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RESEARCH ARTICLE

Correlation of Antibody Levels with Peripheral Lymphocyte Subsets and Routine Hematological Parameters after Vaccination with FMD Vaccine in Young Sires

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A B S T R A C T

High immune response (HIR) cows have balanced and roborant host defences and lower disease, and selecting young sires is more essential than selecting cows. Foot and mouth disease (FMD) is a communicable disease in cattle, but there is no convenient method for selecting HIR young sires to FMD. In this study, 39 young Holstein sires were vaccinated by a trivalent FMD serotypes (A, O and Asia I) vaccine. Blood samples were obtained from Jugular vein before vaccination and at day 51 after primary vaccination (APV), and antibody levels of the three FMD serotypes, T-cell subsets and routine hematological parameters were detected. HIR and low immune response Holstein young sires were classed by the antibody levels of the three FMD serotypes at day 51 APV. The relation of the antibody levels with the T-cell subsets and routine hematological parameters were analyzed. The results showed that the immune response capability were related to the amounts of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes, and the CD4/CD8 ratio was correlated to the antibody titers after vaccination. In conclusion, level of platelet and mid cell count before vaccination, and CD4/CD8 ratio and level of platelet after vaccination may be used to select the HIR young sires.

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INTRODUCTION

Optimal animal health is closely correlated with the highly quality milk production in dairy cattle. The previous breeding purposes are to select dairy cattle with high production traits, and give little attention to bovine health status, which result in an increase in disease incidence (Parker Gaddis *et al.*, 2015). The increase in disease incidence results in decrease in the milk production in dairy cattle (Parker Gaddis *et al.*, 2014). It has been reported that high immune response (HIR) cattle have roborant host defences and lower disease incidence (Wagter *et al.*, 2000; Thompson-Crispi *et al.*, 2013). The cows identified as HIR have balanced and enhanced levels of cell-mediated immune responses (CMIR) and antibody-mediated immune responses (AMIR) comparing to other cows (Wagter *et al.*, 2000; Thompson-Crispi *et al.*, 2014).

Selection of young sires is more important than selection of cows, which can offer highly quality genetics to his partners and customers. It has been reported that the immune response capability can be assessed with the trivalent foot and mouth disease (FMD) serotypes (A, O and Asia I) vaccine in cattle (Chowdhury *et al.*, 2015). We previously reported that the peripheral CD4 and CD8 lymphocyte subsets were related with the expression level of IFN-γ mRNA in peripheral blood lymphocytes (PBLs) after stimulated by lipopolysaccharide (Yang *et al.*, 2018). In this study, the HIR Holstein young sires were identified after vaccination by the FMD vaccine, and the correlation of antibody levels with the PBL subsets and routine hematological parameters was analyzed, which may be used to select young sires.

MATERIALS AND METHODS

Animals and experimental design: Thirty-nine healthy young Holstein sires with approximately 4 months of age

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were housed on a farm in China, free from drugs and other stress stimuli under the same feeding condition. The experimental protocol was approved by the Shandong Academy of Agricultural Sciences Animal Ethical Committee. The inactivated trivalent FMD (serotypes A, O and Asia I) vaccine (Jinyu Biological Pharmaceutical Co. Ltd., China) was used to vaccinate the male calves, and the booster vaccination was performed at day 30 after the primary vaccination. Blood samples were obtained from Jugular vein before vaccination and at day 51 after primary vaccination (APV).

Detection of antibody levels to FMD vaccine: Antibody levels against the FMD virus serotypes A, O and Asia I were detected by an ELISA kit (Lanzhou Institute of Veterinary Research, Chinese Academy of Agricultural Sciences, China) according to the manufacturer's instructions. The young sires were classified into three groups according to the antibody levels at day 51 APV. The young sires with higher (or lower) antibody levels against the three serotypes than the averages were classed as the HIR group (n=8) or LIR group (n=6), and the rest were the medium immune response (MIR) group (n=25).

Assessment T-cell subset: The functional subsets of $CD4^+$ and $CD8^+$ T-cells were assessed by flow cytometer and Ce1l Quest software as described previously (Yang *et al.*, 2018).

Blood routine test: The blood samples from all sires were analyzed on a hematology analyzer (pocH-100iV Diff; Sysmex, Hyogo, Japan) as described in a previous report (Whisler and Dahlgren, 2005).The hematological parameters included white blood cell (WBC) count (WBCC), red blood cell (RBC) count (RBCC), hemoglobin (HGB), lymphocyte count (LYM), mid cell count (MIDC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet, red cell distribution width SD (RDW-SD) and red cell distribution width CV (RDW-CV).

Statistical analyses: Experimental groups consisted of three biological replicates, and the data for the percentage of $CD3^+$, $CD4^+$, $CD8^+$ lymphocytes, CD4/CD8 ratio, and the hematological parameters were analyzed as a completely randomized design using the Proc Mixed models of SAS (Version 9.1; SAS Institute, Cary, NC). For blood samples from different immune response groups or time points, the model contained random effects of the young sires and fixed effects for immune response capability, time point and the interaction of immune response capability and time point. The comparisons among the percentage of $CD3^+$, $CD4^+$, $CD8^+$ lymphocytes, CD4/CD8 ratio and the hematological parameters in different immune response groups were made using the Duncan method and controlling the experiment-wise type ± error equal to 0.05, respectively. Data are present as least squares means. Groups were considered to be significantly different at $P<0.05$.

RESULTS

Antibody titers to FMD vaccine detected by ELISA: The ELISA test showed (Fig. 1) that the antibody titers of the three serotypes of the HIR group were the highest at day 51 APV ($P<0.05$). However, before vaccination, the antibody titer of serotype A of the HIR and LIR groups was lower than that of the MIR group ($P<0.05$) and there was no significant difference in the antibody titers of serotypes O and Asia I among the three groups.

$CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes analysis: The numbers of $CD3^+$ and $CD4^+$ lymphocytes were higher at day 51 APV than that before vaccination (Fig. 2). Before vaccination, only the ratio of $CD4^+/CD8^+$ of the HIR group was higher than that of the LIR group ($P<0.05$; Fig. 3), but that of the MIR group was higher than that of the HIR group. At day 51 APV, the ratio of $CD4^+/CD8^+$ of the HIR group was higher than that of the MIR and LIR groups ($P<0.05$). Furthermore, the data of $CD4^+$ lymphocytes of the HIR group were also higher than that of the LIR group ($P<0.05$), but that of the MIR group was the highest among the three groups. In addition, the data of $CD3^+CD8^+$ lymphocytes of the LIR group were lower than that of the HIR and MIR groups ($P<0.05$), and there was no significant difference between the HIR and LIR groups in the data of $CD3^+$, $CD4^+$,$CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes before vaccination, $CD3^+$ and $CD3^+CD4^+$ lymphocytes at day 51 APV ($P>0.05$).

The hematological parameters analysis: The blood routine test revealed (Fig. 4) that there was no significant difference between the HIR and LIR groups in the data of the RBCC, HGB, LYM, HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV before vaccination, WBCC, RBCC, HGB, LYM, MIDC, HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV at day 51 APV ($P>0.05$). Before vaccination, the data of WBCC and MIDC of the LIR group were the lowest among the three groups ($P<0.05$) (Fig. 4A, 4B). Both before vaccination and at day 51 APV, the level of platelet of the LIR group was the lowest among the three groups ($P<0.05$) (Fig. 4C).

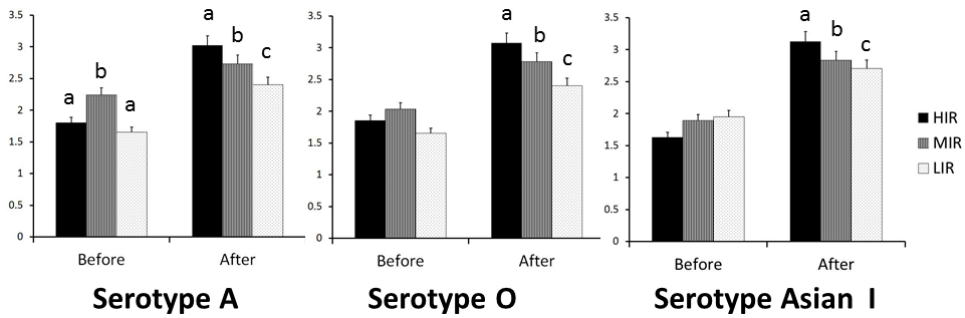

Fig. 1: Antibody titers of foot and mouth disease serotypes (A, O and Asia I) in the high immune response (HIR), medium immune response (MIR) and low immune response (LIR) groups. Note: Before=Before vaccination; After=Day 51 after primary vaccination. Different letters indicate significant differences ($P<0.05$) at the same stage.

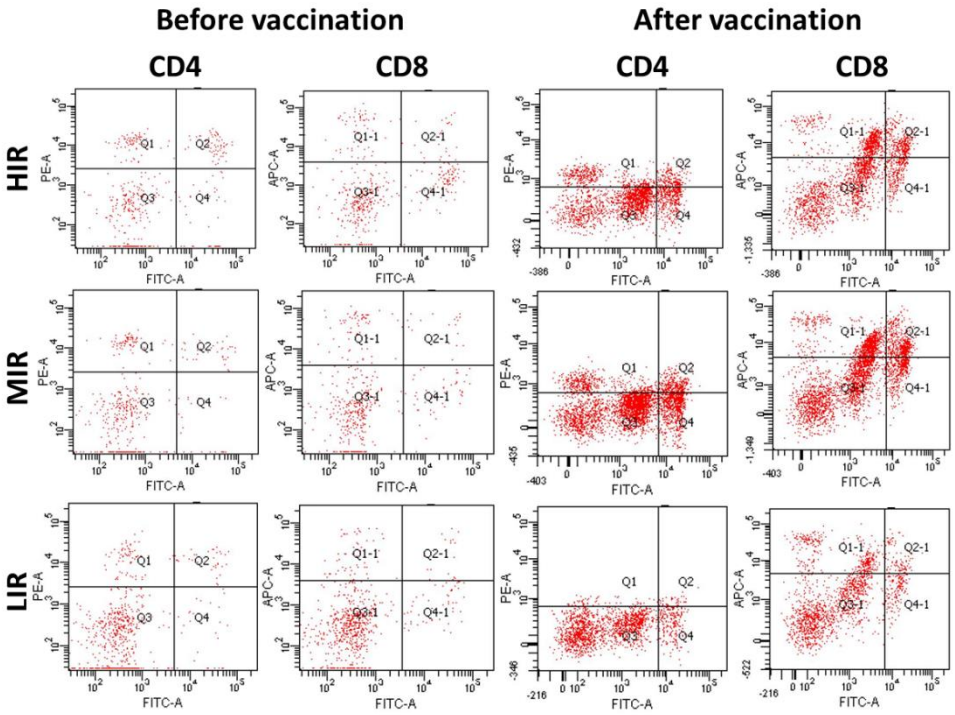

Fig. 2: Representative flow cytometric dot plots of $CD4^+$ and $CD8^+$ in the high immune response (HIR), medium immune response (MIR) and low immune response (LIR) groups. Note: After vaccination = Day 51 after primary vaccination.

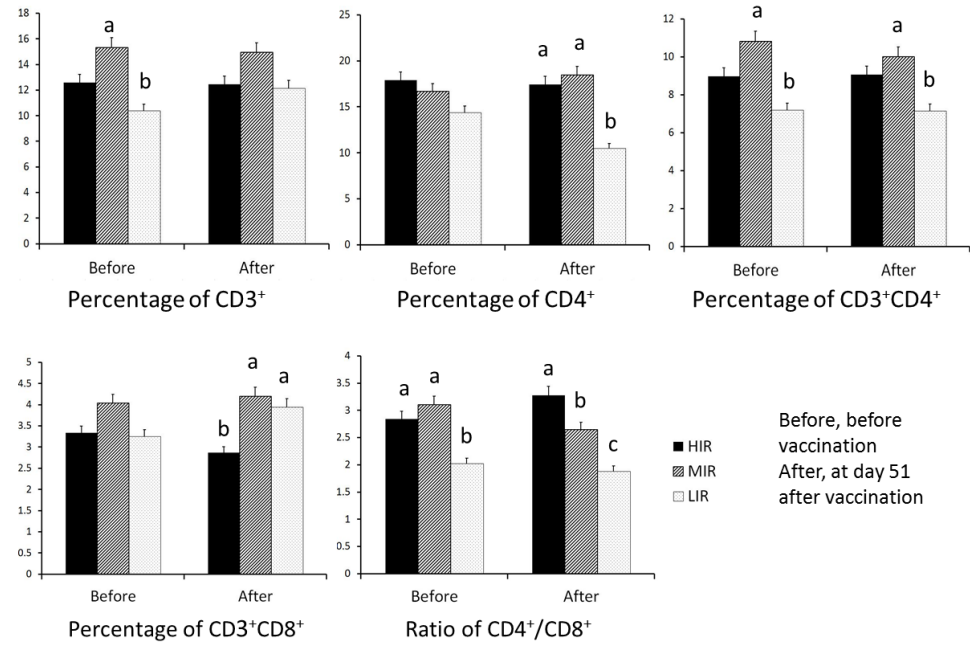

Fig. 3: The percentages of $CD3^+$, $CD4^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes, the ratio of $CD4^+$/ $CD8^+$ in the high immune response (HIR), medium immune response (MIR) and low immune response (LIR) groups. Different letters indicate significant differences ($P<0.05$) at the same stage.

DISCUSSION

$CD4^+$ T-cell is implicated in the differentiation of B-cells, and CD8 T-cell is crucial in regulating multiple immunological mechanisms, and destroys virally infected cells and tumor cells (Bosire *et al.*, 2013). It was showed in Fig. 1 that the antibody titers of the three serotypes at day 51 APV were higher than that before vaccination, and the amounts of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes at day 51 APV were also higher than that before vaccination (Fig. 2). It has been reported that $CD4^+$ and $CD8^+$ T cells are induced by vaccination with nucleoprotein DNA, which are implicated in protection from influenza virus challenge in mice (Ulmer *et al.*, 1998). Therefore, it was suggested that the production of the antibodies of the three serotypes was related to the amounts of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes.

In this study, the antibody titers of the three serotypes in HIR group were higher than that of the MIR and LIR groups at day 51 APV (Fig. 1), and the similar pattern occurred in the CD4/CD8 ratio at day 51 APV (Fig. 3), which indicated that the CD4/CD8 ratio was associated with the antibody titers of the three serotypes. It has been reported that low CD4/CD8 ratio is related to the persistent cytomegalovirus replication and high mortality (Serrano-Villar *et al.*, 2014). As a parameter, CD4/CD8 ratio can be used to assess the ongoing immune activation (Serrano-Villar *et al.*, 2013), and detect danger of disease progression in successfully treated human immuno-deficiency virus infected patients (Serrano-Villar *et al.*, 2014). Low CD4/CD8 ratio is associated with the functional immune disturbance in human immuno-deficiency virus infected patients with successful antiretroviral therapy (Avelino-Silva *et al.*, 2016b), and

human immunodeficiency virus infection can be foretelled via lower CD4/CD8 ratio in humans (Avelino-Silva *et al.*, 2016a). Therefore, CD4/CD8 ratio may be used to select young sires.

White blood cells play a key role in animal immune system, but there is no relationship between the WBCC and humoral immune response induced by dietary energy and protein restriction in broiler chicks (Fanooci and Torki, 2010). The similar results were showed in Fig. 4A that there was no relationship between the WBCC and immune response capability at day 51 APV. However, the WBCC was higher in the HIR and MIR groups before vaccination, which may be owing to that the maternal antibody was high in the HIR and MIR groups.

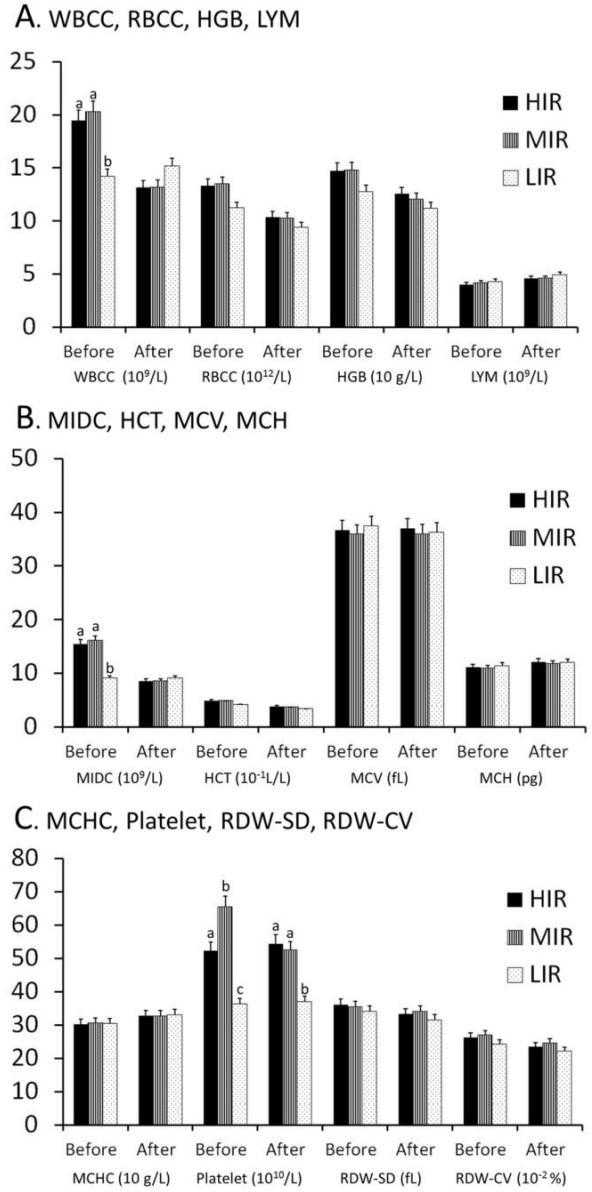

Fig. 4: The blood routine parameters in the high immune response (HIR), medium immune response (MIR) and low immune response (LIR) groups. Note: Before=Before vaccination; After=Day 51 after primary vaccination; WBCC=White blood cell count; RBCC=Red blood cell count; HGB=hemoglobin; LYM=Lymphocyte count; MIDC=Mid cell count; HCT=Hematocrit; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=Mean corpuscular hemoglobin concentration; RDW-SD=Red cell distribution width SD; RDW-CV=Red cell distribution width CV. Different letters indicate significant differences ($P<0.05$) at the same stage.

RBCs contain HGB and carry oxygen to animal tissues, and animal tissues need oxygen to function. In this study, it was found that the RBCC and HGB were higher in the HIR and MIR groups before vaccination, but there was a slight decline in the RBCC and HGB at day 51 APV (Fig. 4A). It has been reported that RBC plays roles in animal immune response, and HGB released from RBCs can destroy the pathogen's cell wall and membrane (Jiang *et al.*, 2007). The high levels of RBCC and HGB in the HIR and MIR groups before vaccination may be partly implicated in the defensive immune response, owing to low antibody levels before vaccination. Furthermore, at day 51 APV, there were high levels of antibodies in young sires, so the high levels of RBCC and HGB may be not necessary for the defensive immune response.

Lymphocytes mainly include T-cells, B-cells and natural killer cells, are related to the individual antiviral capacity in mammals (Wang *et al.*, 2016). In this study, there was an increase in the LYM of the three groups at day 51 APV, which may be due to the high antibody levels in the three groups after vaccination. However, there was no relationship between the LYM and immune response capability (Fig. 4A), so the immune response capability may not be measured by the LYM.

Mid cells include monocytes, eosinophils, basophils and other precursor white cells, are between neutrophils and lymphocytes in size, and the parts of animal innate immune system. It was showed in Fig. 4B that there was a significantly low level of MIDC in the LIR group before vaccination, so low level of MIDC in the blood of young sires before vaccination may be used to select the LIR sires. However, there was no relationship between the MIDC and immune response capability at day 51 APV. The reasons may be that certain amounts of mid cells was changed into other immune cells in the HIR and MIR groups, which led to the high immune response capability in the HIR and MIR groups after vaccination.

Our results showed that there was no relationship between these six parameters (HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV) and immune response capability (Fig. 4B, 4C). HCT is the volume percentage of RBCs, also a reference point of the capability of delivering oxygen of RBCs. An abnormal level of HCT indicates possible diseases in the animals (Birchard, 1997). MCV is a measure of the average volume of a RBC, related with the RDW-SD and RDW-CV and RDW is a powerful marker of cardiovascular disease in humans (Al-Kindi *et al.*, 2017). MCH is average mass of HGB per RBC, and MCHC is related to concentration of HGB. Our results also indicated that RBCC and HGB were not related to the immune response capability (Fig. 4A), so these six parameters may not be used to measure the immune response capability of the sires.

Our results showed that platelet level was lower in the LIR group comparing to that in the HIR and MIR groups at two stages (Fig. 4C). It has been reported that activated platelet can bind to circulating lymphocytes (Diacovo *et al.*, 1996), which suggests that the activated platelet is implicated in regulating the immune response through binding to lymphocytes. Platelet-mediated cell-cell interactions on lymphocyte trafficking and formation of immunologic memory affects a variety of autoimmune and inflammatory conditions (Diacovo *et al.*, 1998).

Therefore, it was suggested that the level of platelet was related with regulation of immune response in the young sires, and platelet level in the blood may be used to select young sires.

Conclusions: The immune response capability of young sires after vaccination were related to the level of platelet and MIDC before vaccination, and the CD4/CD8 ratio and level of platelet after vaccination, which may be used to select the HIR young sires.

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Authors contribution: LJB designed the experiment, and HKL conducted the experiment. YL, MYB, LRL, HMH, GRD and ZJF analyzed the resulting data. YL drafted the manuscript. All authors read, revised and approved the final manuscript.

REFERENCES

Al-Kindi SG, Kim CH, Morris SR, *et al*., 2017. Brief report: elevated red cell distribution width identifies elevated cardiovascular disease risk in patients with HIV infection. J Acquir Immune Defic Syndr 74:298-302.

Avelino-Silva VI, Miyaji KT, Hunt PW, *et al*., 2016a. CD4/CD8 ratio and kt ratio predict yellow fever vaccine immunogenicity in HIV-infected patients. PLoS Negl Trop Dis 10:e0005219.

Avelino-Silva VI, Miyaji KT, Mathias A, *et al*., 2016b. CD4/CD8 Ratio Predicts Yellow Fever Vaccine-Induced Antibody Titers in Virologically Suppressed HIV-Infected Patients. J Acquir Immune Defic Syndr 71:189-95.

Birchard GF, 1997. Optimal Hematocrit: Theory, Regulation and Implications. Integr Comp Biol 37:65-72.

Bosire EM, Nyamache AK, Gicheru MM, *et al*., 2013. Population specific reference ranges of CD3, CD4 and CD8 lymphocyte subsets among healthy Kenyans. AIDS Res Ther 10:24.

Chowdhury MMR, Hossen ML, Amin KB, *et al*., 2015. Assessment of immune response in cattle against experimentally prepared trivalent (O, A, and Asia-1) FMD vaccine in Bangladesh. J Adv Vet Anim Res 2:475.

Diacovo TG, Catalina MD, Siegelman MH, *et al*., 1998. Circulating activated platelets reconstitute lymphocyte homing and immunity in L-selectin-deficient mice. J Exp Med 187:197-204.

Diacovo TG, Puri KD, Warnock RA, *et al*., 1996. Platelet-mediated lymphocyte delivery to high endothelial venules. Science 273:252-5.

Fanooci M and Torki M, 2010.Effects of qualitative dietary restriction on performance, carcass characteristics, white blood cell count and humoral immune response of broiler chicks. Global Vet 4:277-82.

Jiang N, Tan NS, Ho B, *et al*., 2007. Respiratory protein-generated reactive oxygen species as an antimicrobial strategy. Nat Immunol 8:1114-22.

Parker Gaddis KL, Cole JB, Clay JS, *et al*., 2014.Genomic selection for producer-recorded health event data in US dairy cattle. J Dairy Sci 97:3190-9.

Parker Gaddis KL, Tiezzi F, Cole JB, *et al*., 2015. Genomic prediction of disease occurrence using producer-recorded health data: a comparison of methods. Genet Sel Evol 47:41.

Serrano-Villar S, Gutiérrez C, Vallejo A, *et al*., 2013. The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression. J Infect 66:57-66.

Serrano-Villar S, Sainz T, Lee SA, *et al*., 2014. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 10:e1004078.

Thompson-Crispi K, Atalla H, Miglior F, *et al*., 2014. Bovine mastitis: frontiers in immunogenetics. Front Immunol 5:493.

Ulmer JB, Fu TM, Deck RR, *et al*., 1998.Protective CD4+ and CD8+ T cells against influenza virus induced by vaccination with nucleoprotein DNA. J Virol 72:5648-53.

Wang H, Hou Y, Guo J, *et al*., 2016. Transcriptomic landscape for lymphocyte count variation in poly I:C-induced porcine peripheral blood. Anim Genet 47:49-61.

Whisler S and Dahlgren C, 2005. Performance evaluation of the Sysmex pocH-100i automated hematology analyzer. Lab Hematol 11:107-17.

Yang L, Liu Z, Li J, *et al*., 2018. Association of the expression of Th cytokines with peripheral CD4 and CD8 lymphocyte subsets after vaccination with FMD vaccine in Holstein young sires. Res Vet Sci 119:79-84.