https://doi.org/10.17221/64/2023-CJAS

Effects of plant sterol microcapsules on growth performance and serum biochemical indicators in pigs

Bin Song[1], Shengwei Di[2], Yan Zhao[1], Ziwei Li[1], Junjie Jin[1], Fengxiang Hou[1], Yawen Zhang[1], Hongxi Xu[1]*

[1]College of Animal Science, Wenzhou Vocational College of Science & Technology, Wenzhou, PR China
[2]College of Animal Science and Technology, Northeast Agricultural University, Harbin, PR China

**Corresponding author: songbin_418@163.com*

Citation: Song B., Di S.W., Zhao Y., Li Z.W., Jin J.J., Hou F.X., Zhang Y.W., Xu H.X. (2023): Effects of plant sterol microcapsules on growth performance and serum biochemical indicators in pigs. Czech J. Anim. Sci., 68: 497–507.

Abstract: To better explore the effects of adding phytosterol (PS) microcapsules to feed on pig growth performance, nutrient apparent digestibility, and serum biochemical indicators, 200 healthy ternary hybrid Duroc × Landrace × Yorkshire piglets with an average initial weight of 7.53 ± 0.57 kg) were used as experimental subjects, and randomly divided into five groups with four replicates in each group. The control group of pigs was fed the basic diet, while the experimental group received diets supplemented with different PS levels. They were divided into experimental groups 1 to 4 (100 mg/kg to 5 502 mg/kg) according to different dosage added components. The duration of the experiment was 54 days. The results showed that the total triglyceride (TG) index and diarrhoea status in the experimental group were significantly improved compared to the control group ($P < 0.05$). The increase in PS addition levels was more significant in the change of pig average daily gain (ADG) ($P < 0.05$), and the difference in average daily weight gain and feed conversion ratio between PS-400 and PS-550 was significant ($P < 0.05$). Compared with the control group, the experimental group showed significant differences in crude fibre (CF), ether extract (EE), dry matter (DM), organic matter (OM), average digestible energy (ADE), metabolizable energy, xylan, and calcium ($P < 0.05$), with a maximum increase of 7.02% in calcium content. The addition of PS can be effective in making the experimental and control groups show a significant difference in value changes in high-density cholesterol (HDL-C) and low-density cholesterol (LDL-C) indicators ($P < 0.05$), while no significant changes were revealed in the other indicators ($P > 0.05$). From the above, plant sterol microcapsules can effectively improve pig growth performance and nutrient apparent digestibility, and improve their blood lipid status.

Keywords: plant sterols; pig; diarrhoea rate; nutrient digestibility; aspartate aminotransferase; alanine aminotransferase; high-density lipoprotein cholesterol

The improvement in people's living standards and their pursuit of the quality of life have made them pay more attention to the quality of livestock and poultry products. In the process of large-scale breeding, the growth and development of piglets will be affected to some extent due to changes in climate factors such as air humidity and temperature in the surrounding environment, as well as bacteria, pathogens, and anti-nutritional factors in feed. The environmental hygiene conditions and intake of nutrients are im-

Supported by the Wenzhou Science and Technology Plan Project, Study on Castration and Rearing Pattern of Local Chicken Breeds in Wenzhou (No. N20210004).

https://doi.org/10.17221/64/2023-CJAS

portant influencing factors for the disease of piglets. A series of symptoms such as indigestion, diarrhoea, and viral infection can slow down their growth and development (Gravert et al. 2021; Guthrie et al. 2022). Among them, antibiotic therapy is a common prevention and treatment method, which plays an important role in maintaining the animal growth status and protecting animal health. However, long-term use of antibiotics can lead to drug resistance in pig herds, and it can cause a significant burden on their liver and kidney functions, leading to poor body quality. Moreover, it can remain in animal products in the form of metabolites, causing significant damage to human health (Isaac et al. 2019; Vilahur et al. 2019; Yuan et al. 2021). The EU banned antibiotics in 2006, and China also implemented it at the beginning of 2020. The implementation of antibiotic reduction action makes it very important to find a new type of feed additive that is green, safe, non-toxic, and effective.

Phytosterol (PS) is a natural active component isolated from plant oils extracted from e.g. soybeans and corn seeds, characterized by hydroxysteroids. It is generally composed of sitosterol, rapeseed sterol, and stigmasterol, and is mostly distributed in the husk, germ, and other parts of grains (Call et al. 2020; Tian et al. 2020). Plant sterols, sitosterol, and camphor sterols in feed belong to a group of plant sterol compounds. They have similar chemical structures, but there are subtle differences. Plant sterols are a class of naturally occurring substances in plants that play a structural and functional role in plant cell membranes, similar to cholesterol in animals. Sitosterol is a sterol synthesized by plants and widely used as a component of plant hormones and cell walls. Camphor sterol is a sterol compound present in camphor resin, which has antibacterial and insecticidal properties. Plant sterols have a similar structure to animal cholesterol and can inhibit the absorption of animal cholesterol by competing for absorption sites, thereby achieving their degradation metabolism and regulating the permeability and stability of liposomes. Meanwhile, PS can effectively maintain the balance of cholesterol in the body and reduce the risk of cardiovascular disease. Research has shown that adding PS to feed can effectively promote the synthesis of animal proteins, improve the reactive capacity of antioxidant enzymes in the blood, and exhibit good disease prevention and both control and reduce the cancer risk. Its high-dose use also rarely results in adverse toxic side effects (Wang et al. 2020; Mun et al. 2022). Plant sterols, as a common compound in plant feed, are widely present in plant-based foods such as grains and oilseed crops. They exhibit good anti-inflammatory properties and lower total serum cholesterol. Related studies have shown that consuming 2 g of PS does not have any adverse effects on animal health, and long-term testing data also shows that consuming PS is safe and effective, without obvious toxic side effects, and will not affect their biochemical indicators (Gylling et al. 2014). It has been proven in mouse experiments that the input of plant sterols can effectively regulate specific lipid metabolism, and the expression gene network also indicates that it has a certain degree of safety and will not interfere with its normal metabolic process (Zhu et al. 2021). At the same time, a low content of plant sterols was used in the study, which will not cause significant damage to animal metabolism during the experimental process. However, the chemical structure of purified PS does not fully match the metabolic pathways in animals, and it competes with cholesterol for absorption channels. Therefore, the relative amount of PS that can effectively be absorbed during liver work and enter the body's circulation is relatively low. Among them, microcapsules, as a new dosage form, are polymer materials wrapped in a thin film and displayed as sealed vesicular particles. They can effectively reduce the volatility and instability of drugs, control their release degree, and improve their bioavailability (Calkins and Robinson 2020). This study aims to explore the mechanism of the effect of microencapsulated plant sterols on pig growth performance and related physiological characteristics, and design experiments based on ensuring the activity of plant sterols.

MATERIAL AND METHODS

Experimental design

In the study with a single-factor experimental design for analysis 200 healthy ternary hybrid Duroc × Landrace × Yorkshire piglets with an average initial weight of 7.53 ± 0.57 kg were used as the experimental subjects. This pig breed has fast growth performance and high economic benefits, which ensures good research representativeness. The experimental piglets were from the same source with

https://doi.org/10.17221/64/2023-CJAS

similar parity and they were weaned on day 26. The study piglets were randomly divided into five groups, with four replicates per group and 10 pigs per replicate. Pig feed was divided into experimental feed and control feed based on the addition of PS. One control group (corn-soybean meal-based diet) was added different doses of PS in three experimental groups, with doses of 100 mg/kg (experimental group 1), 250 mg/kg (experimental group 2), 400 mg/kg (experimental group 3), and 550 mg/kg (experimental group 4). The experimental period was 60 days, with six days for the pretrial and 54 days for the regular trial. The basic diet was formulated according to the NRC "Pig Nutrition Requirements", and the composition and nutritional level of the diet are shown in Figure 1 (Faubel et al. 2022; Hao et al. 2022).

In Figure 1, digestible energy (DE) is the calculated result, and the remaining nutrient levels are the actual measured values. The pigs were fed a pelleted feed manufactured by an agricultural company, in which the PS microencapsulation was purchased from Shanghai Jinsui Biotechnology Co. Microcapsule preparation was classified according to their different levels of PS for experimental control. The experimental content was approved by Wenzhou Vocational College of Science & Technology.

Feed management

The feeding and management conditions of each group were the same, including feeding conditions and hygiene cleaning conditions. Before the experiment began, the pigsty where the pigs were kept was first cleaned and disinfected, and the feeding and drinking troughs were cleaned, spraying insect repellents. All observed piglets received feed and water *ad libitum*. The pigsty was cleaned regularly and at designated points every day, with a disinfection frequency once a week to ensure that the pigsty was in dry and ventilated conditions. Daily feeding, excretion, and mental state of pigs were observed and recorded (El-Shafei et al. 2022).

Indicator measurement method

Growth properties. All pigs were weighed on an empty stomach on the first and 53rd day of the experiment and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated on a repeated basis. The diarrhoea and mortality status of pigs in each replicate group was evaluated based on diarrhoea rate, diarrhoea score, and mortality rate. The diarrhoea rate refers to the proportion of pigs with diarrhoea in the total number of pigs, and there were slight differences in the degree and duration of diarrhoea. A score of 0–3 indicates the severity of diarrhoea, which is examined using pig faeces. The mortality rate represents the proportion of pigs that die from the total number during the experimental period (Sorelle et al. 2023). Equations 1 and 2 represent the mathematical expression of diarrhoea rate and mortality rate.

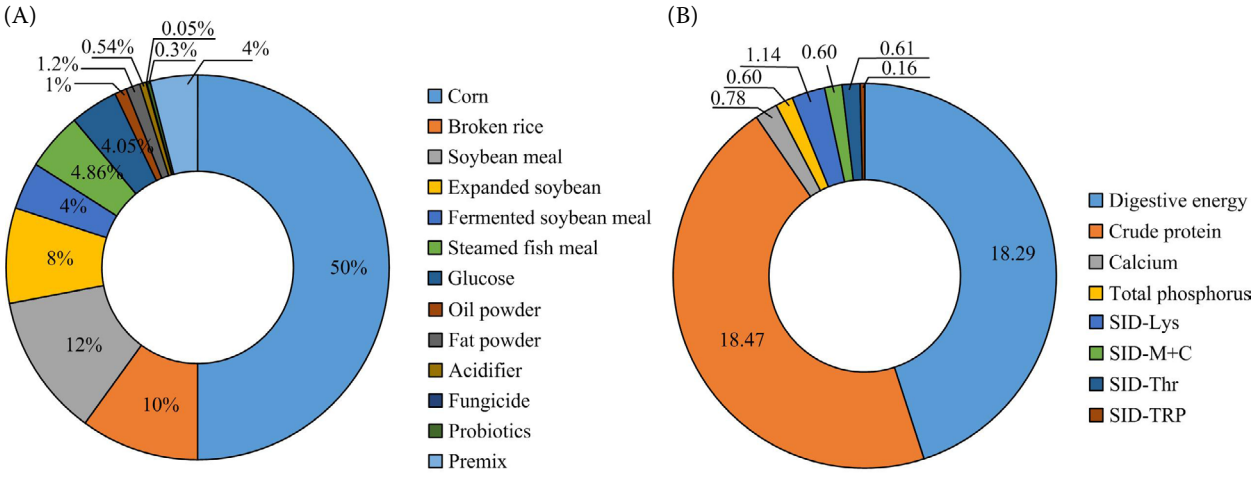

Figure 1. Composition and nutritional level of experimental diet for pigs
(A) Composition structure; (B) nutrition level

https://doi.org/10.17221/64/2023-CJAS

$$\text{Diarrhoea} = (P_{dt} \times D/P_t \times D) \times 100\% \quad (1)$$

where:
P_{dt} – recurrent cases of pigs with diarrhoea;
P_t – total number of pigs;
D – number of experimental days.

$$\text{Death} = (P_d/P_t) \times 100\% \quad (2)$$

where:
P_d – number of dead pigs.

Apparent nutrient digestibility. Three days before the end of the experiment, faecal samples were taken from each replicate group, and a five-point sampling method was used. The midpoint of the diagonal and four locations on the diagonal that were equidistant from the point were selected for sampling. An amount of 200 g of faecal samples was collected and added to 10% sulphuric acid (H_2SO_4) at a ratio of 1 : 10 to achieve the conversion of free nitrogen to combined nitrogen in the air (Suda et al. 2022). The processed samples were stored in the freezer environment at minus 20 °C. An amount of 250 g of feed samples was selected and stored at 4 °C. The collection of feed samples was carried out using the quartering method, which involves mixing the original samples and stacking them into a cone on white paper. Then, they are pressed into a 3-centimeter thick cone shape, and two diagonal portions are taken for the above mixing operation until the final required sample amount is left. The content of crude protein (CP), crude fat, crude fibre (CF), and organic compound (OC) was determined in two experimental samples, and they were detected and classified by the ashing method according to the reaction characteristics of different contents. The experimental operation refers to the "Feed Analysis and Quality Detection Technology" (Sun et al. 2019; Yuan et al. 2021). The mathematical calculation formula is shown in Equation (3).

$$\text{NAD} = 100 - (\text{Fe} - \text{N}/\text{FAC}) \times (\text{FN}/\text{Fe} - \text{AC}) \times 100 \quad (3)$$

where:
NAD – apparent nutrient digestibility;
Fe – N, FN – nutrient status in faecal and feed samples;
Fe – AC, FAC – ash content in the corresponding samples.

Serum collection and biochemical indicator determination. At the end of the experiment, feeding was stopped and the duration was guaranteed to be half a day to reduce the impact of unconsumed feed on serum indicators. Then, venous blood samples were taken from the experimental pigs in the next morning, with a blood sample content of 10 ml. The blood sample was placed in a centrifuge tube and stewed for 2 h, followed by centrifugation at a speed of 3 500 rpm for 10 minutes. The upper layer of the serum was stored after centrifugation at a low temperature of –20 °C. By using the Synchron ISE/CX3 Delta fully automatic biochemical analyzer, blood routine indicators, serum biochemical indicators, and fat metabolism indicators in serum were measured. They included aspartate aminotransferase (AST), alanine aminotransferase (ALT), free amino acids, high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), glucose (GLU), total bilirubin (TBILI), low density lipoprotein-cholesterol (LDL-C), urea nitrogen (UN), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total protein (TP), total triglycerides (TG) and other indicators (Suda et al. 2022).

Testing enzymatic determination of xylan content: xylanase degrades xylan into measurable oligosaccharide and monosaccharide molecules while reducing sugar molecules can undergo the colour reaction with DNS under boiling water bath conditions. The colour depth is directly proportional to the amount of reducing sugar produced by enzymatic hydrolysis, and the amount of reducing sugar produced is directly proportional to the activity of xylanase. Subsequently, the concentration of the reaction product is determined using a spectrophotometer. The determination of polysaccharides generally relies on the anthrone colorimetric method, which is based on the principle that anthrone can react with sugar groups to form a blue-green solution and exhibit the maximum absorption value at a peak of 620 nm. The anthrone ethyl acetate reagent can measure the absorbance value of the sample to obtain the sugar content.

Antioxidant indicators. To evaluate the antioxidant capacity of the blood, the antioxidant is an important manifestation of inhibiting the oxidation reaction of free radicals. As a complex system, the stronger the body's antioxidant capacity, the higher the health level. At the same time, the removal of oxidative free radicals can effectively prevent some cardiovascular diseases. Total superoxide dismutase (T-SOD), catalase (CAT), total antioxidant capacity (T-AOC), and malondialdehyde

https://doi.org/10.17221/64/2023-CJAS

enzyme (MDA) are designed to evaluate the antioxidant capacity of PS in pig blood. Excessive MDA values can lead to changes in the structure and function of cell membranes, which are products of peroxidation reactions (Xie et al. 2022).

For the determination of antioxidant indicators, the first step was to select corresponding measurement methods for different indicators as follows: SOD activity is often measured using the nitrogen blue tetrazole (NBT) method, and the reduction effect of NBT under light can represent the SOD activity. The ground sample was added to a phosphate buffer solution and centrifuged before extracting the crude SOD extract, and the solution with methionine (Met) was subjected to photometry treatment. The determination of catalase involved diluting the standard solution to a certain concentration, then holding the hydrogen peroxide phosphate buffer solution in a water bath in an iodine volumetric flask, and reacting with the enzyme standard solution. The absorbance change of the reaction solution was measured using an ultraviolet absorption method. The T-AOC was measured using an analytical reagent kit which included buffer, substrate, and sample. Thiobarbituric acid (TBA) can be used for the determination of MDA content. The condensation of MDA and TBA can form a red product and exhibit the maximum absorption peak aggregation. Subsequently, after the experiment was completed, blood was collected from the arteries of piglets. The collected blood was placed in a centrifuge tube and allowed to stand for half an hour before centrifugation. The centrifuge speed was 3 000 rpm, and the centrifugation time was 15 minutes. The upper clear liquid was pipetted and transferred into a prelabelled centrifuge tube (with a capacity of 5 ml). The reagents or methods were determined according to the corresponding indicators for analysis. Based on the experimental results, data analysis was conducted and corresponding antioxidant indicators were calculated. The antioxidant capacity of different groups was evaluated and compared based on the value of the indicators.

Data processing and analysis

In the process of data processing and analysis research, Microsoft Excel was first used to organize and process the data. Then, SPSS v22.0 statistical tools (IBM Corp., Armonk, NY, USA) were used to conduct statistical analysis and significant difference analysis of the data during the experimental process. The relationship between the effects of plant sterols at different levels of addition on pig growth was analyzed using one-way ANOVA, Duncan's multiple comparisons, linear models, and other methods. The impact of data processing analysis on the number of experimental repetitions was processed in the form of mean ± standard deviation to reduce experimental errors, and $P < 0.05$ or $P < 0.01$ indicate statistically significant or extremely significant.

RESULTS

Pig growth performance

In this study the experimental time was divided into three stages: 30–37 days, 38–45 days, and 46–54 days. The first 30 days of piglet production were the weaning period, during which piglets experienced rapid growth changes and exhibited small differences in inter-group data, resulting in poor data stability and feasibility. Therefore, during the experimental period, the experimental stage was divided into three time stages, and the growth performance of different groups of piglets was analyzed. The results are shown in Table 1.

The results in Table 1 indicated that in the early stage of the experiment, compared with the control group, the pigs receiving the feed added 100, 250, 400, and 550 mg/kg PS levels showed varying increases in total body weight, with an increased rate of 3.64%, 4.57%, and 5.36%. During days 30–37 and 46–54 of the experiment, the ADG of the four experimental groups showed significant differences ($P < 0.05$) in inter-group and intra-group comparisons, with an increased rate exceeding 2.5%. In the later stage of the experiment, there was a significant difference ($P < 0.05$) in the ADG and feed weight ratio between experimental group 3 and experimental group 4 compared to the control group and experimental group 1. The increase in ADFI value exceeded 6.24% compared to the control group and 3.25% compared to experimental group 1.

The FCR index of experimental group 3 and experimental group 4 decreased by more than 6% compared to the control group and experimental group 1.

SEM represented the average error of data within the group. In Figure 2A, the error results of different groups were generally small, and there was no linear

https://doi.org/10.17221/64/2023-CJAS

Table 1. Growth performance of pigs in different groups

Observation time (days)	Index	Control group	Experimental group (phytosterol addition level)				SEM
			group 1 (100 mg/kg)	group 2 (250 mg/kg)	group 3 (400 mg/kg)	group 4 (550 mg/kg)	
30–37	TG	11.8	12.3	12.4	12.4	12.5	0.12
	ADG	0.53	0.54^{a}	0.55^{ab}	0.58^{ab}	0.66^{ab}	0.01
	ADFI	0.78	0.75^{a}	0.83^{ab}	0.86	0.94^{ab}	0.01
	FCR	1.71	1.6	1.54	1.5	1.46	0.01
38–45	TG	15.9	15.9	15.8	15.8	15.9	0.18
	ADG	0.72	0.71^{a}	0.71^{ab}	0.74	0.82^{ab}	0.01
	ADFI	1.12	1.13^{a}	1.12^{ab}	1.15^{ab}	1.23^{ab}	0.03
	FCR	1.78	1.79^{a}	1.78^{ab}	1.74^{ab}	1.68^{ab}	0.22
46–54	TG	27.7	28.1^{a}	28.1	28.1	28.2	0.01
	ADG	0.62	0.62^{a}	0.63^{ab}	0.66^{ab}	0.74^{ab}	0.10
	ADFI	0.95	0.94^{a}	0.98^{ab}	1.01^{ab}	1.09^{ab}	0.01
	FCR	1.76	1.72^{a}	1.7^{ab}	1.65^{ab}	1.62^{ab}	0.12

ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; TG = total triglyceride

^{a,b}Significant differences from the control group and from the previous experimental group ($P < 0.05$)

relationship between the groups. In Figure 2B, when comparing the experimental groups with different levels of PS, there was a significant difference in TG indicators ($P < 0.05$), indicating that the addition of PS dosage can effectively improve the growth rate of pigs during the growth process.

Changes in diarrhoea by group

The diarrhoea status of pigs was analyzed in each group. The results in Figure 3A indicate that at the beginning of the experiment (30–37 days), piglets in different groups showed varying degrees of diarrhoea, with diarrhoea rates of 2.15%, 1.67%, 1.48%, 1.23%, and 0.77%. There was no significant correlation between differences in the data ($P > 0.05$). In the later stage of the experiment (38–54 days), the diarrhoea status of the experimental pigs improved after adding different doses of PS, and the difference between the experimental group and the control group was significant ($P < 0.05$). The diarrhoea rate of experimental group 4 and experimental group 5 in the later stage was lower than 0.2%. In Figure 3B, as the experimental time progressed, the data error results between groups

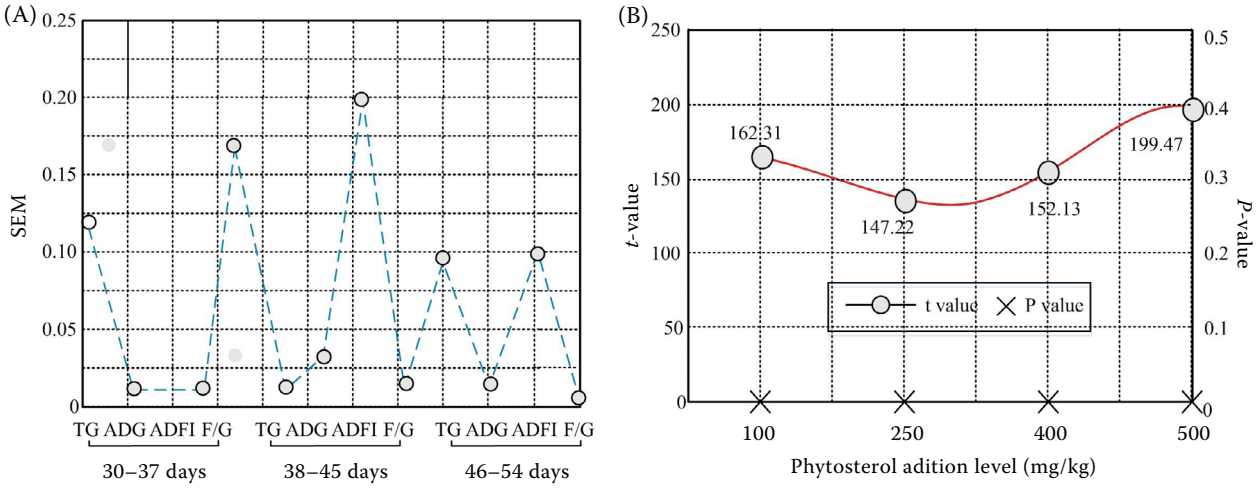

Figure 2. Comparison of SEM values of growth performance among different groups and differences between experimental groups

(A) SEM value of growth performance; (B) comparison of TG indexes between experimental groups

ADFI = average daily feed intake; ADG = average daily gain; F/G = feed conversion ratio; TG = total triglyceride

https://doi.org/10.17221/64/2023-CJAS

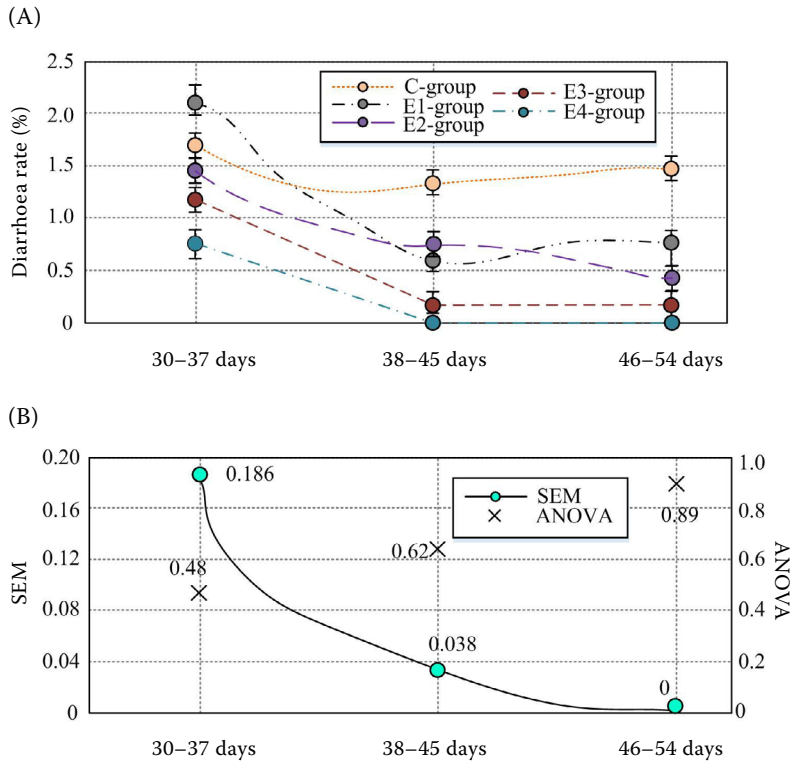

Figure 3. Diarrhoea in different groups of pigs

(A) Comparison of diarrhoea rates among different groups of pigs; (B) error and variance of experimental data from different groups

C-group = control group; E1-group = phytosterol addition level 100 mg/kg; E2-group = phytosterol addition level 250 mg/kg; E3-group = phytosterol addition level 400 mg/kg; E4-group = phytosterol addition level 550 mg/kg

showed a decreasing trend, with significant changes in univariate analysis. The addition of PS can effectively alleviate diarrhoea in pigs ($P < 0.05$).

Nutrient digestibility in pigs receiving different feeds

The nutrient digestion in pigs was analyzed in different groups, mainly measuring CP, CF, crude fat, dry matter (DM), organic matter (OM), and DE. In Table 2, compared with the control group, the four experimental groups showed significant differences in CF, ether extract (EE), DM, OM, DE, metabolizable energy (ME), xylan and calcium ($P < 0.05$). In terms of CP digestion, there were no significant differences between different groups. In terms of CF, EE, DM, OM, calcium, and DE indicators, the experimental group with different doses of PS showed significant statistical differences ($P < 0.05$) in intra-group comparison. The content of PS in experimental group 3 and 4 was higher.

Table 2. Comparative analysis of nutrient digestibility

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	Experimental group 4
CP (%)	83.7 ± 1.34	86.6 ± 2.25	94.9 ± 1.76^{ab}	89.3 ± 2.09	88.2 ± 1.27
CF (%)	50.3 ± 3.28	44.8 ± 5.07^{a}	63.9 ± 8.88^{ab}	44.7 ± 8.09^{ab}	39.9 ± 7.27^{ab}
EE (%)	89.2 ± 1.74	91.9 ± 1.53^{a}	99.1 ± 0.83^{ab}	94.7 ± 1.50^{ab}	92 ± 1.34^{ab}
DM (%)	94.2 ± 0.65	95.4 ± 1.08^{a}	100.2 ± 1.36^{ab}	96.2 ± 1.24^{ab}	96.2 ± 1.24^{ab}
OM (%)	95.3 ± 0.65	96.2 ± 1.04^{a}	100.9 ± 1.18^{ab}	97.5 ± 1.18^{ab}	97.4 ± 0.85^{ab}
ADE (MJ/kg)	17.1 ± 0.18	17.4 ± 0.25^{a}	18.42 ± 0.26^{ab}	17.6 ± 0.26^{ab}	17.4 ± 0.17^{ab}
ME (MJ/kg)	16.8 ± 0.15	16.9 ± 0.22^{a}	17.34 ± 0.18^{a}	17.5 ± 0.39^{ab}	18.1 ± 0.24^{ab}
Xylan (%)	36.1 ± 0.11	42.1 ± 0.32^{a}	43.26 ± 0.47^{a}	46.2 ± 0.25^{ab}	47.3 ± 0.16^{ab}
Calcium (%)	48.5 ± 2.68	51.2 ± 3.20^{a}	54.1 ± 1.89^{ab}	57.3 ± 1.48^{ab}	59.2 ± 1.56^{ab}

ADE = average digestible energy; CF = crude fibre; CP = crude protein; DM = dry matter; EE = ether extract; ME = metabolizable energy; OC = organic compound; OM = organic matter

^{a,b}Significant differences from the control group and from the previous experimental group ($P < 0.05$)

https://doi.org/10.17221/64/2023-CJAS

During the experiment, compared with the control group, the absorption of CF by experimental pigs was significantly reduced. However, there were varying degrees of growth in OM, digestible energy, ME, calcium, and xylan, with a maximum increase of 13.25% (ME). Among them, experimental groups 1–4 showed significant improvements in calcium indicators compared to the control group, reaching 5.74%, 6.29%, 6.87%, and 7.02% with significant data differences ($P < 0.05$).

Serum biochemical indicators

The serum indicator data in the growth process of pigs were organized and analyzed during the experimental process. The results in Table 3 indicated that the experimental group with different levels of PS showed different changes in serum biochemical indicators compared to the control group. In lipoprotein cholesterol, the HDL-C and LDL-C values of the experimental group showed significant statistical differences compared to the control group ($P < 0.05$). The values of TC and TG in experimental group 1 were 3.577 mmol/l and 0.432 mmol/l, respectively, and those in group 2 were 3.563 mmol/l and 0.404 mmol/l. Compared with the mean values of 3.605 mmol/l and 0.433 mmol/l in the control group, there was a statistically significant difference ($P < 0.05$). The addition of PS to the diet did not have any significant impact on the LDH, GLU, ALP, AST, TP, ALB, ALT, and UN indicators in their serum. There was no significant statistical difference between the control and experimental groups ($P > 0.05$).

Antioxidant application effect

The analysis of serum antioxidant effects during pig growth (Table 4) showed that adding different levels of PS to the basic diet did not reveal any significant statistical differences in T-AOC, CAT, MDA, and T-SOD indicators compared to the control group ($P > 0.05$). Specifically, the increase in T-AOC in plant sterols in experimental groups 1–4 was above 15%, and low doses of PS reduced the enzyme activity in pigs by 6.23%.

DISCUSSION

Plant sterols, as a natural steroid compound, are mostly present in plants. Due to their different forms of existence, they can be divided into sterol esters, free sterols, acylated sterol glycosides, etc. β-Glutinosterol is an important component of PS, accounting for over 50%. At the same time, after entering the human body, PS will react with substances such as glucose and fatty acids, resulting in the pro-

Table 3. Serum biochemical indicators of different groups of pigs

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	Experimental group 4
LDH	487.6 ± 85.34	448.6 ± 72.61	517 ± 92.91	479 ± 59	497.93 ± 80.9
GLU (mmol/l)	5.64 ± 0.86	6.26 ± 0.81	5.26 ± 1.04	5.78 ± 1.07	5.3 ± 1.19
ALP (IU/l)	344.1 ± 47.14	339.9 ± 92.98	360 ± 78.36	357.9 ± 73.3	345.5 ± 53.9
AST (IU/l)	46.9 ± 16.48	63.2 ± 11.21	52.1 ± 14.85	54.2 ± 17.5	56.6 ± 14.9
TP (g/l)	44.9 ± 2.3	48 ± 3.34	44.3 ± 3.13	45.2 ± 4.22	46.6 ± 2.69
TC (mmol/l)	3.6 ± 0.57	3.57 ± 0.53[a]	3.56 ± 0.79[a]	3.08 ± 0.42	3.43 ± 0.63
TG (mmol/l)	0.433 ± 0.28	0.432 ± 0.18[a]	0.404 ± 0.22[a]	0.481 ± 0.21	0.484 ± 0.18
ALB (g/l)	26.17 ± 3.93	28.38 ± 3.63	26.4 ± 3.17	28.4 ± 3.27	27.8 ± 2.09
ALT (IU/l)	63.4 ± 13.51	72.91 ± 11.98	70.7 ± 7.18	67.8 ± 15.5	64.9 ± 13.7
UN	24.9 ± 2.87	24.88 ± 3.48	27 ± 3.87	21.43 ± 1.83	23.2 ± 9.38
HDL-C (mmol/l)	7.49 ± 1.61	11.37 ± 3.02[ab]	9.3 ± 1.76[ab]	9.67 ± 1.84[ab]	9.03 ± 1.23[ab]
LDL-C (mmol/l)	1.32 ± 0.42	1.33 ± 0.23[ab]	1.22 ± 0.13[ab]	1.03 ± 0.2[ab]	1.32 ± 0.29[ab]

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GLU = glucose; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low density lipoprotein-cholesterol; TC = total cholesterol; TG = total triglycerides; TP = total protein; UN = urea nitrogen

[a,b]Significant differences from the control group and from the previous experimental group ($P < 0.05$)

https://doi.org/10.17221/64/2023-CJAS

Table 4. Comparison of antioxidation result

	Control group	Experimental group 1	Experimental group 2	Experimental group 3	Experimental group 4
T-AOC (IU/mgprot)	0.85 ± 0.32	0.97 ± 0.17	1.02 ± 0.21	1.05 ± 0.11	1.04 ± 0.14
CAT (IU/mgprot)	42.13 ± 0.4	38.3 ± 0.76	38.54 ± 0.28	37.2 ± 1.07	35.7 ± 0.15
MDA (nmol/mgprot)	0.96 ± 0.32	0.87 ± 0.14	1.47 ± 78.36	1.36 ± 0.36	1.34 ± 0.32
T-SOD (IU/mgprot)	116.2 ± 1.48	104.7 ± 1.07	112.1 ± 1.01	114.3 ± 0.05	115.1 ± 0.96

CAT = catalase; MDA = and malondialdehyde enzyme; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase

duction of various active substances. The changes in the binding position and chemical form of the linkage can cause certain changes in the absorption and chemical properties of plant sterols. Most scholars believe that the hormone effects carried by plant sterols can effectively play a certain promoting role in the field of animal growth. Zhao et al. (2019) studied the effect of PS on the growth performance and meat quality of broilers by designing experimental replicates. The results showed that adding 40 mg/kg PS to the diet resulted in significant differences in daily weight gain and increased antioxidant enzyme activity during the growth process. The data showed significant statistical differences compared to the control group. Zhang et al. (2020) believed that dimethyl PS can effectively exert good disease prevention and control capabilities. The ban on antibiotics makes it crucial to find a new type of feed to improve the immune system and growth and development of pigs. The study is based on the safety and effectiveness advantages of plant sterols to investigate the effects of their microcapsules on pig growth performance, nutrient digestion, and serum biochemical indicators.

In the present study the experimental time was divided into three time stages and the growth performance of pigs under the addition of different plant sterol levels was analyzed. The results showed that in the early stage of the experiment, compared with the control group, the pigs receiving 100, 250, 400, and 550 mg/kg plant sterol levels increased their total body weight by 3.64%, 4.57%, and 5.36%. During days 30–54 of the experiment, there were significant differences ($P < 0.05$) in ADG between the four experimental groups in both inter-group and intra-group comparisons, with an increased rate of over 2.5%. Moreover, there was a significant difference ($P < 0.05$) in ADG and FCR between experimental group 3 and experimental group 4 compared to the control group, with an increase in ADFI values exceeding 6.24%. The improvement in pig diarrhoea was good, but the data difference was not significant. This was mainly due to the lower occurrence of adverse reactions such as diarrhoea during the later growth process of pigs due to the improvement of their body mass and the stability of environmental conditions (Djuissi et al. 2021; Guo et al. 2021). Plant sterols in pigs can inhibit the absorption of animal cholesterol, thereby achieving its degradation and metabolism, regulating the permeability and stability of liposomes, and improving the gastrointestinal function and nutrient absorption ability of pigs, which has a promoting effect on their growth (Bhattacharyya 1979). Concurrently, within plant sterol microcapsules, β-sitosterol, α-glutinosterol, and dehydroergosterol can modulate gastrointestinal hormones, enhance the intestinal barrier function, influence the pig gut microbiota composition and metabolite production, and improve nutrient digestion (Keszthelyi et al. 2012). The above results indicated that the addition of PS can improve the growth performance of pigs to a certain extent, possibly due to the hormone activity in PS being able to synthesize proteins during induced activities, thereby achieving a regulatory effect on pig growth. This result was consistent with the content proposed by Shang et al. (2020). The nutrient expression rate can directly reflect the nutrient absorption in animals. In the experiment, the experimental groups with dose levels of 400 mg/kg and 550 mg/kg showed varying degrees of growth in OM, DE, ME, calcium and xylan, with a maximum increase of 13.25%. Moreover, there was a significant difference in calcium absorption between the PS experimental group and the control group ($P < 0.05$), with a maximum increase of 7.02%. This result was highly similar to the viewpoint proposed by Dawood et al. (2022).

Protein and enzymes in serum biochemical indicators can effectively play an important role in body metabolism, growth and development, etc. Among them, aspartate aminotransferase is an indicator

https://doi.org/10.17221/64/2023-CJAS

for evaluating liver damage. When its value exceeds the normal range, it indicates obvious liver damage (Feng et al. 2022). Total protein, albumin, and urea nitrogen can reflect the absorption and metabolism of proteins. When the body's metabolism is imbalanced, the urea nitrogen products in the blood will significantly increase. Related studies have shown that appropriate levels of plant sterols can regulate the process of protein synthesis, enhance protein synthesis and degradation, and thus affect the cell growth and function. Plant sterols can regulate enzyme activity and catalysis by interacting with enzymes, thereby affecting the rate and efficiency of enzyme-catalyzed reactions and playing a role in cellular metabolism and physiological processes (Valitova et al. 2016). Plant sterols may participate in the regulation of cellular signalling pathways by binding to receptors in the cell membrane, thereby affecting the regulation of cellular function and metabolic pathways. Protein cholesterol with lipids of different density is closely related to the coronary heart disease, with HDL-C being a protective factor (Cheng et al. 2020). It was found that the experimental group with different levels of PS showed significant statistical differences in HDL-C and LDL-C values in various serum biochemical indicators compared to the control group ($P < 0.05$). The values of TC and TG in experimental group 1 and experimental group 2 were significantly different from the mean values of 3.605 mmol/l and 0.433 mmol/l in the control group ($P < 0.05$). However, there was no significant statistical difference in the values of other indicators such as LDH, GLU, ALP, AST, ALB, ALT, etc. ($P > 0.05$). In the comparison of serum antioxidant activity, adding different levels of PS to the basic diet did not show any significant statistical differences in T-AOC, CAT, MDA, and T-SOD indicators compared to the control group ($P > 0.05$). The addition of PS did not affect the antioxidant effect of pig blood. However, the addition of PS can improve T-AOC in the blood, thereby enhancing the resistance of pigs.

The above results indicated that adding different levels of PS to the basic diet can effectively improve growth performance and nutrient digestibility. In the serum indicator test, the addition of PS only had a significant impact on HDL-C and LDL-C indicators, effectively reducing pig blood lipids and reducing the risk of illness. However, further consideration is needed for the impact of the PS microcapsule system on the later growth stage of pigs, and in-depth research should be conducted taking into account factors such as different additive components, environmental hygiene conditions, and individual physical condition of pigs. The effectiveness and safety of PS microcapsules on pig growth performance effectively provide reference value and significance for disease prevention and management of farmers.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bhattacharyya AK. In vitro esterification of plant sterols by the esterifying enzyme of the small intestine of rat. Cell Mol Life Sci. 1979;35(12):1614-5.

Calkins KL, Robinson DT. Intravenous lipid emulsions in the NICU. Neoreviews. 2020;21(2):e109-19.

Call L, Molina T, Stoll B, Guthrie G, Chacko S, Plat J, Robinson J, Lin S, Vonderohe C, Mohammad M, Kunichoff D, Cruz S, Lau P, Premkumar M, Nielsen J, Fang Z, Olutoye O, Thymann T, Britton R, Sangild P, Burrin D. Parenteral lipids shape gut bile acid pools and microbiota profiles in the prevention of cholestasis in preterm pigs. J Lipid Res. 2020;61(7):1038-51.

Cheng Y, Chen Y, Li J, Qu H, Zhao Y, Wen C, Zhou Y. Dietary β-sitosterol regulates serum lipid level and improves immune function, antioxidant status, and intestinal morphology in broilers. Poult Sci. 2020;99(3):1400-8.

Dawood A, Zuberi A, Shi W. Plant-based β-mannanase supplemented diet modulates the gut microbiota and up-regulates the expression of immunity and digestion-related genes in Cyprinus carpio. J Appl Anim Res. 2022;50(1):21-30.

Djuissi NM, Ngoula F, Kouamo J. Reproductive characteristics, serum metabolites, and oxidative status in female guinea pigs (Cavia porcellus) fed with ethanolic extract of dichrostachys glomerata fruit. World Vet J. 2021;11(1):66-72.

El-Shafei AA, Al-Gamal MA, Shams El-Deen AE. Effectiveness of caprylic acid and Yucca schidigera extract on productive and physiological performance of laying hens. Egyptian J Nutr Feed. 2022;25(1):67-83.

Faubel N, Makran M, Cilla A, Alegria A, Barbera R, Garcia-Llatas G. Bioaccessibility of plant sterols in wholemeal rye bread using the INFOGEST protocol: Influence of oral phase and enzymes of lipid metabolism. J Agric Food Chem. 2022;70(41):13223-32.

Feng S, Wang L, Shao P, Sun P, Yang CS. A review on chemical and physical modifications of phytosterols and their

https://doi.org/10.17221/64/2023-CJAS

influence on bioavailability and safety. Critic Rev Food Sci Nutr. 2022;62(20):5638-57.

Gravert TKO, Fauser P, Olsen P, Hansen M. In situ formation of environmental endocrine disruptors from phytosterol degradation: A temporal model for agricultural soils. Environ Sci Proces Impacts. 2021;23(6):855-66.

Guo Z, Chen X, Huang Z, Chen D, Yu B, Chen H, Yu J, Yan H, Zheng P, Luo Y. Dietary dihydromyricetin supplementation enhances antioxidant capacity and improves lipid metabolism in finishing pigs. Food Funct. 2021;12(15):6925-35.

Guthrie G, Stoll B, Chacko S, Mohammad M, Burrin D. Depletion and enrichment of phytosterols in soybean oil lipid emulsions directly associate with serum markers of cholestasis in preterm parenteral nutrition–fed pigs. J Parenter Entereral Nutr. 2022;46(1):160-71.

Gylling H, Plat J, Turley S, Ginsberg HN, Ellegard L, Jessup W, Jones PJ, Lutjohann D, Maerz W, Masana L, Silbernagel G, Staels B, Boren J, Catapano AL, De Backer G, Deanfield J, Descamps OS, Kovanen PT, Riccardi G, Tokgozoglu L, Chapman MJ, European Atherosclerosis Society Consensus Panel on Phytosterols. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular diseas. Atherosclerosism, 2014;232(2):346-60.

Hao L, Wang C, Wang H. Effects of supplementing with Humulus scandens on the growth performance and gut microbiota in piglets. J Appl Microbiol. 2022;133(6):3546-57.

Isaac DM, Alzaben AS, Mazurak VC, Yap J, Turner JM. Mixed lipid, fish oil, and soybean oil parenteral lipids impact cholestasis, hepatic phytosterol, and lipid composition. J Pediatr Gastroenterol Nutr. 2019;68(6):861-7.

Keszthelyi D, Knol D, Troost FJ, van Avesaat M, Foltz M, Masclee AAM. Time of ingestion relative to meal intake determines gastrointestinal responses to a plant sterol-containing yoghurt drink. Eur J Nutr. 2012;52:1417-20.

Mun HS, Rathnayake D, Dilawar MA, Jeong M, Yang CY. Effect of ambient temperature on growth performances, carcass traits and meat quality of pigs. J Appl Anim Res. 2022;50(1):103-8.

Shang Q, Ma X, Liu H, Liu S, Piao X. Effect of fibre sources on performance, serum parameters, intestinal morphology, digestive enzyme activities and microbiota in weaned pigs. Arch Anim Nutr. 2020;74(2):121-37.

Sorelle DN, Narcisse VB, Momo CMM, Herve T, Ferdinand N, Joseph T. Curative effects of spirulina (Spirulina platensis) aqueous extract on reproductive parameters and oxidative stress markers in female guinea pig (Cavia porcellus) exposed to lead. Eur J Vet Med. 2023;3(1):6-11.

Suda Y, Kagawa K, Fukuyama K, Elean M, Zhou B, Aminul Islam M, Rajoka MSR., Kober AKMH, Shimazu T, Egusa S, Terashima Y, Aso H, Ikeda-Ohtsubo W, Villena J, Kitazawa H. Soymilk-fermented with Lactobacillus delbrueckii subsp. delbrueckii TUA4408L improves immune-health in pigs. Benef Microbes. 2022;13(1): 61-72.

Sun HY, Yun HM, Kim IH. Effects of dietary n-6/n-3 polyunsaturated fatty acids ratio on growth performance, apparent digestibility, blood lipid profiles, fecal microbiota, and meat quality in finishing pigs. Canad J Anim Sci. 2019;100(2):272-81.

Tian Z, Deng D, Cui Y, Chen W, Yu M. Diet supplemented with fermented okara improved growth performance, meat quality, and amino acid profiles in growing pigs. Food Sci Nutr. 2020;8(10):5650-9.

Valitova JN, Sulkarnayeva AG, Minibayeva FV. Plant sterols: Diversity, biosynthesis, and physiological functions. Biochemistry. 2016;81(8):819-34.

Vilahur G, Ben-Aicha S, Diaz-Riera E, Diaz E, Badimon L, Padro T. Phytosterols and inflammation. Curr Med Chem. 2019;26(37):6724-34.

Wang K, Zhang H, Han Q, Lan J, Chen G, Cao G, Yang C. Effects of astragalus and ginseng polysaccharides on growth performance, immune function and intestinal barrier in weaned piglets challenged with lipopolysaccharide. J Anim Phys Anim Nutr. 2020;104(4):1096-105.

Xie T, Liu W, Chen Y, Zhou Y. An evaluation of graded levels of beta-sitosterol supplementation on growth performance, antioxidant status, and intestinal permeability-related parameters and morphology in broiler chickens at an early age. Poult Sci. 2022 Nov;101(11): 13 p.

Yuan C, Ding X, Jiang L, Ye W, Wu J, Qian L. Effects of dietary phytosterols supplementation on serum parameters, nutrient digestibility and digestive enzyme of white feather broilers. Ital J Anim Sci. 2021;20(1):2102-9.

Zhang T, Liu R, Chang M, Jin Q, Zhang H, Wang X. Health benefits of 4, 4-dimethyl phytosterols: An exploration beyond 4-desmethyl phytosterols. Food Funct. 2020; 11(1):93-110.

Zhao YR, Chen YP, Cheng YF, Qu HM, Li J, Wen C, Zhou YM. Effects of dietary phytosterols on growth performance, antioxidant status, and meat quality in Partridge Shank chickens. Poult Sci. 2019;98(9):3715-21.

Zhu Q, Wu J, He D, Lian X. Effects of plant sterols intake on systematic and tissue specific lipid metabolism in C57BL/6J mice. Curr Dev Nutr. 2021;5(suppl 2):388.

Received: May 4, 2023
Accepted: November 13, 2023
Published online: December 6, 2023