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Splenic comparative transcriptome analysis reveals the immunological mode of undomesticated *Gayal* (*Bos frontalis*) for adapting to harsh environments

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Abstract

Background The utilization of transcriptome technology in the identification of pivotal regulatory genes associated with immunity is of paramount importance. Previous studies have shown that undomesticated *gayal* (*Bos frontalis*) may have higher humoral responses which is comparable to yaks. However, research on immune function of *gayal* is limited, and comparisons with different breeds are rarely reported. The objective of this study was to inspect the immune status and compare splenic differential expression genes (DEGs) through comparative transcriptome analysis of *gayal* and domesticated local cattle (Yunan yellow cattle).

Results Serum immunological status investigation showed the better humoral immune status and lower levels of pro-inflammatory cytokines of *gayal* when compared to the local cattle. Spleen RNA-seq showed that 708 DEGs (365 up- and 343 down-regulated genes) were obtained between the *gayal* and local cattle. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis showed that immune system pathways, immune disease pathways, and chemotaxis-related molecular function of *gayal* were significantly enriched, whereas T cell-related cellular component and biological process were downregulated in the *gayal*. Correlation analysis shown that *CD1*, *CD36*, *CD38*, *CD179a*, *CD179b*, *CXCL8*, *IGCGAMMA*, *IGH*, *IGHG1*, *IGLL1*, *IL1R2*, *SERPINB*, and *SERPINB4* had positive correlations with splenic IgA, IgD, IgE, IgG, and IgM, respectively ($R > 0.5$, $P < 0.05$). *ANPEP*, *BVD1.23*, *CD1E*, *CD3D*, *CD3E*, *CD3G*, *CD5*, *CD8 A*, *HBB*, *IDO1*, *LCK*, *MGC126945*, *MHC1*, *TRAV*, *TRBV*, and *ZAP70* had negative correlations with splenic IgA, IgD, IgE, IgG, and IgM, respectively ($R < -0.5$, $P < 0.05$).

Conclusions Our results reveal the immunological mode of *gayal* with high-level humoral immunity and enhanced splenic immunoglobulin gene expression and B cell differentiation, which may enable *gayal* to adapt to the harsh environments.

Keywords *Gayal*, Spleen, Anti-oxidant, Immune function, Transcriptome

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Introduction

Gayal (*Bos frontalis*) is one of the least researched and undomesticated ruminants because of the low accessibility of their habitats [1, 2]. As the unique semi-wild rare bovine specie, *gayal* inhabits the unique geographical environment that contains subtropical rain forests and alpine valleys of the Dulong and Nujiang river basins of Gongshan, Fugong, and Lushui county (Yunnan Province, China) [3]. *Gayal* resembles *Bos gaurus* and has 29 pairs of chromosomes that differs *gayal* from *Bos gaurus* (28 pairs of chromosomes) and *Bos taurus* (30 pairs of chromosomes) [4–6]. Therefore, it was concluded that the *Gayal* is neither a domesticated type of bison nor a hybrid offspring of bison and yellow cattle, but a species of the genus *Bos*. The polymorphic analysis of partial sequences of the Cytb gene of *Gayal* by Ma et al. (2007) also confirmed that it is a separate species in the genus *Bos* [7]. *Gayal* feeds on the leaves and branches of grasses (such as bamboo, reeds, etc.) and other plants.

The complex geographical and climatic conditions shape the unique physiological features and enhanced adaptability of *gayal*. Previous studies have shown that *gayal* has the enhanced hemoglobin synthesis and similar level of red blood cells to the yak to adapt with the lower oxygen partial pressure [7, 8]. The similar level of white blood cells and expression of splenic chemokines, tumor necrosis factors, and interleukins indicating that *gayal* has the similar ability to resist pathogens and inflammation as yak in harsh environment [7]. Whole-genome sequence also exhibited 143 specific gene families (915 genes), including complement component 4 (*C4*), immunoglobulin heavy locus (*IgH*), lysozyme C (*LZC*), interferon alpha, toll-like receptor (*TCR*), in *gayal* [3]. Generally, wild animals' immune responses, which are shaped by multiple factors such as genetics, resources, seasons and ecological pressures, contribute to their capacity of adapting to harsh environment. Humoral (antibody) responses are highly elevated of wild animals, such as wild yaks, rodents, which may result from the selection to be appropriate to the continuously exposure to multiple pathogens in the harsh environment [9, 10]. However, considering the complexity of immune regulation, the knowledge of immune function of *gayal* is still unclear. Therefore, we hypothesized that *gayal* has better humoral immune or cellular immune function when compared with other domesticated bovine species.

The spleen, the largest immune organ in the animal organism, plays a pivotal role in disease control [11, 12]. It is responsible for both specific and non-specific immune functions, with the majority of the body's immune response occurring in this organ [13]. Transcriptome sequencing technology, as an efficient and rapid high throughput sequencing method, can provide insights into various complex physiological pathways, including epidemics and immune responses [14]. Transcriptome sequencing of the spleen is an effective method to study the molecular mechanisms of spleen development, analyze the effects of stress on immune functions, and uncover potential disease resistance genes [15]. In present study, we inspected the level of serum and splenic immune factors and anti-oxidant capacity, and performed comparative splenic transcriptome analysis to explore the immunological mode of *gayal* adapting to complex wilderness environment.

Materials and methods

Ethical statement and location

The protocol and details of the present study were approved by the Animal Care Committee at the Faculty of Animal Science and Technology, Yunnan Agricultural University (Kunming, P. R. China).

The present study was conducted from June to August, 2023 at the trail pasture of Fenghuang Mountain *Gayal* Breeding and Expansion Base (altitude 2700 m, Lushui, Yunnan, China), the *Gayal* and local cattle (Yunnan yellow cattle) are on this farm. The dominant plant species of the trail pasture were bamboo, bamboo shoots, clover, and ryegrass.

Experimental design, animals, and managements

According to the age (2-year-old) and body weight (240 ± 7.0 kg; mean ± SEM), eight male and healthy *gayal* (*Bos frontalis*) and eight male and healthy local cattle (Yunnan yellow cattle, *Bos taurus*) were used in this experiment and divided into two groups (*gayal* group and local cattle group). The selection of *gayal* continued for more than a month because they are semi-wild animals, making the process more challenging. All the cattle grazed trail pasture all day with free access to water and salt blocks during the whole observation period. All the cattle healthily completed the entire observation period (30 d) with no major symptoms affecting the results.

Sample collection

At the end of the experiment, after 16 h fasting and not receiving water, the serum samples were collected from jugular and stored at −80 °C. Then, eight *gayal* (*Bos frontalis*) and eight local cattle were euthanized after anesthesia with 0.3 mg/kg·BW xylazine hydrochloride injection according to the manufacturer's instructions. The splenic tissues from eight *gayal* (*Bos frontalis*) and eight local cattle were harvested at fixed position and washed immediately in ice-cold phosphate-buffered saline solution 3 times, and then stored at −80 °C for subsequence analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

The serum immune factors (IL1β, IL2, IL4, IL6, IL8, IL10, IL13, TGF-β1, TGF-β2, IFN-γ, TNF-α, IgA, IgD, IgE, IgG, and IgM), splenic antioxidant capacity (total antioxidant capacity, malondialdehyde, glutathioneperoxidase, and superoxide dismutase), and splenic immunoglobulins (IgA, IgD, IgE, IgG, and IgM) were determined by using ELISA kits (Additional file Table S1). The serum and spleen samples were processed according to the manufacturer's instructions.

Splenic transcriptome

Total RNA purification, reverse transcription, library construction, and sequencing were conducted at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions. Total RNA of splenic tissues was extracted using QIAzolLysis Reagent (Qiagen, German). RNA quality was determined by 5300 Bioanalyser (Agilent Technologies, Inc., USA) and NanoDrop ND-1000 (Thermo Fisher Scientific, Inc., USA). Only high-quality RNA sample (OD260/280 = 1.8 ~ 2.2, OD260/230 ≥ 2.0; RQN ≥ 6.5) was used to construct sequencing library. The splenic RNA-seq transcriptome library was prepared following Illumina® Stranded mRNA Prep, Ligation (San Diego, CA) using 1 μg of total RNA. The sequencing library was performed on NovaSeq X Plus platform (Illumina, Inc., USA) using NovaSeq Reagent Kit, and using 2× 150 bp paired-ended sequencing. After quality control (removing low-quality reads, reads with adaptor sequences, and reads with more than 5% unknown bases in raw reads) by fastx_toolkit v0.0.14, the clean reads were separately aligned to *Bos_taurus* (reference genome version: GCF_002263795.3, reference genome source: https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002263795.3/) with orientation mode using HISAT2 v2.0.1 [16]. The mapped reads of each sample were assembled by StringTie in a reference-based approach [17]. The expression level of each transcript was calculated according to the fragments per kilobase of exon per million mapped reads (FPKM) using StringTie v1.2.2 and Ballgown package in R [17]. Only expressed genes (FPKM > 5 in all samples) in at least 50% of cattle within each group were used in subsequent analyses. KEGG functional-enrichment analysis was performed by Python scipy software [18]. GO term annotation and enrichment analysis were conducted by using the Blast2GO and goatools software [19, 20].

Quantitative Real-Time PCR (RT-qPCR) for Validation of RNA-Seq Data

Total RNA was reverse transcribed by using NovoScript®Plus All-in-one 1 st Strand cDNA Synthesis SuperMix (gDNA Purge) (E047-01B; Novoprotein, Shanghai, China) to obtain cDNA following the manufacturer's procedures. Q-Tower^3 (Analytik Jena AG, Jena, Germany) with NovoStart®SYBR qPCR SuperMix Plus (E096-01 A; Novoprotein, Shanghai, China) was used to perform quantitative real-time PCR (qRT-PCR) of the target genes, including *BCR*, *CD3D*, *CD3E*, *CD8A*, *CD38*, *IDO*, *IgA*, *IgH*, *IgM*, *IL8*, *LCK*, *MHC1*, *TRBV*, *ZAP70*, and *β-actin*. The data of the mRNA expression were normalized to the housekeeping gene (β-actin or GAPDH) using the $2^{-\Delta\Delta CT}$ method [21]. The primers and amplicon sizes of genes are shown in Additional file (Additional file Table S2).

Statistical analysis

Grubbs' test was used for detecting outliers form nonomics data [22]. The results of serum and spleen immune factors, anti-oxidant, splenic immunoglobulins, splenic antioxidant capacity, and splenic mRNA expression were analyzed by using the independent samples t-test in SPSS 20.0 (SPSS INC, USA). $P < 0.05$ was considered as statistically significant. For splenic transcriptomic data, P value was corrected by FDR [23]. Reads data was analyzed by using the Mann–Whitney U test in SPSS 20.0 (SPSS INC, USA). Mapping results of RNA-seq data, which presented as percentage, were analyzed by using the Chi-square test in SPSS 20.0 (SPSS INC, USA). Differential expression genes (DEGs) with |log2 FC| ≥ 1 and FDR < 0.05 were considered to be significant by DESeq2 [24]. DEGs were subjected to KEGG and GO enrichment analysis and FDR < 0.05, rich factor > 0.1 were considered to be significant metabolic pathways. Relationships between phenotypes and DEGs were explored by Spearman's rank correlation test. |R| > 0.5, $P < 0.05$ was used to identify significant correlations. Cytoscape 3.6.1 was used to construct the correlation network.

Results

Comparison of serum immune factors level between the gayal and local cattle

As shown in Fig. 1A, compared with the local cattle, *gayal* had lower level of pro-inflammatory cytokines, such as IL1β ($0.05 < P < 0.10$), IL8 ($P < 0.05$), IFN-γ ($P < 0.05$), and TNF-α ($P < 0.05$), in serum. However, the level of serum anti-inflammatory cytokines, such as IL4, IL10, IL13, TGF-β1, and TGF-β2, had no significant difference between 2 bovine species ($P > 0.10$; Fig. 1B). Serum immunoglobulins concentrations, including IgA ($0.05 < P < 0.10$), IgG ($P < 0.05$), and IgM ($P < 0.05$), were higher in *gayal* when compared to local cattle (Fig. 1C).

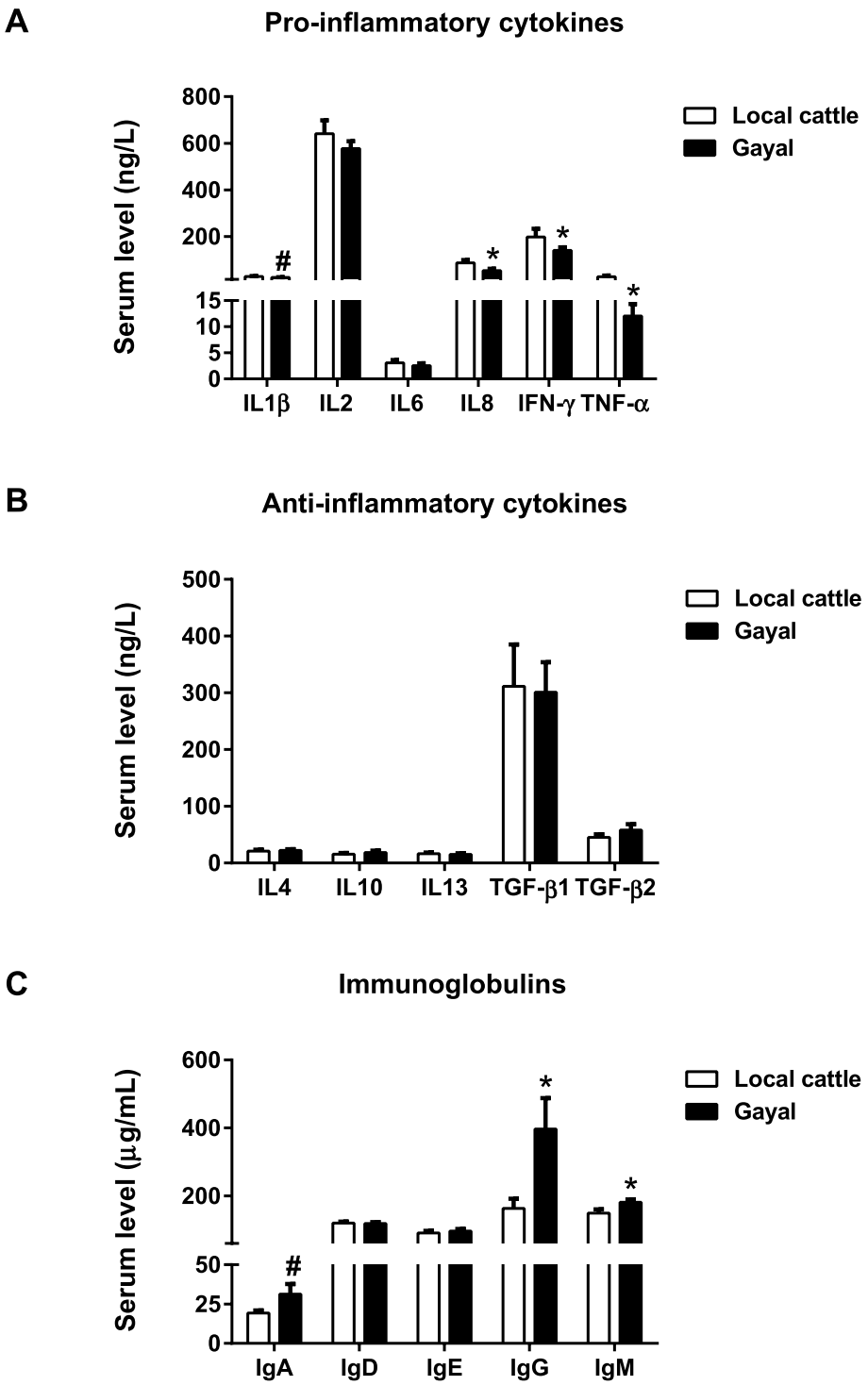

Fig. 1 Comparison of serum immune factors level between *gayal* and local cattle. **A** Pro-inflammatory cytokines. **B** Anti-inflammatory cytokines. **C** Immunoglobulins. Note: The results were analyzed by using the independent samples t-test in SPSS 20.0 (SPSS INC, USA). Results are shown as mean ± SEM, symbols indicate significance (∗, $P < 0.05$; #, $0.05 < P < 0.1$)

Comparison of splenic transcriptome profile between the gayal and local cattle

A total of 118.05 Gb of clean data were obtained from 16 samples with an average of 6.33 Gb per sample. 48,975,829 ± 1,059,400 and 49,595,919 ± 1,610,582 clean reads were respectively obtained from *gayal* and local cattle, and Q30 scores of clean bases were more than 91.75% for all 16 splenic samples (Table S3). Based on the *Bos taurus* reference genome (domestic cattle; GCF_002263795.3, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_002263795.3/), the mapping ratio ranged from 82.90% to 93.85% and shown no differences between two group (Table S3). Detailed information of RNA-seq quality and mapping for single sample was listed in Additional file Table S3.

A total of 10,991 effective genes with 365 upregulated and 343 downregulated differential expression genes (DEGs) were identified between *gayal* and local cattle (Fig. 2A; Additional file Table S4). As shown in the Fig. 2B, KEGG Enrichment analysis identified 17 pathways based on DEGs (FDR < 0.05; Table S5). Among these pathways, 5 immune-related pathways, including primary immunodeficiency (ko05340), amoebiasis (ko05146), african trypanosomiasis (ko05143), hematopoietic cell lineage (ko04640), and allograft rejection (ko05330), were identified according to the DEGs (FDR < 0.05, rich factor > 0.1; Fig. 2C; Table S5). We also performed GO terms enrichment analysis that were enriched in the set of 708 DEGs. A total of 21 GO terms were significantly enriched (FDR < 0.05, rich factor > 0.1; Fig. 2D; Table S6). Among these GO terms, 9 GO terms, including G protein-coupled chemoattractant receptor activity (GO:0001637), serine-type endopeptidase inhibitor activity (GO:0004867),

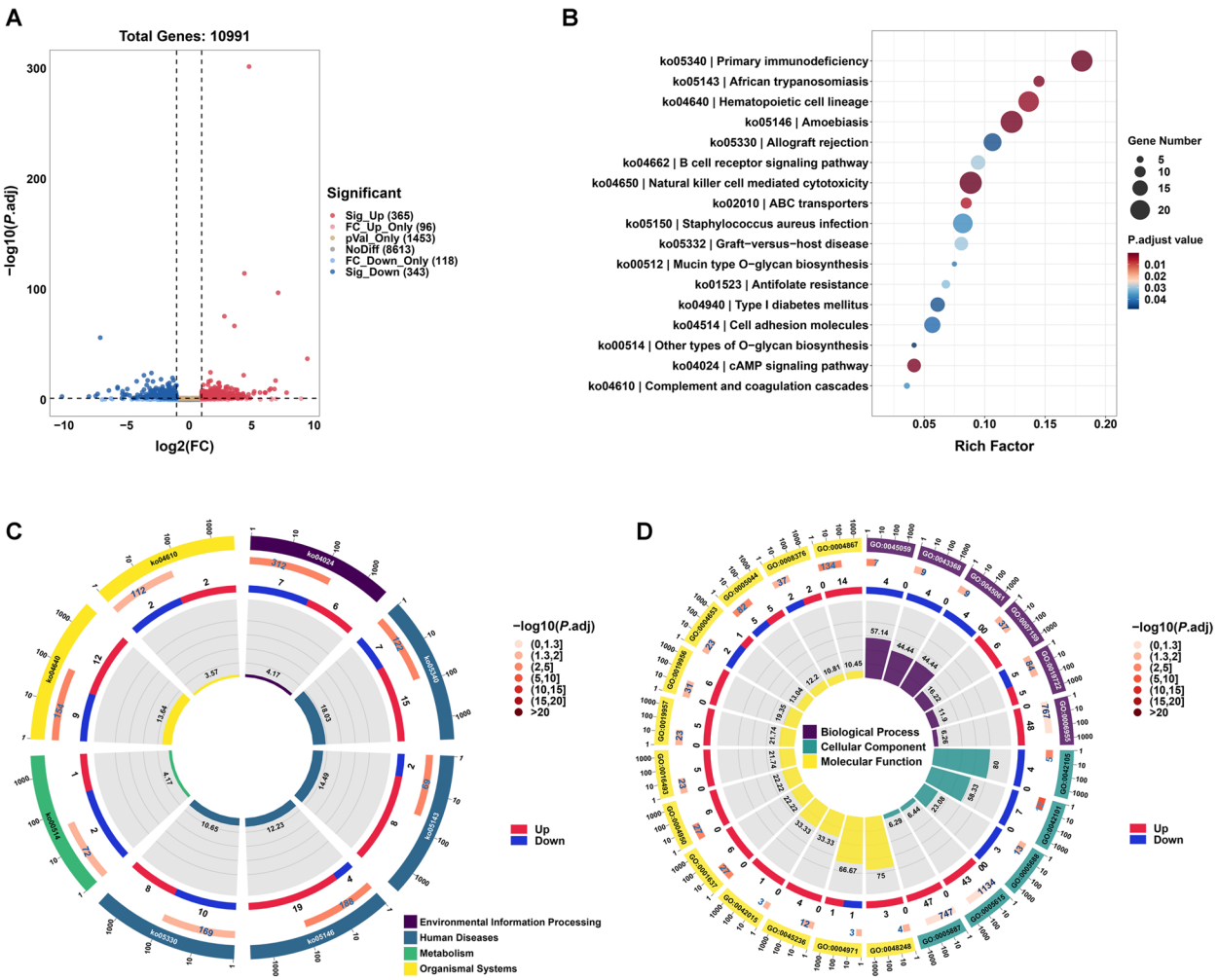

Fig. 2 Comparison of splenic transcriptome profile between *gayal* and local cattle. **A** Volcano plot of splenic gene expression. **B** KEGG pathway enrichment analysis of differential expression genes (DEGs) (FDR < 0.05). **C** Enrichment circos plot of KEGG pathways (FDR < 0.05, rich factor > 0.1). **D** Enrichment circos plot of GO pathways (FDR < 0.05, rich factor > 0.1)

chemokine receptor activity (GO:0004950), leukocyte cell–cell adhesion (GO:0007159), C–C chemokine receptor activity (GO:0016493), chemokine binding (GO:0019956), C–C chemokine binding (GO:0019957), CXCR chemokine receptor binding (GO:0045236), and CXCR3 chemokine receptor binding (GO:0048248), were significantly enriched according to the 362 upregulated DEGs. 6 GO terms, including U6 snRNP (GO:0005688), T cell receptor complex (GO:0042101), alpha–beta T cell receptor complex (GO:0042105), positive T cell selection (GO:0043368), positive thymic T cell selection (GO:0045059), and thymic T cell selection (GO:0045061), were significantly enriched according to the 343 downregulated DEGs.

10 DEGs in these pathways had been validated by qPCR (Fig. 3A). The expression of *CD38* and *IL8* were significantly upregulated in *gayal* ($P < 0.05$). The expression of T cell receptor beta chain V region (*TRBV*), *CD3D*,

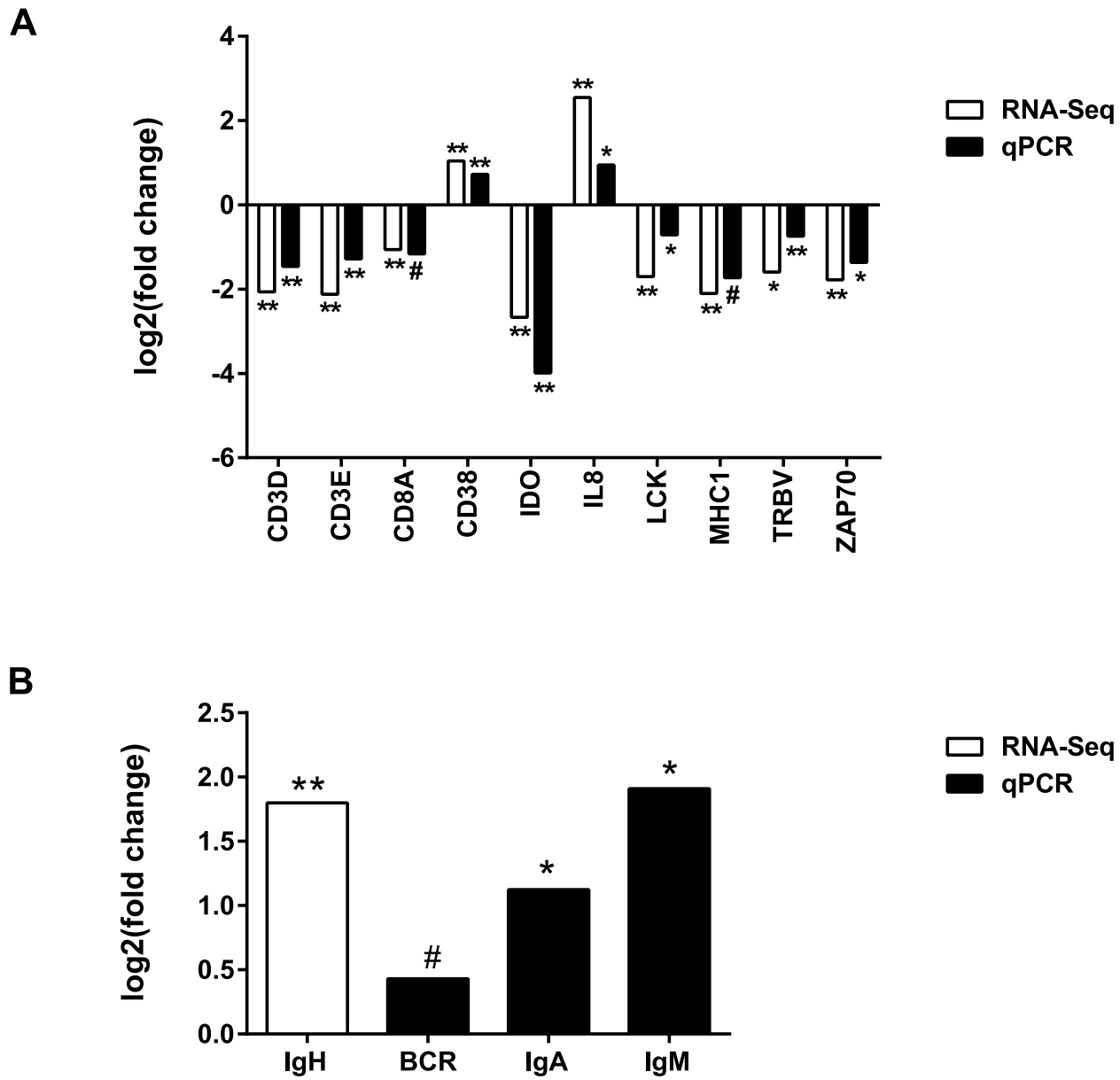

Fig. 3 Validation of differential expression genes (DEGs) expression. **A** Validation of immune-related DEGs expression. The results were analyzed by using the independent samples t-test in SPSS 20.0 (SPSS INC, USA). Results are shown as mean ± SEM, symbols indicate significance (∗∗, $P < 0.01$; ∗, $P < 0.05$; #, $0.05 < P < 0.1$). **B** Validation of immunoglobulin heavy locus gene related DEGs expression. Fold change = *gayal*/local cattle; symbols indicate significance (∗∗, $P < 0.01$; ∗, $P < 0.05$; #, $0.05 < P < 0.1$)

CD3E, indoleamine-2,3-dioxygenase (*IDO*), lymphocyte cell-specific protein tyrosine kinase (*LCK*), and tyrosine-protein kinase (*ZAP70*) were significantly downregulated in *gayal* ($P < 0.05$). The expression of MHC class I antigen (*MHCI*) and *CD8 A* showed the same trends with RNA-seq results ($0.05 < P < 0.1$). Immunoglobulin heavy locus (*IGH*), on the other side, had the similar trends with the mRNA expression of B cell receptor (*BCR*) ($0.05 < P < 0.1$), *IgA* ($P < 0.05$), and *IgM* ($P < 0.05$) in the *gayal* (Fig. 3B).

Comparison of splenic immunoglobulins levels and antioxidant capacity between the gayal and local cattle

We also inspected the level of splenic immunoglobulins (IgA, IgD, IgE, IgG, and IgM) and found that the level of IgA ($P < 0.05$), IgE ($P < 0.05$), IgG ($0.05 < P < 0.10$), and IgM ($P < 0.05$) were higher in *gayal* when compared to local cattle (Fig. 4). As shown in Fig. 5, there was no significant difference in the splenic antioxidant capacity, such as total antioxidant capacity, malondialdehyde, glutathioneperoxidase, and superoxide dismutase, between 2 bovine species ($P > 0.05$). As shown in Fig. 6, correlation analysis shown that serum IgG had positive correlation with splenic IgE, IgG, and IgM, while serum IgA had positive correlation with splenic IgM ($R > 0.5$, $P < 0.05$). *CD1*, *CD36*, *CD38*, *CD179a*, *CD179b*, *CXCL8*, *IGCGAMMA*, *IGH*, *IGHG1*, *IGLL1*, *IL1R2*, *SERPINB*, and *SERPINB4* had positive correlations with splenic IgA, IgD, IgE, IgG, and IgM, respectively ($R > 0.5$, $P < 0.05$). *ANPEP*, *BVD1.23*, *CD1E*, *CD3D*, *CD3E*, *CD3G*, *CD5*, *CD8 A*, *HBB*, *IDO1*, *LCK*, *MGC126945*, *MHC1*, *TRAV*, *TRBV*, and *ZAP70* had negative correlations with splenic IgA, IgD, IgE, IgG, and IgM, respectively ($R < -0.5$, $P < 0.05$). Finely, splenic immunological mode of *gayal* was summarized in Fig. 7.

Discussion

Wild animals' immune responses, which are shaped by multiple factors such as genetics, resources, seasons and ecological pressures, contribute to their capacity of adapting to harsh environment. Humoral (antibody) responses are highly elevated of wild animals, such as wild yaks, rodents, which may result from the selection to be appropriate to the continuously exposure to multiple pathogens in the harsh environment [9, 10]. The

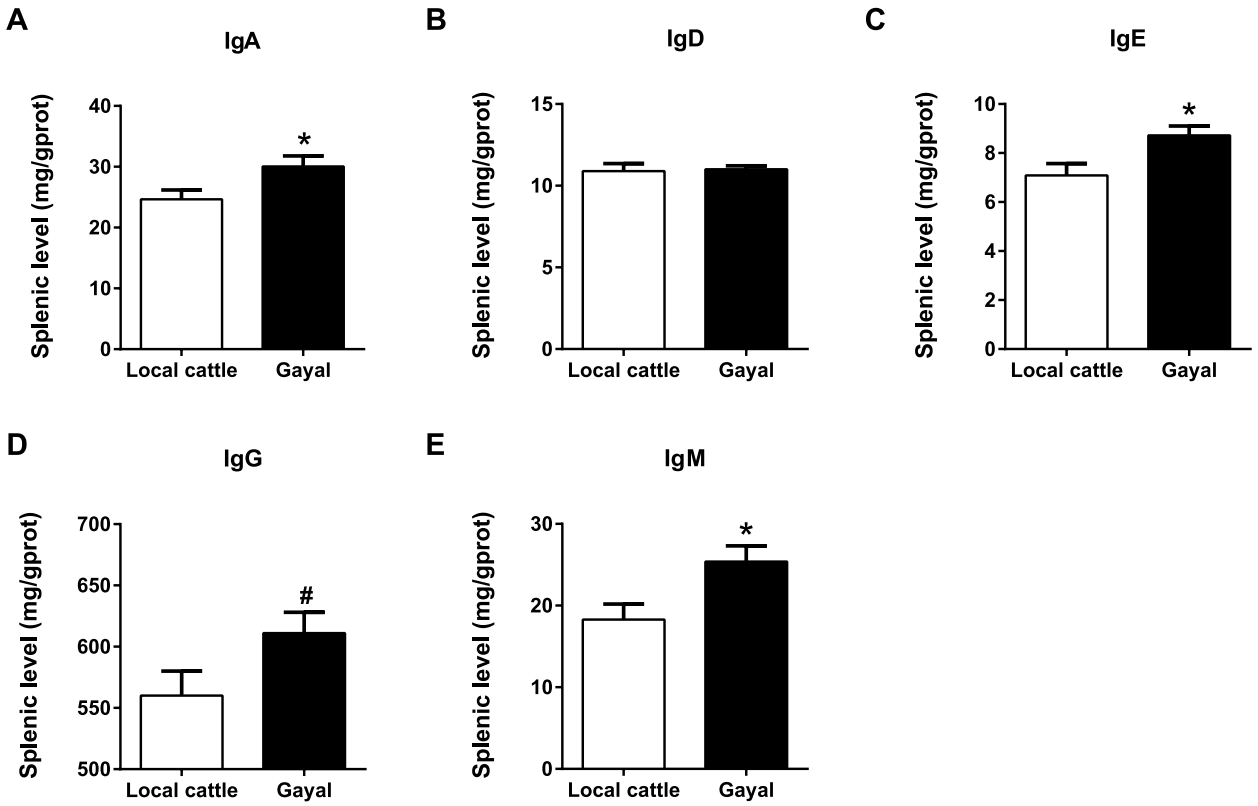

Fig. 4 Comparison of the levels of splenic immunoglobulins (A, IgA; B, IgD; C, IgE; D, IgG; E, IgM) between *gayal* and local cattle. The results were analyzed by using the independent samples t-test in SPSS 20.0 (SPSS INC, USA). Results are shown as mean ± SEM, symbols indicate significance (*, $P < 0.05$; #, $0.05 < P < 0.1$)

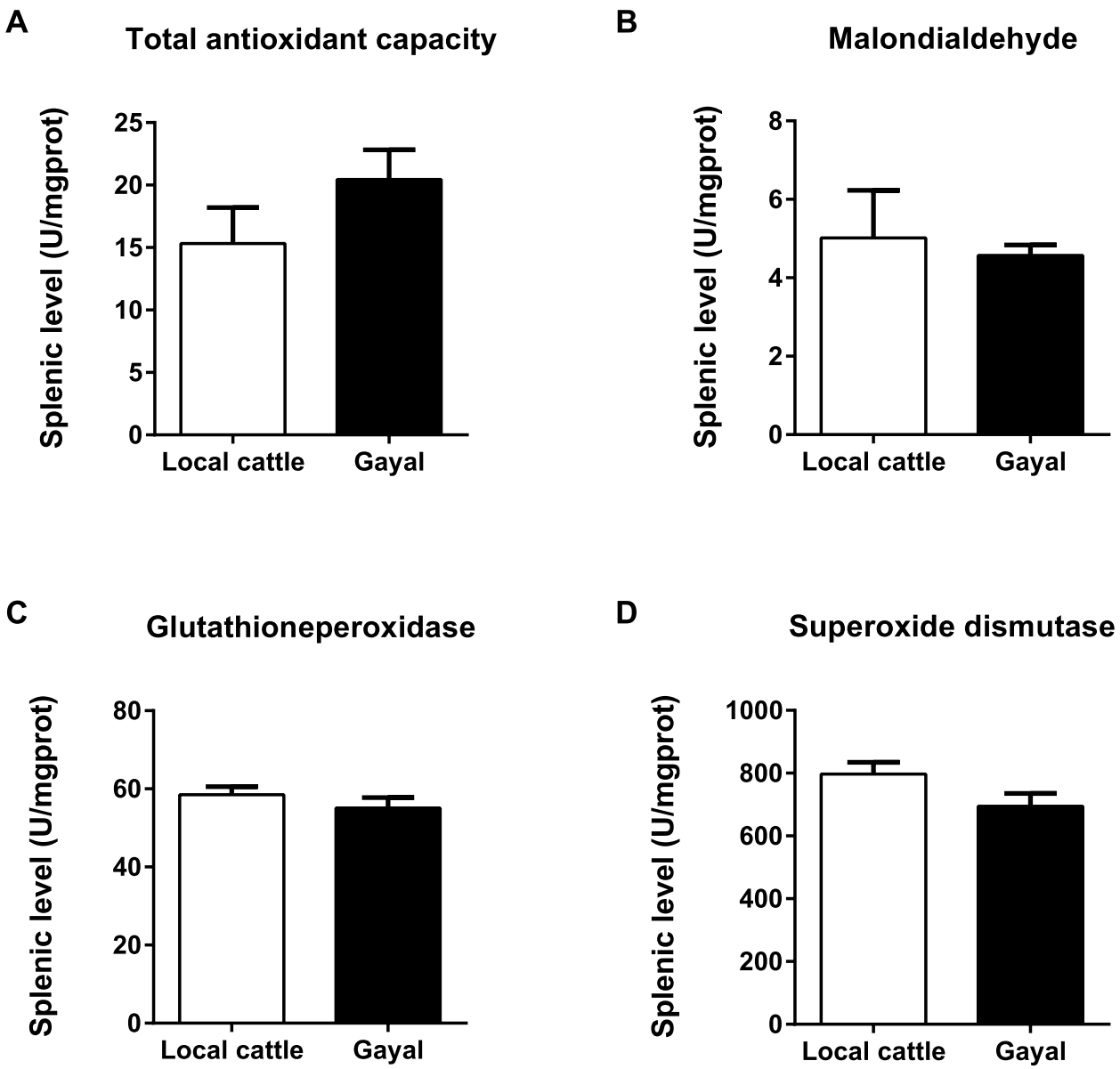

Fig. 5 Comparison of splenic antioxidant capacity (**A**, total antioxidant capacity; **B**, malondialdehyde; **C**, glutathioneperoxidase; **D**, superoxide dismutase) between *gayal* and local cattle. Note: The results were analyzed by using the independent samples t-test in SPSS 20.0 (SPSS INC, USA). Results are shown as mean ± SEM

present study is the first to investigate and compare the immune status and splenic transcriptome profiles between the *gayal* and local cattle. We demonstrated that *gayal* had high-level humoral immune status and low-level of cell-mediated immune function. Our data support the hypothesis that *gayal* may have high-level of humoral responses to adapt to harsh environment.

We evaluated the levels of serum immune factors and found that *gayal* had better humoral immune status and lower levels of pro-inflammatory cytokines when compared to local cattle, which may be one of the reasons for their adaptation to the harsh environment. Similarly, Yaks are characterized by high altitude and harsh environmental adaptations [25, 26], but whether they have a better humoral immune status than *gayal* needs to be further explored.

Spleen is the largest lymphatic organ of animals, including a variety of immune cells, such as B cells, T

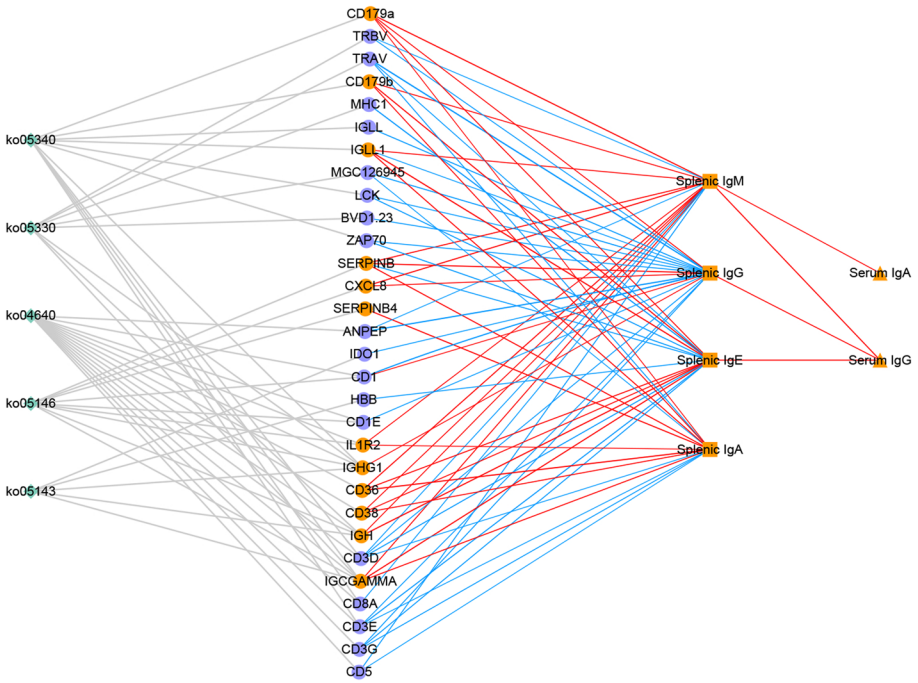

Fig. 6 Relationships between DEGs and phenotypes. The width of edges is proportional to the correlation strength. The color of edges: red, positive; blue, negative. The color of nodes: orange, significantly enriched in *gayal*; purple, significantly enriched in local cattle; green, pathway. The shape of nodes: diamond, pathway; ellipse, DEGs; rectangle, splenic immunoglobulins; triangle, serum immunoglobulins. ko04640, hematopoietic cell lineage; ko05143, african trypanosomiasis; ko05146, amoebiasis; ko05330, allograft rejection; ko05340, natural primary immunodeficiency. Only strong correlations were displayed ($|R| > 0.5$, $P < 0.05$)

cells, natural killer (NK) cells, etc., and plays a vital role in the immune system. In present study, we identified 5 KEGG pathways and 21 GO terms according to 708 DEGs. DEGs of the spleen in *gayal* were significantly enriched in immune system pathways, immune disease pathways, and chemotaxis-related molecular function, this is consistent with the study of the spleen transcriptome of Kazakh and Suffolk sheep, which identified adaptation-related immune response genes [27]. Whereas T cell-related cellular component and biological process were downregulated, suggesting that *gayal* and local cattle differed significantly on the immune functions.

Among these DEGs, The B cell receptor, which encoded by *BCR* gene, control B cell activation, proliferation, differentiation, apoptosis, homeostasis, and B cell-related immune responses. Activation and regulation of B cells is induced by the binding the cognate antigens with BCR, and thus initiates signal cascades to result in the differentiation to the memory B cells and plasma cells and production of antibodies [28, 29]. For ruminants, the *MHC* gene is linked to resistance or susceptibility to disease. Evidence shown that the major histocompatibility complex (MHC) proteins, which encoded by *MHC* gene, play a role in regulating antigen presentation to participate in the innate and adaptive immune responses in the cattle [30]. The typical feature of CD8 + T cell response is the high affinity binding the pathogen derived peptides with T cell receptors (TCR), which presented by MHC proteins [31, 32]. T cell receptor β variant (*TRBV*) are antigen receptors of CD4 +/8 + T cells. *CD3D* and *CD3E*, which are respectively known as CD3-δ and CD3-ε, are essential for T cell activation and ontogeny [33–35]. As a multimeric protein complex, CD3 binds with TCR to activate T cell immune response and induces the activation of NF-κB, NFAT, and AP-1 via being phosphorylated by Src family tyrosine kinase (LCK) and other tyrosine-protein kinase (ZAP70) [9, 36]. As the *CD8* gene encoded glycoprotein, CD8 can assist TCR in recognizing antigens and activate CD8 + T cell to start gene expression and metabolic programs to satisfy the rapid population expansion and differentiation with metabolic reprogramming [37]. CD8 + T cells can eliminate pathogens through the cytokines expression or killing the target cells and maintain the homeostasis by differentiating into long-lived memory CD8 + T cell [38]. CD38 is a single chain glycoprotein, which is encoded by *CD38* gene and expressed in the immune cells, and mediates the production of IL-1, IL-6, IL-10, IFN-γ, and TNF-α [39]. Previous studies shown that CD38 can consume NAD in CD4 +/CD8 + T cells to downregulated the function of mitochondria

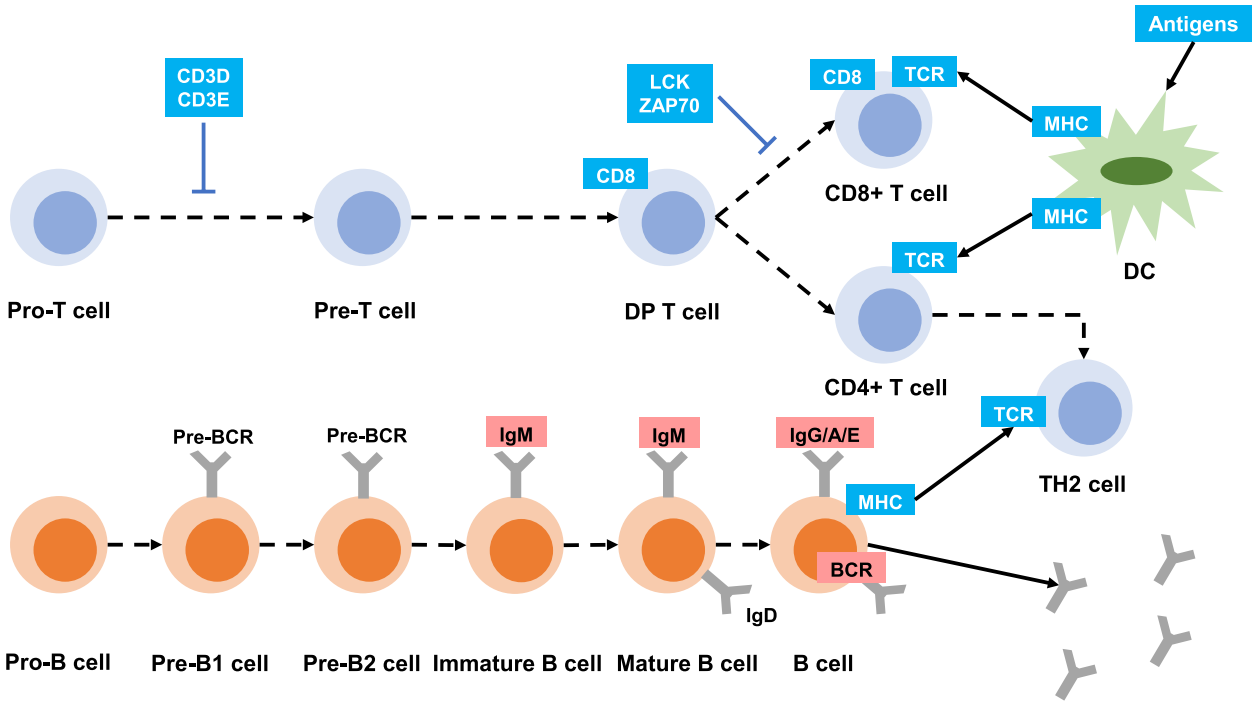

Fig. 7 Immunological mode in spleen of *gayal*. *Gayal* upregulated the gene expression of B cell differentiation and activation, whereas downregulated the gene expression of antigen presentation and T cell differentiation and activation in the spleen. Red, upregulation; blue, downregulation. Abbreviations: DC, dendritic cells; MHC, major histocompatibility complex proteins; TCR, T cell receptor; LCK, Src family tyrosine kinase; ZAP70, tyrosine-protein kinase

[40]. As a member of the chemokines superfamily, IL8 is produced by immune cells, epithelial cells, and histiocyte, and released during inflammation [41, 42]. IL-8 is chemotactic for neutrophils into inflammatory sites and release cellular contents to generate superoxide [43]. Our data shown the lower splenic gene expressions of *MHC* and *TCR* in the *gayal*, indicating that *gayal* might have lower level of splenic antigen recognition and presentation of cellular immunity. Furthermore, reduced splenic gene expressions of *CD3*, *CD8*, *LCK*, and *ZAP70*, which involved in allograft rejection (ko05330) and primary immunodeficiency pathway (ko05340), were observed in the *gayal*. This finding suggested a downregulation of T cell differentiation within spleen. Our results provided evidence that *gayal* kept the splenic cellular immune activation in a lower level when compared to local cattle, implying that *gayal* might exhibit a greater redundancy of splenic cellular immune response and less susceptibility to antigens.

In addition, immunoglobulin genes are usually expressed in B cells. Immunoglobulins play an essential role in the adaptive immune system in jawed vertebrates (mammals, birds, amphibians, etc.) and have been become the hallmark of the adaptive immune system [44, 45]. Immunoglobulin heavy locus genes (*IgH*) has been experienced large-scale structural renovation and evolution to produce a diverse antibody library [46]. *IgH* can express many isotypes. 5 *IgH* isotypes, including IgA, IgD, IgE, IgG, and IgM, are expressed by the majority of mammals [45]. IgA, which has monomeric structure (serum) and dimeric structure (secretory), plays a crucial role in passive and active immunity [47]. Monomeric IgA is easier to recognize antigens, while secretory IgA participates in the mucosal defense and has superiority status in maintaining microbiome-host homeostasis [45, 48]. IgD can support the development and maturation of B cell and its function, which has a long and flexible hinge region to bind antigen [45]. IgE and IgG only exists in mammals. IgE is the key antibody in resisting parasitic infections [47]. IgG, which has 4 subclasses (IgG1, IgG2, IgG3, and IgG4), is the key effector in humoral immune system by activating leukocyte and inducing inflammation [49]. IgG has 2 same antigen binding loci, resulting in the high sensitivity and affinity antigen recognition and connecting adaptive immune system and innate immune system [47, 50]. IgG is also crucial for the elimination of pathogenic microbes [51]. IgM is the primordial isotype that participates in the formation of BCR and expression of other IgH isotypes, and is involved in the regulatory process of infection, inflammation, B cell survival,

and B cell homeostasis [52, 53]. Previous study identified 143 specific gene families (915 genes), which contain *IgH* gene, in the *gayal* by whole-genome sequence [3]. Immunoglobulins of wild animals are not only a direct tool to resist pathogens, but also an important indicator of environmental adaptability. Previous study shown that 20- and 200-fold higher levels of serum IgG and IgE were observed in wild mice [54]. Wild wolves had higher IgE levels when compared to domestic dogs [55]. Splenic transcript levels of IgE of wild platypus were higher than that in domestic platypus [56]. Higher humoral (antibody) responses in wild animals might be attribute to the natural selection and multi-pathogen exposure. Our data shown the higher level of serum immunoglobulins and splenic immunoglobulins in the *gayal*, confirming that *gayal* had higher level of humoral antibody when compared to local cattle. We also observed that splenic *IgH*, *IGHG1*, *IGLL1*, *BCR*, *IgA*, and *IgM*, which involved in primary immunodeficiency pathway (ko05340), were enriched in the *gayal*, suggesting that *gayal* enhanced B cell antigen recognition and differentiation within spleen. Furthermore, correlation analysis strengthened the evidence linking the gene expression of immunoglobulins with immunoglobulins' level. Our findings revealed potential immunological mechanism of *gayal*. *Gayal* promotes the B cell differentiation and enhances the immunoglobulin gene expression, thereby raising the levels of spleen and circulating immunoglobulins. The improvement of humoral immune status, which accompanied by a higher redundancy in splenic cellular immunity, may enable *gayal* to be more adaptable to the harsh environment.

Our data could help in advancing our understanding of immunological mechanisms of *gayal* adapting the harsh environments. However, some limitations should be noted. Due to the difficult sampling conditions (remote geographical position, inconvenient transportation, and no power), blood and spleen lymphocyte subsets were not assessed by flow cytometry experiments. Thus, further systematic research is needed to complement our findings.

Conclusions

The present study provides a systemic insight into the immune mode and function of *gayal*. Our results reveal the potential immunological mode of *gayal* with high redundancy in splenic cellular immunity and enhanced splenic gene expressions of immunoglobulin and B cell differentiation, which may enable *gayal* to adapt to the harsh environments. Furthermore, the present study also provides fundamental information and a novel approach to the breeding of cattle with higher disease resistance.

Abbreviations

OM	Organic matter
CP	Crude protein
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
T-AOC	Total antioxidant capacity
CAT	Catalase
GSH-Px	Glutathioneperoxidase
SOD	Superoxide dismutase
FRKM	Fragments per kilobase of exon per million mapped reads
DEGs	Differential expression genes
HSC	Hematopoietic stem cells
DC	Dendritic cells
MHC	Major histocompatibility complex proteins
TCR	T cell receptor
BCR	B cell receptor
LCK	Src family tyrosine kinase
ZAP70	Tyrosine-protein kinase
IgH	Immunoglobulin heavy locus

Supplementary Information

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Additional file 1.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Authors' contributions

J.L. and R.F. conceived and designed the study; L.H., B.F., and Q.L. performed the research; Y.Y. and C.J. analyzed, interpreted the data and wrote the manuscript; J.L., Y.C., and C.J. gave advice during the experiments and revised the manuscript.

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Data availability

The raw sequence data reported in this paper is available in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA020464) at https:// bigd.big.ac.cn/gsa/browse/CRA020464.

Declarations

Ethics approval and consent to participate

The protocol and details of the present study were approved by the Animal Care Committee at the Faculty of Animal Science and Technology, Yunnan Agricultural University (Kunming, P. R. China). Animal experiments were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, 2004). Sample collection comply adhered to the guidelines of the Institutional Management Committee and Laboratory Animal Ethics Committee, Yunnan Agricultural University (Kunming, P. R. China).
Human participants were not involved in this research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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