Effects of Diets with Different Ratios of Whole-Plant Corn Silage on Production Performance, Nutrient Utilization, Hematological Parameters, and Serum Oxidative Stress Markers in Growing Pigs: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of diets containing different proportions of whole-plant corn silage on production performance, nutrient utilization, hematological indices, and serum oxidative stress indices in growing pigs. Thirty-two growing pigs (Duroc × Landrace × Large White) with similar parity and body weight [(33.16±3.49) kg] were selected and randomly allocated into 4 groups with 8 replicates per group. The control group was fed a basal diet with nutrient levels supplied according to the feeding standards for lean-type pigs in NY/T 65-2004, while the experimental groups were fed experimental diets containing 20%, 30%, and 40% whole-plant corn silage, respectively. The nutrient contents (on a dry matter basis) of these four diets were not significantly different ($P>0.05$), with crude fiber contents of 3.0%, 3.5%, 4.0%, and 4.5%, respectively. The experimental pigs were individually housed in metabolism cages, with a 7-day preliminary period and a 42-day formal experimental period. The results showed that with increasing dietary whole-plant corn silage proportion, final body weight, average daily gain, apparent digestibility of nutrients (dry matter, organic matter, crude protein, crude fat, and gross energy), protein biological value, net protein utilization, gross energy metabolic rate, hemoglobin concentration, and serum ceruloplasmin content in growing pigs decreased linearly ($P<0.05$), whereas average daily feed intake on an as-fed basis, average daily feed intake on a dry matter basis, average daily crude fiber intake, feed-to-gain ratio on an as-fed basis, and feed-to-gain ratio on a dry matter basis increased linearly ($P<0.05$). Compared with the control group, the 20% and 30% whole-plant corn silage groups showed no significant differences in average daily gain, average daily feed intake on a dry matter basis, feed-to-gain ratio

on a dry matter basis, protein biological value, net protein utilization, hematological indices, and serum oxidative stress indices (P>0.05); however, the 40% whole-plant corn silage group exhibited significantly reduced final body weight, average daily gain, nutrient apparent digestibility, blood hemoglobin concentration, and serum ceruloplasmin content (P<0.05). It was concluded that whole-plant corn silage can be used in the formulation of low-nutrient-level diets for growing pigs, and inclusion levels of 20%–30% can maintain production performance and improve health status.

Full Text

Effects of Diets with Different Proportions of Whole Plant Corn Silage on Growth Performance, Nutrient Utilization, Hematological Indexes and Serum Oxidative Stress Indexes of Growing Pigs

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Abstract: This experiment was conducted to investigate the effects of diets with different proportions of whole plant corn silage on growth performance, nutrient utilization, hematological indexes, and serum oxidative stress indexes in growing pigs. Thirty-two growing pigs (Duroc × Landrace × Large White) with similar parity and initial body weight [(33.16±3.49) kg] were randomly allocated into 4 groups with 8 replicates per group. The control group was fed a basal diet with nutrient levels formulated according to the NY/T 65-2004 standard for lean-type pigs. The three experimental groups were fed test diets containing 20%, 30%, and 40% whole plant corn silage, respectively. The nutrient contents (on a dry matter basis) of these four diets were not significantly different (P>0.05), while the crude fiber contents were 3.0%, 3.5%, 4.0%, and 4.5%, respectively. All experimental pigs were housed individually in metabolic cages. The experiment consisted of a 7-day preliminary period followed by a 42-day formal experimental period. The results showed that as the proportion of whole plant corn silage in the diet increased, the final body weight, average daily gain, apparent digestibility of nutrients (dry matter, organic matter, crude protein, ether extract, and gross energy), biological value of protein, net protein utilization, gross energy metabolizable rate, hemoglobin concentration, and serum ceruloplasmin content of growing pigs decreased linearly (P<0.05). Conversely, feed intake on an as-fed basis, dry matter intake, average daily crude fiber intake, and feed-to-gain ratio (both as-fed and dry matter basis)

increased linearly (P<0.05). Compared with the control group, the 20% and 30% whole plant corn silage groups showed no significant differences in average daily gain, dry matter intake, dry matter feed-to-gain ratio, protein biological value, net protein utilization, hematological indexes, or serum oxidative stress indexes (P>0.05). However, the 40% whole plant corn silage group exhibited significantly reduced final body weight, average daily gain, nutrient apparent digestibility, blood hemoglobin concentration, and serum ceruloplasmin content (P<0.05). It is concluded that whole plant corn silage can be used in formulating low-nutrient-level diets for growing pigs, and inclusion levels of 20%–30% can maintain production performance while improving health status.

Keywords: whole plant corn silage; crude fiber; growing pigs; performance; nutrient digestion and metabolism; hematological indexes; serum oxidative stress indexes

Introduction

Feeding appropriate proportions of roughage is fundamental for maintaining microbial digestive function in pigs. Green and succulent feeds can improve palatability and nutrient utilization in swine. However, the shortage of green succulent feeds and high-fiber ingredients makes it difficult to implement low-nutrient-level diets in China' s pig industry. Although corn stalks after grain harvest contain abundant crude fiber, their fiber structure is very compact and difficult for monogastric animals to digest. Research has shown that fermenting whole corn plants at the milk stage reduces crude fiber content and increases nutrient utilization efficiency [1]. Dietary fiber is a complex compound composed of polysaccharide carbohydrates and various non-carbohydrate components. Fiber components in diets generally cannot be digested by animal digestive enzymes but can be utilized by intestinal microorganisms to produce volatile fatty acids [2]. As typical monogastric omnivores, pigs possess well-developed colons and ceca harboring abundant microorganisms capable of utilizing dietary fiber [3]. Studies have confirmed that green succulent feeds and appropriate amounts of quality fiber can improve pig performance, enhance health, and maintain gastrointestinal microecological balance [4], while also regulating intestinal nutrient absorption and strengthening immune function [5-6]. However, Lü et al. [7] demonstrated that high-fiber diets negatively affect energy and nutrient utilization in growing pigs, increasing fecal nitrogen excretion and impacting nitrogen metabolism. As dietary fiber levels increase, dietary energy density decreases, and animals may increase digestive organ weight to expand nutrient absorption area to meet growth requirements [8]. Previous studies on dietary fiber levels in pigs have primarily focused on performance and carcass traits, with fiber sources mainly being processing by-products such as beet pulp, distillers' grains, and wheat bran. Against the backdrop of China' s national "Grain to Forage" policy, whether whole plant corn can utilize its succulent and high-fiber characteristics to improve growth performance and health status in growing pigs has not been reported. Therefore, this study investigated the effects of diets with different

proportions of fermented whole plant corn silage on growth performance, nutrient utilization, hematological indexes, and serum oxidative stress indexes in growing pigs, aiming to provide a theoretical basis for implementing the national "Grain to Forage" policy.

1.1.1 Fermentation Strains

The compound bacteria for whole plant corn silage fermentation were provided by Beijing Keweibo Biological Technology Co., Ltd., containing lactic acid bacteria (*Lactobacillus*) at 5.0×10 CFU/g, yeast at 4.6×10 CFU/g, and *Bacillus subtilis* at 1.9×10¹ CFU/g.

1.1.2 Preparation of Whole Plant Corn Silage

Corn was harvested at the milk stage with a stubble height of approximately 30 cm, chopped to 0.5–1.0 cm, mixed with 0.8 kg/t of compound bacteria, and quickly packed into 1,000 kg plastic-lined nylon bags. The material was compacted, sealed under anaerobic conditions, and stored for more than 4 weeks until pH stabilized at 3.8–4.0. The whole plant corn silage contained (on a fresh sample basis): digestible energy 1.83 MJ/kg, crude protein 2.27%, crude fiber 3.33%, lysine 0.04%, methionine 0.02%, threonine 0.04%, tryptophan 0.01%, calcium 0.01%, phosphorus 0.03%, and dry matter 22.20%. On a dry matter basis, it contained: digestible energy 6.54 MJ/kg, crude protein 8.12%, crude fiber 11.88%, lysine 0.13%, methionine 0.08%, threonine 0.15%, tryptophan 0.02%, calcium 0.02%, and phosphorus 0.11%.

1.2 Experimental Design and Management

A single-factor experimental design was employed. Thirty-two growing pigs (Duroc × Landrace × Large White) with similar parity and body weight [(33.16±3.49) kg] were randomly divided into 4 groups with 8 replicates per group (one pig per replicate). The control group was fed a basal diet without fermented whole plant corn silage, with nutrient levels formulated according to the NY/T 65-2004 standard for lean-type pigs, containing 3.0% crude fiber on a dry matter basis. The experimental groups were fed test diets containing 20%, 30%, and 40% whole plant corn silage, with nutrient levels approximately 98%, 96%, and 94% of the control group on a dry matter basis, respectively, and crude fiber contents of 3.5%, 4.0%, and 4.5% on a dry matter basis, respectively. Diet composition and nutrient levels are shown in Table 1 . Experimental pigs were housed individually in cages (100 cm × 200 cm) equipped with feeders and automatic drinkers, with free access to feed and water. Diets were prepared in one batch, with whole plant corn silage mixed with concentrate, sealed in woven bags, and fed as wet mash. The experiment included a 7-day preliminary period and a 42-day formal experimental period, during which daily feed intake was recorded and body weight was measured at the beginning and end. Pig behavior, health status, and diarrhea incidence were monitored throughout the trial.

1.3.1 Performance Measurements

Based on recorded feed intake and body weight data, average daily gain (ADG), average daily feed intake on an as-fed basis (FB-ADFI), average daily feed intake on a dry matter basis (DM-ADFI), average daily crude fiber intake (ADCFI), feed-to-gain ratio on an as-fed basis (FB-F/G), and feed-to-gain ratio on a dry matter basis (DM-F/G) were calculated.

1.3.2 Digestion and Metabolism Trial and Measurements

During days 21–27 of the formal experimental period, all pigs were subjected to a total feces and urine collection trial. Daily feed intake was recorded accurately, and all feces and urine were collected, measured, and stored at 4°C. At the end of the trial, feces and urine from the 7 consecutive days were each mixed thoroughly. One-fifth of the total feces and urine samples were acidified (10 mL of 10% sulfuric acid added per 100 g fresh feces or 100 mL urine) for nitrogen fixation to determine crude protein content on a fresh basis. Another two-fifths of the urine sample was used to determine gross energy in urine, while the remaining two-fifths of fresh feces was dried at 65°C to prepare air-dried samples for determination of dry matter, organic matter, crude protein, ether extract, crude fiber, calcium, phosphorus, and gross energy (GE) content.

Nutrient contents in diets and feces were determined according to AOAC (2012) [9] methods: dry matter by oven drying at 105°C to constant weight, organic matter by ashing at 550°C, crude protein by Kjeldahl semi-micro method, ether extract by Soxhlet extraction, crude fiber by acid-alkali digestion, calcium by potassium permanganate titration, and phosphorus by colorimetry. Gross energy in diets, feces, and urine was determined using an oxygen bomb calorimeter.

Nutrient digestion and utilization indexes were calculated according to reference [10] as follows:

- Digestible energy intake (MJ/d) = Gross energy intake (MJ/d) -Fecal energy (MJ/d)
- Apparent digestibility of gross energy (%) = 100 × Digestible energy intake / Gross energy intake
- Apparent digestibility of a nutrient (dry matter, organic matter, crude protein, ether extract) (%) = 100 × (Nutrient intake -Fecal nutrient excretion) / Nutrient intake
- Digestible nitrogen (g/d) = Nitrogen intake -Fecal nitrogen
- Biological value (BV) of protein = 100 × (Nitrogen intake -Fecal nitrogen -Urinary nitrogen) / Digestible nitrogen
- Net protein utilization (NPU) = (Crude protein intake -Fecal crude protein -Urinary crude protein) / Crude protein intake × 100
- Gross energy metabolizable rate (%) = (Digestible energy intake -Urinary energy) / Gross energy intake × 100

1.3.3 Blood Sample Collection and Measurements

On day 42 of the formal experimental period, approximately 30 mL of blood was collected via jugular venipuncture from each pig after overnight fasting. About 10 mL of whole blood was used for hematological measurements, while the remaining 20 mL was centrifuged at 3,000 rpm for 10 min to separate serum for oxidative stress index determination.

Hematological indexes: Measured using a KX-21 hematology analyzer, including white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), neutrophil percentage (NEUT%), lymphocyte percentage (LY%), hemoglobin concentration (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Serum oxidative stress indexes: Total superoxide dismutase (T-SOD) and copper-zinc superoxide dismutase (CuZn-SOD) activities were determined by xanthine oxidase method (hydroxylamine method). Glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) and ceruloplasmin (CER) contents were determined by colorimetric method. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4 Statistical Analysis

Experimental data were analyzed using SAS 8.2 software. One-way ANOVA was used for significance testing, and Duncan' s multiple range test was used for inter-group comparisons. Data are expressed as mean ± standard deviation, with significance level set at $P<0.05$. Orthogonal polynomial contrast was used for linear regression analysis of treatment effects with different crude fiber levels.

Results

2.1 Comparison of Performance Indexes

Throughout the experimental period, all pigs remained healthy with no diarrhea or mortality. Performance results (Table 2) indicated that as the proportion of whole plant corn silage in the diet increased, final body weight and average daily gain of growing pigs decreased linearly ($P<0.05$), while feed intake on an as-fed basis, dry matter intake, average daily crude fiber intake, and feed-to-gain ratio (both as-fed and dry matter basis) increased linearly ($P<0.05$). Compared with the control group, the 20% and 30% whole plant corn silage groups showed no significant differences in final body weight, average daily gain, or dry matter feed-to-gain ratio ($P>0.05$). However, the 40% whole plant corn silage group exhibited significantly reduced final body weight and average daily gain ($P<0.05$) and significantly increased dry matter feed-to-gain ratio ($P<0.05$). The 20%, 30%, and 40% whole plant corn silage groups had significantly higher feed intake (as-fed basis), dry matter intake, average daily crude fiber intake, and feed-to-gain ratio (as-fed basis) than the control group ($P<0.05$).

2.2 Comparison of Nutrient Digestion and Metabolism Indexes

As shown in Table 3 , apparent digestibility of dry matter, organic matter, crude protein, ether extract, and gross energy, as well as biological value of protein, net protein utilization, and gross energy metabolizable rate in growing pigs decreased linearly with increasing whole plant corn silage proportion ($P<0.05$). Compared with the control group, the 20%, 30%, and 40% whole plant corn silage groups showed significantly reduced apparent digestibility of dry matter, organic matter, crude protein, ether extract, and gross energy, as well as gross energy metabolizable rate ($P<0.05$). The 40% whole plant corn silage group also exhibited significantly reduced protein biological value and net protein utilization ($P<0.05$), indicating that 20%-30% whole plant corn silage did not affect protein utilization efficiency.

2.3 Comparison of Hematological Indexes

Table 4 shows that hemoglobin concentration in hematological indexes decreased linearly with increasing whole plant corn silage proportion ($P<0.05$). Compared with the control group, the 40% whole plant corn silage group had significantly reduced hemoglobin concentration ($P<0.05$), while no significant differences were observed among other groups ($P>0.05$).

2.4 Comparison of Serum Oxidative Stress Indexes

As shown in Table 5 , serum ceruloplasmin content decreased linearly ($P<0.05$), while serum total superoxide dismutase activity increased linearly ($P<0.05$) with increasing whole plant corn silage proportion. The 40% whole plant corn silage group had significantly lower serum ceruloplasmin content than the control group ($P<0.05$), though no significant differences were found among the control, 20%, and 30% groups ($P>0.05$).

Discussion

3.1 Effects of Different Proportions of Whole Plant Corn Silage on Growth Performance

Research has shown that feeding green succulent feeds to pigs can improve palatability and nutrient utilization [11]. A certain proportion of roughage in the diet is essential for maintaining microbial digestive function, and dietary crude fiber content of 5%-7% does not affect performance or growth rate of growing-finishing pigs [12]. When dietary energy concentration is adequate, pigs can tolerate relatively high levels of crude fiber [13]. Dietary fiber can stimulate gastrointestinal development, maintain normal peristalsis, and accelerate digesta passage through the digestive tract, making it an indispensable component in pig diets [14]. Studies have indicated that within a certain range, as dietary crude fiber content increases and dietary digestible energy concentration decreases, pigs typically increase feed intake through physiological regulatory

mechanisms to meet their energy requirements [15-16]. In this experiment, feed intake on an as-fed basis in the 20%, 30%, and 40% whole plant corn silage groups increased by 23%, 39%, and 49% compared with the control group, respectively, while dry matter intake increased by 5%, 8%, and 5%, respectively. Therefore, although the 20%, 30%, and 40% whole plant corn silage diets replaced the basal diet at equivalent proportions and had nutrient levels of 98%, 96%, and 94% of the control group on a dry matter basis, growing pigs could adjust feed intake to meet their energy and nutrient requirements. In this study, no significant differences in final body weight and average daily gain were observed among the control, 20%, and 30% whole plant corn silage groups. Corn harvested at the milk stage has succulent characteristics. Although corn stalks contain considerable fiber, dietary crude fiber content was less than 4% on a dry matter basis and below 5% on an air-dry basis, which would not affect nutrient digestibility. Moreover, the low dietary nutrient level on a fresh sample basis due to high moisture content in fresh corn could be compensated by increased feed intake to meet pig requirements. Therefore, feeding diets containing 20%–30% whole plant corn silage can maintain normal performance in 30–60 kg growing pigs. However, when dietary crude fiber content increased to 4.5% (i.e., 40% whole plant corn silage), average daily gain and dry matter intake decreased to varying degrees, possibly because the adaptive increase in digestive tract volume could not accommodate the large volume of high-fiber diet, leading to reduced daily gain [17].

3.2 Effects of Different Proportions of Whole Plant Corn Silage on Nutrient Digestion and Metabolism

Increased dietary crude fiber content reduces digestibility of dry matter, crude protein, and ether extract, thereby decreasing energy digestibility. The present results showed that nutrient apparent digestibility, protein biological value, net protein utilization, and gross energy metabolizable rate decreased linearly with increasing whole plant corn silage proportion. Related studies have confirmed that increasing dietary crude fiber content reduces digestibility of other nutrients and digestible energy intake [18]. This may be attributed to the lack of fiber-degrading enzymes in the digestive tract, preventing digestive enzymes from breaking down nutrients encapsulated by fiber. Additionally, soluble dietary fiber increases digesta viscosity, affecting enzyme-substrate binding and thereby reducing digestibility of dry matter, crude protein, and ether extract [19-20]. Moreover, increased dietary crude fiber content enhances parasympathetic nerve excitability, accelerating intestinal peristalsis and digesta passage rate, reducing contact time between digesta and digestive enzymes, and consequently decreasing nutrient digestibility [21-22]. Wilfart et al. [23] found that high-fiber wheat bran diets reduced retention time of solid digesta in the small intestine. Another study reported that each 1% increase in dietary neutral detergent fiber content corresponded to a 1% decrease in gross energy digestibility [24]. However, in this experiment, no significant differences were observed in nutrient apparent digestibility, protein biological value, net protein utilization,

or gross energy metabolizable rate between the 20% and 30% whole plant corn silage groups. Furthermore, protein biological value and net protein utilization in the 20% and 30% groups did not differ significantly from the control group, indicating that 20%–30% whole plant corn silage did not affect protein metabolism, though the molecular mechanism by which whole plant corn silage affects energy metabolism requires further investigation.

3.3 Effects of Different Proportions of Whole Plant Corn Silage on Hematological Indexes

Blood components involved in immunity mainly include neutrophils and lymphocytes [25]. Total white blood cell count indicates animal susceptibility and microbial virulence. Red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration can be used for anemia diagnosis [26]. No previous studies have examined the effects of whole plant corn silage on hematological indexes in growing pigs. The present study found that hemoglobin concentration decreased gradually with increasing whole plant corn silage proportion, possibly due to reduced biological utilization of iron or impaired absorption of certain nutrients required for hemoglobin synthesis as whole plant corn silage proportion increased. However, the molecular mechanism requires further investigation.

3.4 Effects of Different Proportions of Whole Plant Corn Silage on Serum Oxidative Stress Indexes

When animals are under stress, antioxidant enzyme activities gradually decline, reducing the capacity to eliminate oxidative metabolites and breaking the dynamic balance between free radical production and clearance, leading to free radical accumulation and oxidative damage. Serum malondialdehyde content is the most commonly used indicator for measuring lipid peroxidation [27]. Superoxide dismutase and glutathione peroxidase in serum can strongly scavenge reactive oxygen species and free radicals [28], and higher values within the normal range indicate stronger antioxidant function [29]. Studies have shown that active substances such as vitamin C, vitamin D, and carotenoids in alfalfa meal can promote antioxidant capacity, and alfalfa leaf protein can increase serum superoxide dismutase and glutathione peroxidase activities in mice [30]. In this experiment, serum total superoxide dismutase activity increased linearly with increasing whole plant corn silage proportion, possibly due to the abundant vitamins and carotenoids in whole plant corn silage diets enhancing antioxidant capacity. Serum ceruloplasmin content decreased linearly with increasing whole plant corn silage proportion. Research indicates that after normal copper requirements are met, serum ceruloplasmin content is less affected by dietary copper and may be regulated by iron metabolism. Iron deficiency can stimulate increased ceruloplasmin synthesis to promote iron transport and maintain intracellular iron homeostasis [31-32]. However, the molecular mechanism by which whole plant corn silage regulates serum antioxidant enzyme activities requires

further investigation.

Conclusions

1. Compared with the control group, diets containing 20% and 30% whole plant corn silage did not affect growth performance, while 40% whole plant corn silage reduced growth performance in growing pigs.
2. Diets containing 20% and 30% whole plant corn silage did not affect protein metabolism or significantly impact hematological indexes and serum oxidative stress indexes. Feeding diets containing 20%–30% whole plant corn silage to growing pigs at the 30–60 kg stage is feasible.

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Note: Figure translations are in progress. See original paper for figures.