9 antigens was carried out as reported in OIE's procedure [2].

Humoral Immunolgy

To assess the antibodies produced after H9 vaccination and to monitor the evolution of maternal derived antibodies (MDA), serological tests were conducted as follow:

Ten (10) birds from each group were selected and bled weekly from day 1 to 35, the samples were collected from the same labeled birds. Antibody titers were analyzed from the sera by using a commercial kit for indirect ELISA (IDEXX AI MultiS-Screen Ab Test). This test revealed only IgY isotypes, and was conducted following the instructions of the manufacturer.

In parallel of this test, an Haemagglutination inhibition (HI) assay against H9 virus was performed [2] to assess the hemagglutinin antibodies present in the serum.

Cellular Immunity

To evaluate the cell mediate immunity response, total RNA was extracted from each collected spleen (Five per group), at 21 days after vaccination, according to the study design.

The extraction was done by using Monarch total RNA Miniprep Kit of Biolab, thus, lysis of samples, binding and elution of RNA were performed following the manufacturer's instructions. Quantification of RNA samples of each run of extraction was carried out.

Table 2: Protocol of the Rt PCR Conducted, using Syber Green Luma Universal-One Step RT-qPCR

	20µl Reaction	Final concentration
One step Reaction Mix (2X)	10µl	1X
Enzyme Mix (10X)	1µl	1X
Forward primer (10µM)	0,8µl	0,4µM
Reverse primer (10µM)	0,8µl	0,4µM
RNA sample	1µl	--
Nuclease free water	6,4µl	--

The genes cytokine levels of Th1 (IFN-g, Il-18) and Th2 (IL-6 and Il-10) were measured by using rtPCR expression of cytokine. Rt PCR was conducted by using Syber green Luma Universal-One Step RT-qPCR kit- according to the following protocol (Table **2**).

Primer sequences for interferon gamma (IFN-g) and interleukin IL-6, IL-10 and IL-18, were obtained from gene bank (Table **3**).

Table 3: Cytokine Primer (Gen Bank)

Genes	Sequence (5'->3')
IFN-g	Forward: TTACACTGGCCTTGGAGCTG
	Reverse: TACACAGCCCGTGATGACGAA
IL 6	Forward: GCAGGACGAGATGTGCAAGA
	Reverse: CAGAGGATTGTGCCCGAACT
IL 10	Forward: TGCTGCGCTTCTACACAGAT
	Reverse: ATCCCGTTCTCATCCATCTTCTC
IL 18	Forward: AGAGGCAGCAAGGAACAATC
	Reverse: CAGTGTTCTCTTGTGGTGGC

The PCR reaction was performed on 42 cycles in Applied biosystem 7500 fast real-time PCR system using the thermal cycling conditions: Reverse Transcription: 55°C for 1 minute, Initial denaturation for 1 min at 95°C, denaturation for 10s at 95ºC, and primer extension for 30s at 60 º C, followed by Melt curve analysis according to real time instrument.

The genes cytokine levels of Th1 (IFN-g, Il-18) and Th2 (IL-6 and Il-10) expressed by their by Ct (Threshold cycle) and Melt curve.

Statistical Analysis

Data were analyzed by one-way ANOVA followed by post hoc Duncan's test, using SPSS v14.0 to compare the means with significance level of 5%.

RESULTS

Clinical Observations

During the first week of the trial, three mortalities were recorded respectively in group G2, G3 and G4 (One mortality per group), after necropsy, no gross lesions were observed and no treatment was administrated.

At 35 days post-vaccination, no lesions were observed on the sites of subcutaneous injection in birds sacrificed for sampling.

Zootechnical Performances

Zootechnical performance results are showed in the Table **4**. The feed intake was not significantly different (P > 0.05) among the groups. The body weight gain was significantly higher (P <0.05) in the negative control group (G1) during the starter phase; however, after D28, birds in group 6 (i.e. 20ug/ml of MCWF) had a noticeable rise in body weights.

The final weight was not significantly different between the experimental groups (P > 0.05). But, the weights of birds in the groups given MCWF adjuvant tended to be higher.

The FCR of the MCWF groups was between 1.86 and 2.08. Although it is not significant, the lowest FCR corresponded to G3 and G6, indicating it may be the most efficient groups at converting the feed intake into live body weight.

Mucosal Immunity

The levels of mucosal antibodies were measured in the BAL and the IF collected following the protocol. Compared to those of broilers injected with commercial vaccine without MCWF, the measurements of HI antibodies in BAL and IF of the adjuvant immunized groups showed significantly higher titers throughout the 35days of the trial (p<0,05).

In fact, at Day 7 a significant difference was noted between the negative control group and other groups, the highest level of HI antibodies in BAL was detected in G6, the same result was noted for HA antibodies detected in IF collected on the same time. This trend was maintained during all over the trial. At Day 14, the negative group had a level of 1Log2 and 1,4 log2 of HI antibodies in the BAL and in the IF respectively. The positive group and G3 have showed a level of 8.4 and 7 Log2 respectively of HI antibodies in the BAL, whereas the adjuvant immunized groups (G4, G5 and G6) registered levels of 10.6, 10 and 10 Log2 respectively.

The HA antibodies detected in IF at Day 14, have showed a significant difference between adjuvanted groups, in fact, the highest level were observed in G4, G5 and G6. This difference is still persistent in day 21.

At Day 35 of the trial, the negative group, the positive group and G1 have showed no measurable titers of mucosal antibodies, meanwhile the adjuvant immunized groups G4, G5 and G6 had respectively levels of 2.2, 3.4 and 4.8 of HA antibodies for the BAL, and 4, 4 and 3.2 for the intestinal HI antibodies (Tables **5** and **6**).

No dose response relationship was clearly identified between the 4 adjuvanted groups. But what is clearly noticeable in the graph of the evolution of the antibodies titers (Figure **1**) is that for both BAL and IF, the margin of HI antibodies levels of the groups G4, G5 and G6 merely going under 8log2 HI for the first 21 days of the trial, then still keeping a protective threshold until the end of the trial.

Humoral Immunity

For the monitoring of antibodies induced by vaccination and also to measure the evolution of MAD, all of collected serum was tested by using a commercial kit of Indirect ELISA (IDEXX). These antibodies present in the serum are IgY.

Table 4: Body Weight (Gramme) and FCR of the Groups during the Trial

Days	G1	G2	G3	G4	G5	G6
D1	51,2	50	48,4	51,2	45,6	50,8
D7	180,4	175,2	190,2	183,2	177,6	190,7
D14	476,6	468,2	499,8	480,6	474,4	486,8
D21	892	833	854,9	821	816,6	897
D28	1447,8	1224,8	1286	1285,5	1230	1392,8
D35	2049,9	1909,5	1945,8	1884,4	1864	2128
D42	2653,5	2540,4	2734,1	2525,4	2471	2766,6
FCR	1,94	2,02	1,88	2,03	2,08	1,86

Table 5: The HI Antibodies Titers Against H9 (Log2) in BAL

BAL (Log2)	G1	G2	G3	G4	G5	G6
	NV	OV	2ug	5ug	10ug	20ug
D7	1,4±0,24[a]	6,6±1,63[b]	6,8±0,37[b]	6±0,44[b]	6,2±0,37[b]	9,8±0,73[c]
D14	1[a]	8,4±0,75[b]	7±0,89[b]	10,6±0,24[c]	10±0,32[c]	10±0,63[c]
D21	0,6±0,24[a]	6,8±0,20[b]	6,6±0,60[b]	7,8±1,32[b]	8,8±0,49[b]	10,8±0,20[c]
D35	0[a]	0,4±0,24[b]	0,4±0,24[b]	2,2±0,73[c]	3,4±0,60[c]	4,8±1,71[c]

[a]05 samples were analyzed to calculate each mean (n=05).
[b]Abbreviations D (Day of collection).
[c]Data are presented as the average± standard error.
[d]Different superscript letters in the same row indicate a significant difference (P<0, 05).

Table 6: The HI Antibodies Titers Against H9 (Log2) in IF

IF (Log2)	G1	G2	G3	G4	G5	G6
	NV	OV	2ug	5ug	10ug	20ug
D7	3±0,31[a]	5,4±0,40[b]	6,2±0,49[b]	6,2±0,53[b]	8,8±0,97[c]	8,8±0,92[c]
D14	1,4±0,24[a]	6±0,55[b]	7,8±0,73[c]	9,4±0,51 [cd]	9,4±0,51 [cd]	10,2±0,49[d]
D21	1[a]	5,8±0,80[b]	6,6±0,24[b]	8,8±0,86[c]	9,8±0,37[c]	10,2±0,49[c]
D35	0[a]	0,6±0,24[b]	0,6±0,24[b]	4[c]	4[c]	3,2±1,46[c]

[a]05 samples were analyzed to calculate each mean (n=05).
[b]Abbreviations D (Day of collection).
[c]Data are presented as the average± standard error.
[d]Different superscript letters in the same row indicate a significant difference (P<0, 05).

The results of the monitoring of all of 6 experimental groups and the evolution of maternal antibodies from day 1 to Day 35 is presented by the Figure **2**.

The evolution of the maternal antibodies in the serum shows the typical consumption of MDA in the negative control group (G1). In fact, titers dropped from 27386 at Day 1 to 259 at D35. For the positive control group (G2), they decrease from 27386 to 290 in 35 days of trial. The same evolution of IgY was noted in adjuvanted groups

In fact, no significant difference in the humoral responses between the MCWF immunized chickens and the group immunized with the commercial H9 vaccine.

The results of the Hemaglutination Inhibition Assay confirm the indirect ELISA results. The graph in the Figure **3** shows a consumption of the maternal antibodies present in the serum of the 6 groups. The decrease of the adjuvant immunized groups is similar to the only vaccinated group, going from approximately 9 Log2 in the first day of the trial, to 4 Log2 in D21, then lowering to 0.8 Log2 in D35. The negative group G1 shower a rapid decrease of these titers dropping from 6 Log2 in D7 to 1 Log2 in D21.

Cellular Immunity

Results show increased levels of Il-18 in the adjuvanted groups at day 21, although not statistically significant.

In comparison with IFNg, where no significant difference has been noted. All the vaccinated groups showed a high level of IL6 when compared with the negative control group (G1). However, a significant difference was revealed between negative control and adjuvanted groups ($p<0,05$). No dose-dependent relationship was noted between results of cytokines levels of MCWF immunized groups and doses of MCWF (Figure **4**).

For a better evaluation of the immunomodulation effect on the balance of Th1/Th2, and since IL-10 is an inhibitor of IFN-g production, ratios of IFNg/IL-10 was established in the Figure **5**.

Although it is not significative, the results show that the groups G5 and G6 show highest ratios compared control groups and G3 and G4.

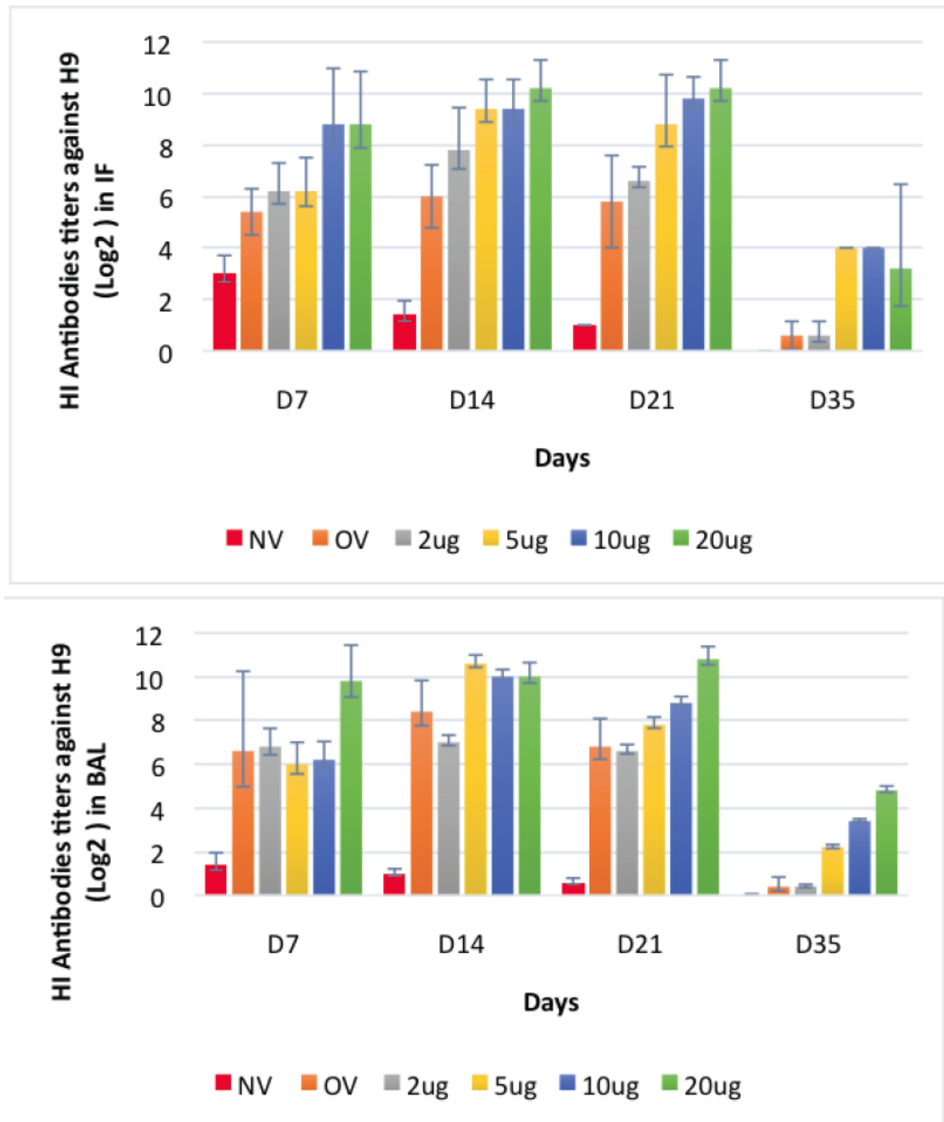

Figure 1: The evolution of mucosal antibodies in both tracheal (BAL) and intestinal fluid (IF) during the trial.

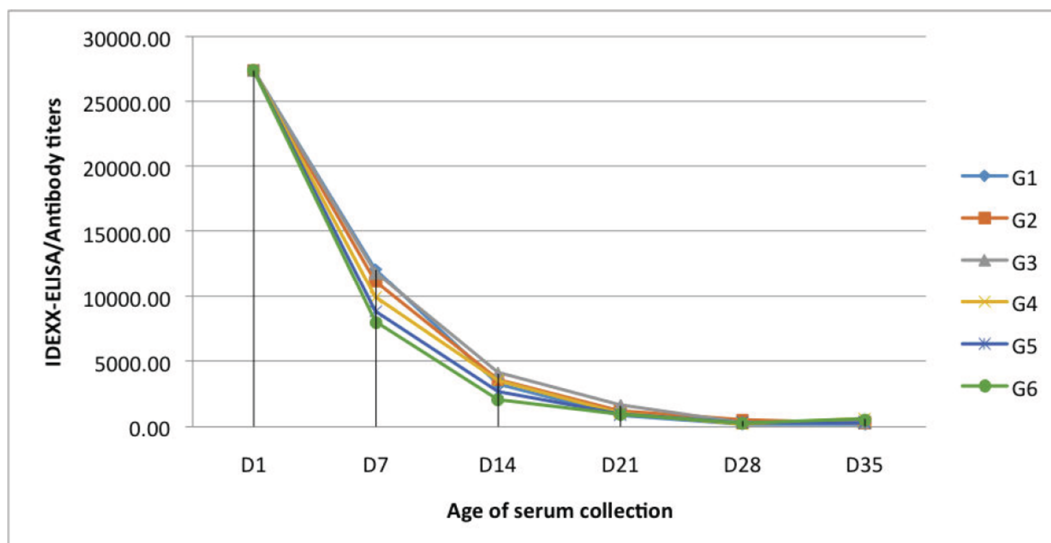

Figure 2: Evolution of iELISA titers for the 6 experimental groups following the age of sampling. (IDEXX software calculation).

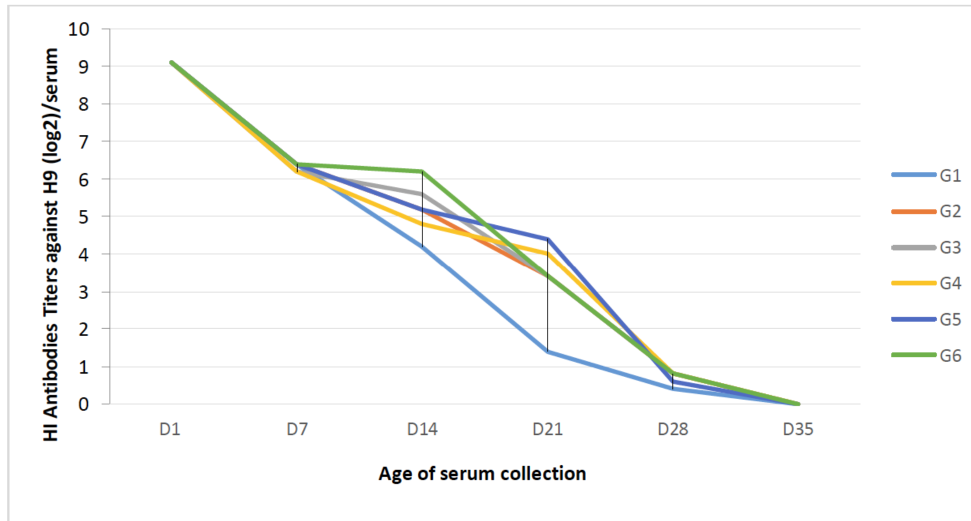

Figure 3: Evolution of antibodies titers against H9 of the 6 groups of the experimentation (using Hemagglutination inhibition Assay).

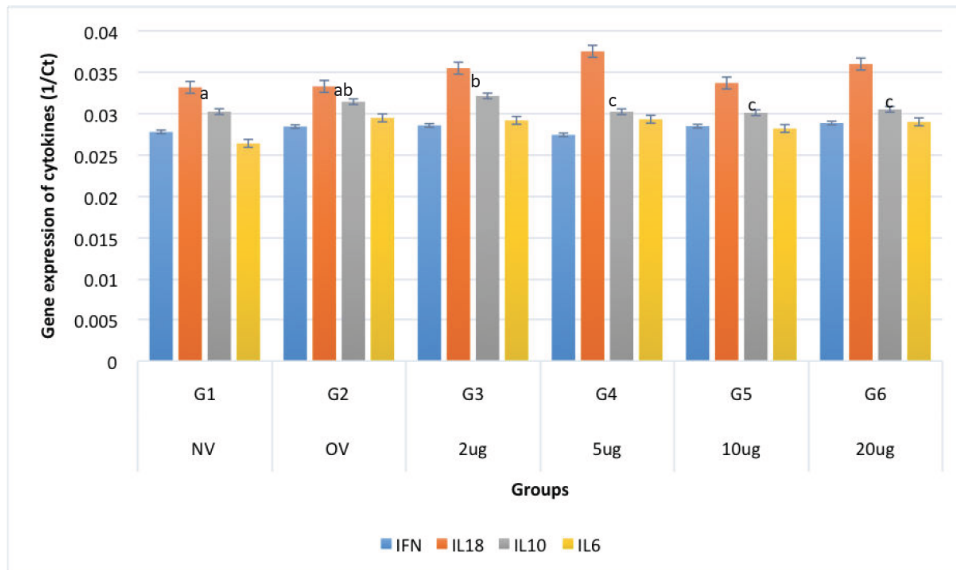

Figure 4: The average gene expression of cytokines (IFNg, IL-18, IL-10 and IL-6) from spleen collected at day 21.

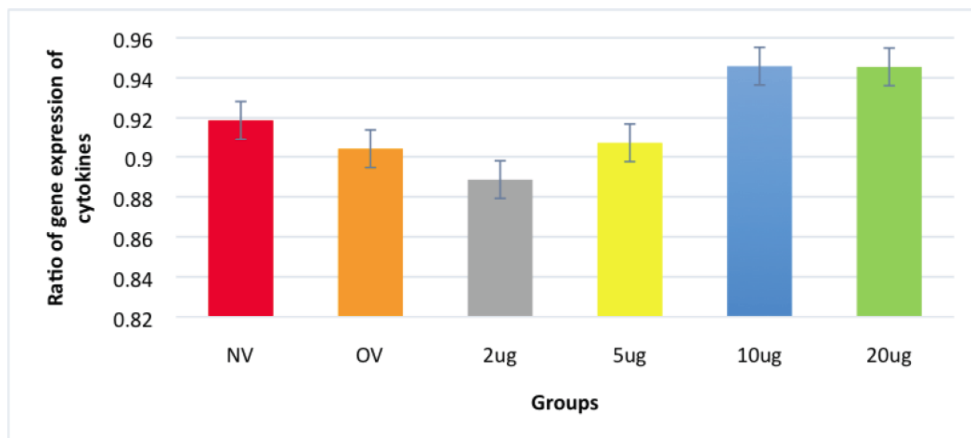

Figure 5: Ratio of the gene expression of IFNg/IL-10.

DISCUSSION

Poultry vaccines are broadly used to prevent and control contagious poultry diseases in the field. Adjuvants are compounds of vaccines, used to increase the speed and magnitude of the immune response to a vaccine, and they are incorporated to vaccines with poor immunogenicity such as the inactivated vaccines used in current prophylactic strategies [12]. Immunomodulators of different nature can be used as adjuvants to enhance vaccines, efficacy or in their own right to increase immune activity and protect against infections.

In our study, we aimed to investigate the safety and immune responses of 4 concentrations of Mycobacterium Cell Wall Fraction of *M.phlei* in broiler chickens, monitoring the local immunity and assess the humoral and cell-mediated immunity in broiler chickens vaccinated with a commercial vaccine against AI H9 associated with MCWF within 4 concentrations.

The data from the trial show that MCWF have no adverse effects on the health of broiler birds as evidenced by clinical observation and body weight gain.

It is especially important for broilers to have good FCR because they are often processed at a targeted live weight and customers want to get as much saleable meat as possible.

It is interesting to see how the MCWF has an effect on the Feed Conversion Ratio, especially for immunized chicken with a commercial H9 adjuvanted to a dose of 20ug of MCWF. Previous studies have demonstrated the positive impacts of the immunostimulants on bodyweight. Chicken feed supplemented with essential oils and other natural immunomodulators have shown an increase in the body-weight, explained by the accrual digestive enzyme activity [16]. Hashemipour and his colleagues have proved it by analyzing the level of trypsin and amylae after adding the stimulants to the food program [17].

It has already been submitted that the increased immune response toward some immunostimulants help boosting zootechnical performances by decreasing the load infectious causes [18].

In contrast to that, the immunostimulation activity of Lipopolysaccharide (LPS) from *E.coli* administrated to broiler chicken has shown a decrease of bodyweight and FCR as a consequence of inflammatory response [6].

Whereas the subcutaneous administration of Mycobacterium Cell Wall Fractions during our trial shows an increase of the growth rate in broiler chicken, demonstrating an interesting interaction between free lipid fraction present in the adjuvant and maximizing the performances of the broiler chicken.

In rodent models and humans, resistance to influenza infection correlates with the induction of IgA antibody in the respiratory tract [19].

Mucosal surface represents the first barrier between AI viruses and internal milieus. Swayne and al., have observed that in experimental studies, most AI vaccines provide consistent protection against clinical disease and death, but they do not always provide absolute protection against mucosal infection or shedding of the virus from oropharynx and cloaca [7].

IgA isotype is the predominant form of HI antibody in the mucosal immunity [20]. In fact, our results indicate that MCFW generate a significant higher level of HI antibodies in the both the intestinal and tracheo-brachial mucosal surfaces. An enhanced antibody response in the BAL after the subcutanious administration of MCWF adjuvant means the induction of immunity at the natural point of entry for the virus. The elevated level of HI antibodies in intestinal secretions can suppose a decrease of virus shedding. Few studies have assessed the mucosal antibody-mediated response using immunostimulants.

It was demonstrated that the intramuscular delivery of an inactivated AIV vaccine adjuvanted with poly (D,L-lactic-co-glycolic acid) encapsulated CpG ODN did not induce a lachrymal IgA antibody response, although the levels of lachrymal IgY recorded were high [21]. Even if IgA does not fix complement, the immunoglobulin possesses a number of effector functions including viral neutralization, inhibition of bacterial adherence and acting as an opsonic for mucosal phagocytes [20]. It could be suggesting that following the results of the trial, the MCWF can shorten the invasive phase of a potential viral infection by enhancing the production of local HI antibodies specifically against H9. Renegar and al found out that in some cases, mucosal IgA possess more cross-reactivity than serum IgG antibody and so, it may contribute to cross-protection observed in mucosal vaccinated animals [22]. Procuring to MCWF the

potential ability to enhance a cross protection against multiple stains of AIV.

Our analysis revealed no significant difference in the humoral responses between the MCWF immunized chickens and the group immunized with the commercial H9 vaccine.

For the purpose of evaluating the immuno-modulating effect of MCWF on the protective immunity against H9, both Th1 (IFNg, IL-18) and Th2 (IL-10) in addition to pro-inflammatory IL-6 were investigated, using the rt-PCR, and evaluate the cytokine network. Results have showed an interesting evolution of the balance Th1/Th2.

Concerning the IL-6, all the immunized groups showed increased levels of IL-6 in the spleens. The IL-6 is a pro-inflammatory cytokine produced early after infection as part of the induced innate immune response [21]. Studies about the delivery of recombinant vectors expressing IL-6 induced substantial increases in IgA responses at the mucosa [23].

These results are concordant with both our records of mucosal HI antibodies and IL-6. Immunized groups and adjuvanted with MWCF seems to have a better preparation for a potential viral infection which first entrance is the mucosal barriers. Other adjuvants have been proved to improve the IL-6, such as poly(A) and poly(U) [24].

The Th2 cytokine measured was IL-10, but no significant differences were noted. Interestingly, the ratio of expression of IFNg and IL-10 are intriguing, since IL-10 is an inhibitor of IFNg, we noted an increase of this ratio in the groups immunized with the doses of 10 and 20ug of MCWF, this result indicated the possibility that a Th2-dominated immune response.

We can suggest that MCWF induce a more solid levels of Th2, Il-6 conferring an availability of local antibodies and/or IgA, in addition IL-10 that inducing the production and the development of macrophages [25]. The IL-10 plays an important role in the Th1/Th2 paradigm, indeed it has a down regulating effect on IFNg [20].

The absence of significant difference in the IFNg recorded during this trial can be explained by the fact that there has been no infection occuring.

It was interesting to note that the adjuvanted groups show also significantly improvment levels of IL-18 compared to the positive control group, the enhance of IL-18 could cause an increased Th1-type response in turn leading to the production of IFN and macrophage activation [25].

Our investigations showed that the MCWF enhance the balance of Th1/Th2, whereas the vaccination by an inactivated vaccine only enhances the IFNg production. This will supposedly potentionalize a response to an infection.

The immune response depends on factors that exist prior to the advent of infection and are capable of a rapid response to AI Viruses [7]. Since the capability of reduction of virus excretion depends on both a reduction in titer of the virus excreted and the shortened duration of viral shedding [7], we suggest a virus challenge to metric the vaccinal protection induced by MWCF used with commercial H9 vaccine and to confirm the predicted high level of cytokines modulating the adaptive immune system as lymphocyte activation, proliferation, differentiation, survival, apoptosis and angiogenesis, but also able to initiate, mediate and propagate numerous cellular inflammatory responses.

CONCLUSION

In this study we asset the safety and the immune response to Mycobaterium Cell Wall Fraction associated to a commercial vaccine against H9. MWCF have showed better zootechnical performances compared to the non treated groups. And no lesions were observed. The restults have shown a significal boost of the HI antibodies in the brancoalveolaire and intestinal mucus, the natural point of entry for the H9 virus. The elevated level of HI antibodies in the intestinal fluids can suppose a decrease of virus shedding. However no difference in humoral immunity was noted ; in fact, humoral immunity cannot be asset if there is no viral challenge. Concerning the cytokines levels, we noted higher levels of pro-inflammatory, in addition to a modification of the Th1/Th2 network, by enhancing the levels IL-18 cytokine.

ABBREVIATION

AI = Avian Influenza

WOAH = World Organisation for Animal Health

OIE = Office International des Epizooties

IL = Interleukin

INF = Interferon

MCWF = Mycobacterium Cell Wall Fraction

MAD = Maternal Derivated Antibodies

FCR = Feed Gonversion Ratio

BAL = BrancoAlveolaire Liquids

IF = Intestinal Fluids

PBS = Phosphate Buffer Solution

HI = Hemagglutinin Inhibitor

ELISA = Enzyme-Linked Immunosorbent Assay

PCR = Polymerase Chain Reaction

Ct = Threshold cycle

Th = T cell helper

Ig = Immunoglobulin

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