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ORIGINAL PAPER

Rice and Cassava Distillers Dried Grains in Vietnam: Nutritional Values and Effects of Their Dietary Inclusion on Blood Chemical Parameters and Immune Responses of Growing Pigs

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Abstract

This work investigated the nutritional values of rice and cassava distillers dried grains (DDG) collected in Vietnam and effects of their supplementation on several blood chemical parameters and immune responses of growing pigs. These two DDGs were determined for approximate moisture, protein, lipid, ash, starch, calcium phosphorous, and amino acids. For the effects of dietary DDGs supplementation on chemical parameters of growing pigs, 30 crossbred TOPIG pigs were randomly assigned to one of the three experimental dietary treatments (control, 7% rice DDG or 4% cassava DDG). Cassava DDG was very rich in crude fiber (32.8%) and low in protein (12.0%) while rice DDG was highly rich in protein (70.4%) and low in fiber (2.9%). At the end of the feeding trial pig performance (average daily gain, feed intake and feed/gain ratio) were under the normal limits and no effect of dietary DDG was found. By contrast, a statistically significant decreasing effect of cassava DDG diets on glucose (− 12.55%) and iron (− 23.81%) concentration and a tendency to decrease triglycerides (− 21.87%) were found. The production of IgM and IgG also increased significantly by 26.5 and 18.7% in plasma of pigs fed with rice DDG diet. DDGs diets did not influence in a significant manner the gene expression of pro- or anti-inflammatory cytokines.

Keywords Cassava · Rice · Dried distillers grains · Nutritional values · Pig feeding · Performance · Health

Introduction

Vietnam is a traditionally agricultural country in Southeast Asia, known for a variety of its agricultural products. Among them, rice and cassava are the most popular crops, playing an important role in Vietnam's agricultural structure. According to data reported by the Bureau of Statistics and the Vietnamese Ministry of Agriculture and Rural Development, rice and cassava production in Vietnam in 2015 was 45.1 and 10.7 million tons, respectively. The rate of rice and cassava production becomes increasingly important raw materials in Vietnamese agricultural export structure. Besides being used as rich in starch foods, rice and cassava are considered attractively as raw materials for ethanol production with many advantages: (i) low price; (ii) relatively stable quality with high starch-containing raw materials; (iii) "all year round" availability, including beverage alcohol and fuel bioethanol production. Indeed, the Vietnamese Ministry of Industry and Trade declared that bio-fuel production would achieve 1.8 million tons

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in 2025 accounting for 5% of country's demand. Moreover, the government also adapted policy to improve the beverage ethanol industry in Vietnam. The strategy of development of beverage ethanol production in Vietnam from 2007 to 2025 proposes that by 2025 approximately 188 million liters of beverage/food ethanol will be produced [1]. Overall, the beverage ethanol and bio-ethanol industry have a great potential in Vietnam in the future. Indeed, the policy of E5 gasoline usage has been implemented in Vietnam from January 1, 2018. Most of the rice/cassava-based ethanol plants in Vietnam have been using conventional technology for the production from raw materials including four consecutive processes, i.e. liquefaction, saccharification, fermentation and distillation.

In recent years, feed industry in Vietnam has to annually import more than 70% of ingredients, mostly corn-based dried grain distillers with solubles (DDGS), by-products from ethanol plants from Europe and America. As a consequence, the cost of feed production becomes excessively high. Therefore, by-products from food industry and available local cheap materials have been suggested as the most desirable alternative for livestock. In fact, cassava (roots, pomace, and peels) and rice (bran, broken rice) wastes are commonly used with other ingredients for pig feeding in Vietnam [2, 3]. Due to their high contents of starch and fibers, these crops and their waste are excellent sources of energy for pig [4]. For example, cassava root waste supplemented at 25% in the diet improved growth performance for pig without affecting carcass quality, and resulted in the better economic benefit [5]. There is less data on the use of cassava or rice by-products obtained as distillers dried grains with soluble (DDGS) from ethanol production processing in the diet of pigs. Because of the high fiber and low lysine level compared to other feed sources typically for pigs, DDGS has been widely fed to ruminants, but the attractiveness for using them in swine diets has been increased lately due to the increase number of new ethanol plants [6]. In goats, for instance, the inclusion of 10% cassava bioethanol waste in the diets did not alter feed intake and the digestibility coefficients of dried and organic matter, crude protein and fiber when compared with control [7]. Also, up to 30% cassava bioethanol by-products can be used to improve carcass characteristics (lean carcass) of yearling heifer cattle [8]. Similar results were obtained with rice by-products. The replacement of corn with brown rice in total mixed ration silage had no adverse effect on feed intake and milk production and reduced urinary N loss [9]. With the aim to valorize rice and cassava-originated DDG (RDDG and CDDG) from the ethanol industry in Vietnam for pig production, the evaluation of nutritional values and the effects of feeding diet containing these by-products on animal health were investigated in this work.

Materials and Methods

Sample Collection

Two wet distiller grain (WDG) samples, one from a rice-based beverage ethanol distillery (Saigon-Dongxuan Beer and Alcohol JSC) located in Phu Tho province (North Vietnam) and the other from a cassava-based bioethanol plant (BSR-BF) located in Quang Ngai province (Center of Vietnam) were collected and transported immediately after production directly to Hanoi University of Science and Technology located in Hanoi. These WDG samples were dried at 90 °C for 30 min, then 80 °C for 2.5–3.0 h and finally 70 °C for 1.0 h in a circulating dryer, resulted in rice dried distiller grains (RDDG) and cassava dried distiller grains (CDDG), respectively. RDDG and CDDG samples were packed in a plastic bag and stored at room temperature in dry place for further analysis.

Analytical Methods

RDDG and CDDG (sample weights ranging from 1.0 to 5.0 g) were analyzed for moisture (AOAC 927.05), protein (ISO 05983-1:2005), crude fibers [10], fats (ISO 6492:1999), ash (AOAC 930.30), calcium (ISO 06490:1985), phosphorous (ISO 6491:1998), starch was determined by the method described by Le-Thanh et al. [11].

Gross Energy

Gross energy was measured by Bomb calorimeter E2K system. Digestible and metabolizable energy (DE and ME) values were calculated using the following formulas [12].

$$\text{DE kcal/kg} = 4151 - (122 \times \%\ \text{Ash}) + (23 \times \%\ \text{Crude Protein}) + (38 \times \%\ \text{Fat}) - (64 \times \%\ \text{Crude Fiber})$$

$$\text{ME kcal/kg} = \text{DE} \times (1.2003 - (0.0021 \times \%\ \text{Crude Protein}))$$

Determination of Amino Acids

RDDG and CDDG (20–40 mg of sample) were hydrolyzed in vapor phase of 1 mL HCl 6M, 0.5% phenol for 24 h at 120 °C. Afterward, the hydrolyzed DDGs were re-suspended in deionized water (Merck, Germany), neutralized with NaOH to pH 7.0 and brought up to 10 mL of total volume. These solutions were filtered through 0.45 µm membrane before applying for HPLC analysis.

The HPLC analysis of amino acid were performed with Agilent 1200 series (Germany) with DAD detector. Amino acid samples were derivatized with OPA reagents (Sigma, Switzerland) in auto-sampler before being injected for separation in C18 Elipse Zorbax column 5 µm, 4, 6 × 150 mm

(Agilent, US). The gradient elution was performed with buffer A (sodium phosphate 40 mM, pH 7.8) and buffer B (methanol: acetonitrile: deionized water = 45:45:10). The buffer A was changed during elution as following 100% (0–1.9 min); to 50% (1.9–15.5 min), to 43% (15.5–21 min), to 0 (21–22 min), 0% (22–26 min); to 100% (26–27 min); 100% (27–31 min). The analysis time for each sample was 31 min at flow rate of 1 min/mL. The column was maintained at 30 °C for separation.

Animals and Sampling

A total of 30 cross bred TOPIG pigs with an initial body weight of 13.15 ± 0.15 kg were used in this study for a period of 5 weeks. Animals housed in floored indoor pens were divided into three groups and fed one of the three experimental diets: control, 7% rice dried distiller grains (treatment 1-RDDG) or 4% cassava distiller grains (treatment 2- CDDG) for approximately 35 days (Table 2). The rate (%) of dietary DDGs inclusion was in accordance with their protein and fiber content. Thus, the high protein content of RDDG (DDG1) allowed the total replacement of corn gluten (59% protein) and a part of soybean meal (44% PB) from control diet. The percentage of 7% RDDG balanced both the energy and protein level as well as the essential amino acids of treatment 1. CDDG (treatment 2) has a high fiber level and protein of low biological value (reflected by the concentration of lysine and met + Cys). This did not allow an inclusion level higher than 4% taking into account the age and nutritional requirements of the pigs used in our experiment. All diets were supplemented with 1% vitamin-mineral premix including: 10,000 IU vitamin A; 2000 IU vitamin D; 30 IU vitamin E; 2 mg vitamin K; 1.96 mg vitamin B1; 3.84 mg vitamin B2; 14.85 mg pantothenic acid; 19.2 mg nicotinic acid; 2.94 mg vitamin B6; 0.98 mg folic acid; 0.03 mg vitamin B12; 0.06 biotin; 24.5 mg vitamin C; 40.3 mg Mn; 100 mg Fe; 100 mg Cu; 100 mg Zn; 0.38 I; 0.23 mg Se.

Pigs were given ad libitum access to water and feed and were weighed individually at the beginning and at the end of the trial. Feed consumption was recorded daily per pen. Average daily gain (ADG), average daily feed intake (ADFI), and gain-feed ratio (G:F) were calculated. At the end of the trial (day 35), blood samples from each pig (10/group) were aseptically collected into 9-mL Vacutainer tubes containing 14.3 U/mL of lithium heparin (Vacutest®, Arzergrande, Italy), centrifuged (3500×*g*) for 25 min to obtain plasma, which was stored at − 20 °C until analysis for immunoglobulin concentration, cytokines and biochemistry parameters. Animals were observed twice daily and cared for in accordance with the Romanian Law 43/2014 for handling and protection of animals used for experimental purposesand the EU Council Directive 98/58/EC concerning the protection of farmed animals. The study protocol was approved by the Ethical Committee of the National Research-Development Institute for Animal Nutrition and Biology, Balotesti, Romania (Ethical Committee No. 52/2014).

Plasma Biochemical and Immunoglobulin Parameters Measurement

Biochemical parameters (glucose, cholesterol, triglycerides, Ca, Mg, total protein, albumin, bilirubin, urea and the activity of alkaline phosphatase-ALKP, glutamate pyruvate transaminase-TGP and glutamate oxaloacetate transaminase-TGO) from plasma of blood collected at the end of the experiment were determined on a clinical BS-130 Chemistry autoanalyzer (Bio-Medical Electronics Co., LTD, China). Total concentration of immunoglobulin (Ig) subsets (A, M, G) was measured in diluted plasma by ELISA (Bethyl, Medist, Montgomery, TX, USA) as described by [13].

Detection of Pro-Inflammatory Genes Expression

The expression of pro- and anti-inflammatory cytokines (TNF-α IL-1β, IL-8, IL-6, IFNγ, IL-4, IL-10) was measured on whole blood cells after in vitro incubation as already described [14]. Briefly, blood was diluted 10 fold in RPMI 1640 containing 2 mM L-glutamine, 1 mM pyruvate, 100 U/mL penicillin and 0.1 mg/mL streptomycin (Sigma, St Louis, MO, USA). After incubation for 24 h with 5% CO_2 at 37 °C, blood samples were centrifuged and cell pellets were resuspended in 1 mL Trizol (Sigma, St Louis, MO, USA) then frozen at − 80 °C until analysis. Total RNAs were extracted by using Qiagen RNeasy midi kit (QIAGEN GmbH, Germany), according to the manufacturer's recommendations and cDNA synthesis was prepared as reported by Pistol et al. [15]. Quantitative real-time PCR was used to evaluate the expression of cytokine mRNA as described by Pistol et al. [15]. The relative product levels were quantified using the $2^{(-\Delta\Delta CT)}$ method [16]. The expression levels of five endogenous control genes, Cyclophilin A (CYPA), β2-microglobulin (β2m), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin (ACTB) and Ribosomal Protein L32 (RPL32) were determined and the best combination (CYPA and RPL32) in terms of gene stability identified by using http://www.gnome.org/gnome-3 were used for data normalisation. The results were expressed as fold change (Fc) after normalisation of target gene expression to the average level of expression of selected reference genes (CYPA and RPL32).

Statistical Analysis

Results were expressed as mean ± standard error. ANOVA *t* test analysis was performed to determine the statistical differences between groups for all parameters analysed. Further differences between means were determined by the least square difference Fisher procedure by using Stat-View, SAS Inst., Inc., Cary, NC. Values of $p < 0.05$ were considered significant.

Results

Nutritional Values of Rice and Cassava Dried Distillers Grains

RDDG and CDDG were analyzed for moisture, protein (amino acid profile), crude fiber, fat, ash, calcium, phosphorous and starch. Results were presented in Tables 1 and 2. It was observed that the protein content of RDDG was 70.4% being in a great extent higher (six times higher) than that of CDDG (12.0%). The individual amino acid content in RDDG was also higher compared to that of CDDG, especially for essential amino acids such as lysine (four times higher) or valine and methionine (mostly six times higher) (Table 2). The nutritional composition of diet included RDDG and CDDG is presented in Table 3. The diets were isocaloric and isoproteic.

Effect of Cassava and Rice DDG in Animals

Pigs fed with DDG1 or DDG2 diets for a period of 5 weeks appeared clinically normal during the whole experiment period; at the end of the feeding period neither average

Table 1 Nutritional values of rice and cassava dried distillers grains

Composition	RDDG	CDDG
Crude protein (% dry matter)	70.4 ± 2.4	12.0 ± 0.7
Starch (% dry matter)	10.1 ± 1.2	25.4 ± 2.2
Crude fibers (% dry matter)	2.9 ± 0.2	32.8 ± 2.1
Ash (% dry matter)	2.9 ± 0.2	8.3 ± 0.3
Fats (% dry matter)	9.7 ± 0.7	2.3 ± 0.1
Calcium (% dry matter)	0.03 ± 0.01	0.50 ± 0.08
Phosphorus (% dry matter)	0.37 ± 0.03	0.19 ± 0.03
DE (kcal/kg)	5791 ± 231	1408 ± 87
ME (kcal/kg)	4952 ± 175	1377 ± 61

RDDG rice dried distiller grains, *CDDG* cassava dried distillers grains

Table 2 Composition of amino acids in rice and cassava dried distillers grains

Amino acids (% dry matter)	RDDG	CDDG
HIS	1.58 ± 0.08	0.25 ± 0.02
ARG	3.37 ± 0.16	1.19 ± 0.04
THR	2.09 ± 0.06	0.45 ± 0.03
VAL + MET	2.45 ± 0.11	0.42 ± 0.02
PHE	3.29 ± 0.16	0.56 ± 0.03
ISOLEU	2.19 ± 0.05	0.51 ± 0.03
LEU	4.46 ± 0.22	0.90 ± 0.04
LYS	3.60 ± 0.18	0.88 ± 0.03
GLY	1.44 ± 0.06	0.09 ± 0.01
ASP	8.16 ± 0.41	2.42 ± 0.50
GLU	8.53 ± 0.43	1.41 ± 0.05
SER	2.38 ± 0.12	0.55 ± 0.02
ALA	3.28 ± 0.16	1.04 ± 0.06
TYR	2.55 ± 0.12	0.14 ± 0.03
CYS	2.00 ± 0.09	0.20 ± 0.03

RDDG rice dried distiller grains, *CDDG* cassava dried distillers grains

Table 3 Nutritional composition of control and experimental diets for growing pigs

Items	Control diet	RDDG diet	CDDG diet
Ingredients (%)			
Corn	48.97	51.44	34.52
Wheat	20.00	20.00	30.50
Soybean meal	14.52	10.00	13.58
Sunflower meal	4.00	4.00	6.00
DDG	–	7.00	4.00
Milk powder	3.00	3.00	3.00
Gluten	5.00	–	3.00
Monocalcium phosphate	1.25	1.35	1.20
Limestone	1.55	1.55	1.49
NaCl	0.20	0.20	0.20
Methionine (99%)	0.07	0.07	0.07
L-lysine-HCl (80%)	0.34	0.36	0.34
Choline premix	0.10	0.10	0.10
Mineral vitamin-premix[a]	1.00	1.00	1.00
Analyzed nutrient content			
Crude protein (%)	19.68	18.96	18.91
Lipids (%)	1.66	2.31	1.70
Crude fiber (%)	4.25	4.80	5.50
ME (kcal/kg)	3173	3137	3188
Lysine (%)	1.05	1.05	1.05
Digestible lysine (%)	0.90	0.90	0.90
Met + Cys (%)	0.68	0.68	0.68
Calcium (%)	0.90	0.90	0.90
Phosphorus (%)	0.65	0.65	0.65

[a]Mineral-vitamin premix (1%) supplied per kg diet

daily gain nor feed intake or feed to gain ratio was influenced by dietary treatments (data not shown).

Effect of DDGs Diet on Plasma Biochemical Parameters

Plasma biochemistry parameters determined as health indicators showed a statistically significant decreasing effect of cassava DDG diets on glucose (90.56 mg/dL vs. 103.56 mg/dL, $p = 0.04$, − 12.55%) and iron (107.86 µg/dL vs. 141.55 µg/dL, $p = 0.036$, − 23.81%) concentration and a tendency to decrease triglycerides (56.19 mg/dL vs. 71.92 mg/dL, $p = 0.116$, − 21.87%) was found (Fig. 1a, b). The other plasma biochemistry parameters were not affected by this by-product. Also, no effect of RDDG diet on aforementioned biochemical parameters was observed.

Effect of DDGs Diet on Total Plasma Immunoglobulin (IgG, IgA, IgM) Concentration

Figure 1 showed that the inclusion of DDGs in pig diet exerted differentially effect on non-specific immunoglobulin subsets concentration measured as humoral immune response indicators. The production of IgM and IgG increased significantly by 26.5 and 18.7% in plasma of pigs fed with RDDG diet ($p < 0.05$) and had tendency to increase also in pigs fed with CDDG diet (+ 16.3 and 9.7% respectively) without significant difference (Fig. 2).

Effect of DDGs Diet on Cytokines

The ability of the two different by-product diets to modulate cytokine gene expression was investigated by qPCR in the whole blood cells. DDGs diets did not influence in a significant manner the gene expression of pro- or anti-inflammatory cytokines. However, a tendency in decreasing IL-1β mRNA expression was produced by the DDG rice diet and in increasing in that of IFN-γ mRNA for both RDDG and CDDG diets was found (Table 4). CDDG diet tended also to higher the IL-8mRNA.

Discussion

Nutritional Potentials of Test DDGs for Feeding

In fact, there was very little information on nutrient composition of rice or cassava—spent grain from distilleries in contrary with the available data about corn-based spent grain. This study might be the first report in nutritional composition of DDG from rice and cassava based distillery. Data in Table 1 demonstrated that RDDG contained significantly higher protein than CDDG. That could be explained by the fact that the raw material and ethanol making technology for alcohol production of two distilleries were different. BSR-BF used cassava, which contained 1–3% protein [17] or 1–5% [18] mixed with cassava pulp for alcohol production, while the other used rice, which contained 7.4–15.4% protein [19]. The higher in protein content of RDDG could indicate that the fermentation as well as alcohol processing period did not

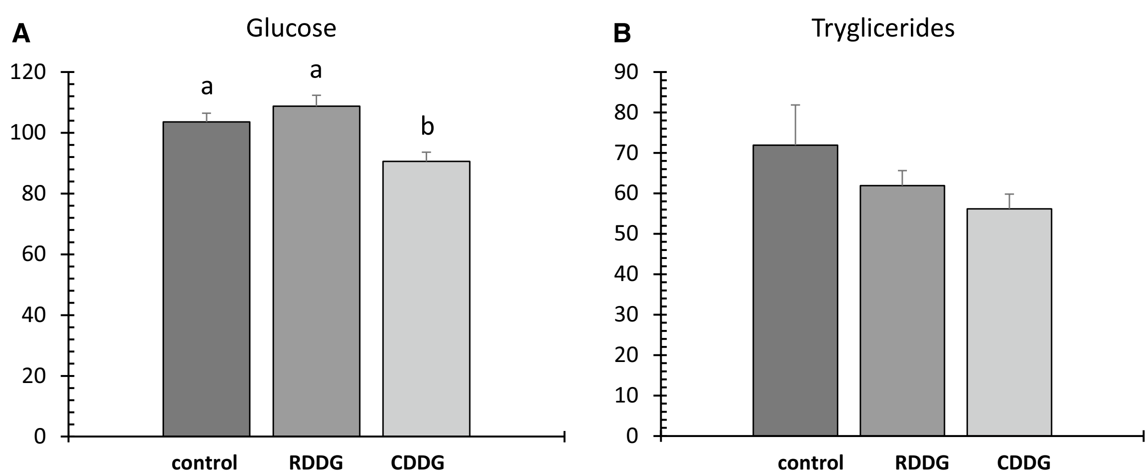

Fig. 1 Effect of dietary DDGs on plasma biochemistry parameters (glucose and tryglicerides). At the end of the experiment plasma from 10 pigs/group was prepared to measure the plasma biochemical parameters using a BS-130 Chemistry autoanalyzer (Bio-Medical Electronics Co., LTD, China). All values are represented as mean with their standard errors. ANOVA (one-way) followed by Fisher's tests were performed to analyze the effect of treatment on biochemical parameters [*$p < 0.05$, diet-control (dark shaded bar) versus RDDG diet—(shaded bar), and CDDG diet-(light shaded bar)]. **a** and **b** Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$)

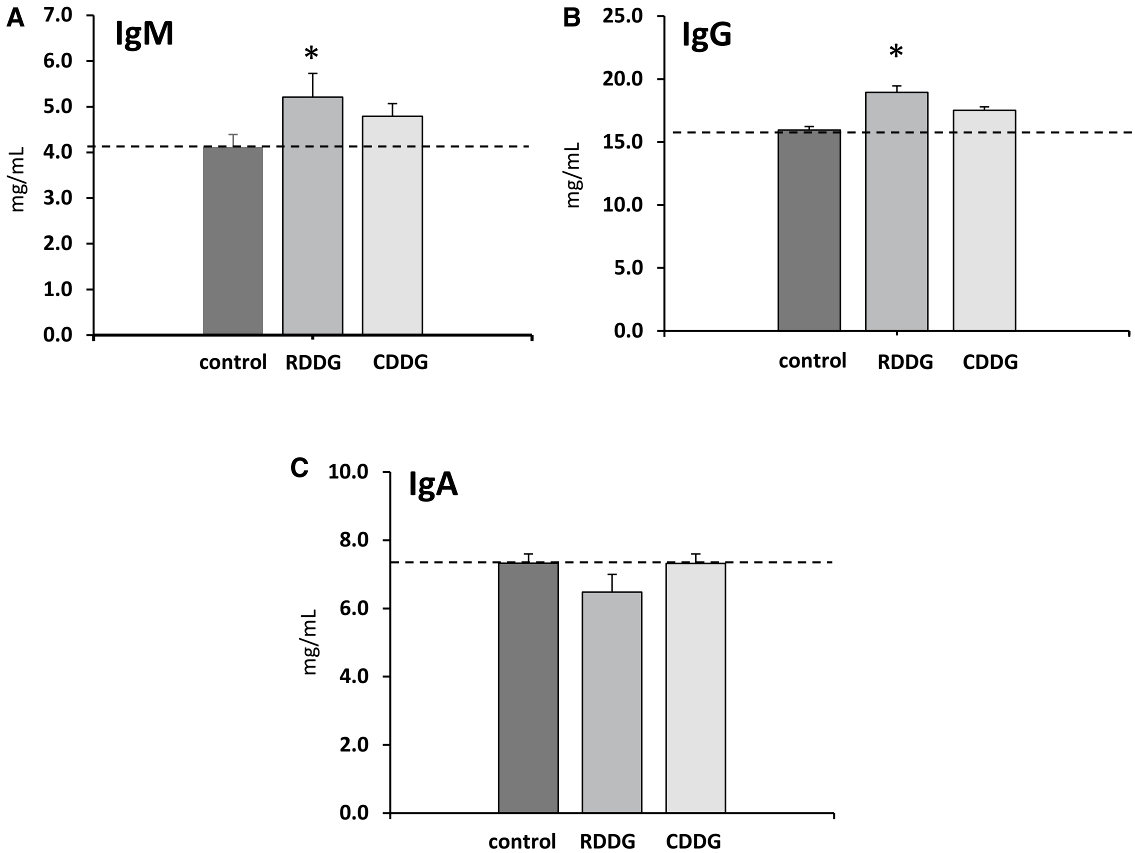

Fig. 2 Effect of dietary DDGs on immunoglobulin production in blood plasma. At the end of the experiment, plasma from 10 pigs/group was prepared and the total concentration of Ig subsets was determined by ELISA after plasma dilution: 1: 10,000 (IgA), 1:10,000 (IgM) and 1: 120,000 (IgG). Ig absorbance was measured at 450 nm using a microplate reader (Tecan Infinite 200Pro, Austria). All values are represented as mean with their standard errors. Statistical analysis was performed using one-way ANOVA followed by Ficher test [*$p < 0.05$, diet-control (dark shaded bar) versus RDDG diet—shaded bar), and CDDG diet-(light shaded bar)]

Table 4 Effects of rice and cassava dried distiller grains diets on pro-and anti-inflammatory cytokine gene expression in whole blood cells

Cytokines	Diets					
Fold change (Fc)	Control		RDDG		CDDG	
	Mean	SEM	Mean	SEM	Mean	SEM
IL-1β	1.00	0.0	0.60	0.1	1.01	0.1
IL-8	1.00	0.0	0.86	0.1	1.53	0.1
TNF-α	1.00	0.0	0.86	0.2	0.87	0.2
IL-6	1.00	0.0	1.04	0.2	0.79	0.1
IFN-γ	1.00	0.0	1.44	0.2	1.59	0.1
IL-4	1.00	0.0	1.04	0.1	0.90	0.1
IL-10	1.00	0.0	1.07	0.2	1.21	0.1

Pigs received two different dietary DDGs: 7% RDDG and 4% CDDG for 5 weeks. At the end of the experiment, blood samples were collected and analysed for the mRNA expression of cytokines using quantitative real-time PCR

Results were expressed as fold change (Fc) after normalisation of target gene expression to the average level of selected internal reference genes (CYP and RPL32) expression. Mean values with their standard errors of the mean (SEM), n = 10. ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of treatment on cytokine gene expression change much protein content of rice. Even though, the thin stillage was not taken in to account, the RDDG contained more protein than the corn DGGS which ranged from 26 to 32% [12, 20–23]. Yeast biomass have been known as a rich source of protein. In uncertain estimation, Liu et al. [23] shown that yeast contribute approximately 5.3% of protein in DDGS, however this is much more lower than number of protein in RDDG in this study.

As mentioned above, the CDDG was mixed with cassava pulp from starch extraction processing. This spent contains up to 50% of residue starch [24]. Therefore the starch content in CDDG was obviously higher than that in RDDG. The starch in both CDDG and RDDG in this study was higher in comparison with the starch content in corn DDGS, ca. 5% [23, 25].

Cassava is a high fiber cereal (7.4–8.5%) [26] meanwhile in rice the fiber is 0.4% [26, 27]. On the other hand, the addition of the cassava pulp resulted in the fiber of CDDG was 32.8% which 10 times higher than that of RDDG (Table 1) and almost 3–4 time higher than crude fiber of corn DDGS (7–10%) [12, 25, 28].

Moreover, ash content of CDDG (8.3%) was significantly higher compared to that of RDDG (2.9%) (Table 1). It could be explained that cassava root itself contained 1.5–2.7% of ash, whereas the ash in rice was about 0.6% [27]; and the cassava roots may get impurities coming from harvesting, transporting and storage. According to different authors, the ash varied from 3.9 to 6.3% of dry matter in corn DDGS [12, 20–22, 25, 28].

It is well known that maize contains more lipid (3.72%) than rice (1–2%) and cassava (0.5–1.0%) [29–31]. This might be an explanation for the observation in this work that the fat in RDDG and CDDG were lower than in the corn DDGS reported in range from 10.2 to 14.5% [12, 22, 23, 25]. Again, the addition of cassava pulp might result in a lower fat content of CDDG than in RDDG, even though the fat in the two raw materials is comparable.

Calcium and phosphorus content in DDG were also considered in this work because of their necessity for animal feeding. It was showed in Table 1 that calcium content of RDDG was 0.03%, while that of CDDG was equal to 0.50%. It was also indicated that phosphorous of RDDG was 0.37%, higher than CDDG (0.19%). Calcium and phosphorous contents of rice ranged from 0.07 to 0.11% and 0.52–0.54% [32] of cassava mosaic disease resistant varieties ranged from 700 to 900 µg/g and 900 to 1200 µg/g respectively [33]. Thus, calcium and phosphorous contents varied depending on different DDG sources and production technology. In addition, in the production process manufacturers may add some micronutrients including phosphorous for the development of yeast cells. Besides, phosphorous digestibility could be improved by the addition of microbial phytase to diets fed to pigs [34]. In corn DDGS, the calcium content also varied in wide range from 0.03 to 0.3%, meanwhile, phosphorus was reported rather close to 0.7–0.9% [12, 21–23, 35].

Gross energy of CDDG (2479 kcal/kg) was lower than that of RDDG (5791 kcal/kg). Moreover, digestible and metabolizable energy of RDDG was 3–4 times higher than that of CDDG. It could be due to the higher in protein and lipid content of RDDG. Based on the chemical composition of a large number of samples of corn DDGS, values of DE and ME in corn DDGS were found to be of approximately 3990 and 3750 kcal/kg dry matter respectively [12]. The average concentration of gross energy of DDGS from corn was 5434 kcal/kg dry matter [36].

In agreement to the protein composition, the amino acid was lower in CDDG than in RDDG. Most of essential amino acids with significant amount were determined in RDDG. Each of amino acid such as leucine, phenyl alanine, arginine, lysine contributed higher than 3% of dry matter in the RDDG (Table 2). However, these amino acids amounted much lower in CDDG. The DDG samples contained also most of non-essential amino acids. Among aspartic acid and glutamic acid were found as the highest amino acids in both RDDG and CDDG.

Lysine, an important amino acid for growth of swine and poutry, is considered as the first limiting in cereals such as maize, cassava and even in some varieties of rice. The rather high level of lysine content in RDDG found in this study (approximately 3.6% of dry matter) suggested that the RDDG is highly potential for animal feeding, together with the balance of the profile of amino acids in the RDDG between essential and non-essenstial amino acid.

Animal Response to DDG Diets

In this study, no daily gain nor feed intake or feed to gain ratio change in animals fed with both RDDG and CDDG diets in comparison with control was observed. However, in the literature, data showed that pig performance was affected by change in fiber diets. In a study conducted in Vietnam by Ngoc et al. [3] to investigate the effect of fiber intake and fiber source on growth performance, digestibility and gastrointestinal tract development of local (Mong Cai) and exotic (Landrance X Yorkshire, LY) breed pigs. It was shown that the increased level of dietary fiber reduced the average daily gain in both breed. Nevertheless, the local Mong Cai breed has been better adapted to digest fibers by larger gastrointestinal tract and longer retention time of digesta. In another experiments it was reported that including cassava pomace (up to 30%) or cassava dried by-products in pig diets from the starter to finishing period gave acceptable performance [37, 38]. Weaned pigs fed diets with increasing inclusion of up to 200 g wheat DDGS/kg and supplemental amino acids to replace soybean meal had reduced gain to feed ratio due to reduced average daily gain, however, feed intake was maintained [39].

The decrease in plasma glucose level of pigs fed diet with cassava based DDG (Fig. 1a, b), was in accordance with previous studies on animal laboratory and human which have been demonstrated the hypoglicemic and antidiabetic effect of cassava [40]. This decreasing effect might be due to the higher content of fibers and composition of these fibers from CDDG diet. It is known that fibers slow down the carbohydrates absorption through their structural network glucose capture, regulating glycaemia and diminishing the insulin secretion [41]. The mechanism has not been elucidated, but the fibers capacity to delay the nutrients absorption has a certain influence on glucidic metabolism [42]. Nutrition studies reported that fibers are also responsible for the reduction of dietary fat absorption, being able to decrease the level of blood lipids [43]. Similarly, diets containing 30% cassava, acha, maize and plantain reduced the increased lipids level in plasma of obese rats in an animal model of obesity study aiming to investigate the influence of some fiber-enriched diets on tissue lipids [44]. The hypocholestolaemic effect of fibers depends on fiber solubility and rate of dietary inclusion. It seems that the fibers solubility and viscosity in solution lead to the steroid excretion and alteration of lipid absorption. That is why the insoluble fibers are not efficacies in reducing the plasma lipid level [45].

The production of IgM increased significantly by 26.5% in plasma of pigs fed with RDDG diet ($p < 0.05$) and had tendency ($p < 0.08$) to increase also in pigs fed with CDDG diet (+ 16.3%). A tendency of increasing was observed as well for IgG concentration for both RDDG and CDDG diets (18.7 and 9.7% respectively) (Fig. 2).

Similarly, previous studies reported the efficacy of rice to enhance the humoral immunity. Thus, Sadeghi et al. [46] showed that diet supplemented with an equal combination (30 g/kg) of sugar beet pulp and rice hull improved humoral immunity by significant increase of antibody titer against Newcastle disease virus in broiler [46]. Also, Oryzanol, an important bioactive component of rice bran oil, evoked a significant increase in antibody titre values in the haemagglutination test and potentiated the delayed type hypersensitivity reaction induced by sheep red blood cells in an experimental rat model [47]. By contrast, Karasawa et al. [48] demonstrated that a soybean protein fraction digested with peptidase R produced by *Rhizopus oryzae* decreased serum total IgG and intestinal IgA level in mice together with the expression of genes related to immunoglobulin production [48]. The enhanced humoral immunity is also associated with the high concentration in essential amino acids in RDDG diet, while it was shown that dietary deficiency of these amino acids impairs animal immunity and elevate animal susceptibility to infectious diseases [49]. No effect on IgA level was found for both DDGs diets.

Interleukin − 8 is a key chemokines known as neutrophil chemotactic factor which induce cells migration toward the site of infection and the study of Milo et al. [50] which tested a baby diet formula supplemented with soya polysaccharides fibers in *Salmonella* Typhimurium infected pigs demonstrated that neutrophils migration was higher than in control pigs [50]. Phytochemical studies indicated that beside fibers cassava contains several bioactive compounds such as flavonoids which are traditionally used for their effect against inflammation and pain [51] by decreasing inflammatory mediators (e.g., TNF-α, IL-1β and NO). In the current study, the modulatory effect either of RDDG or CDDG on cellular immune response was insignificant compared to control; no reduction in pro-inflammatory or increase in anti-inflammatory cytokines was occurred.

Conclusion

In this work, the proximate and nutritional composition of two typical DDG obtained from a rice-based beverage ethanol distillery (RDDG) and a cassava-based bioethanol plant (CDDG) in Vietnam were determined. RDDG was characterized by a very high content of protein (70.4%) and low content of fiber (2.9%) and on the contrary CDDG was characterized by high content of fiber (32.8%) and low content of protein (12.0%). The amino acids of RDDG were significantly higher than that of CDDG. The findings from this study indicated that both RDDG and CDDG possessed sufficient potential for modulating mainly the humoral immune activity in pigs by significantly increasing the immunoglobulins production.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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