

## Abstract

Hesperidin is a member of flavonoids and has beneficial effects on immune function and oxidative stress. Therefore, this study was conducted to investigate the effects of hesperidin supplementation on growth performance, immune function, and antioxidant activity of growing pigs. Total of 24 pigs weighed  $20.4 \pm 0.6$  kg were randomly allotted to 3 treatments with 8 replicates for 9 wk. Dietary treatments included 1) basal diet (CON), 2) basal diet + 0.01% hesperidin (Hes-1), 3) basal diet + 0.02% hesperidin (Hes-2). Pigs fed Hes-1 and Hes-2 diets had higher ( $P < 0.05$ ) gain:feed (G:F) ratio compared to CON group. Blood creatinine concentration was lower ( $P < 0.05$ ) in Hes-1 treatment than that in CON group. In vivo antioxidant activity, represented by serum SOD activity, was increased ( $P < 0.05$ ) by Hes-2 compared to CON. To further confirm the immune function, pigs were i.p. challenged with lipopolysaccharide (LPS; 50ug/kg BW) and blood was analyzed. After 24h LPS challenge, platelet concentration was lower ( $P < 0.05$ ) in hesperidin treatment group compared to CON group. However, immunoglobulin levels were not different among treatment groups. The results of current study indicate that administration of hesperidin has **beneficial effects on growth performance and antioxidant activity** in growing pigs. Furthermore, dietary supplementation of hesperidin reduced levels of serum creatinine as well as LPS induced platelet. However, further studies need to be performed to confirm the effect of hesperidin on stimulation of immune response in pigs.

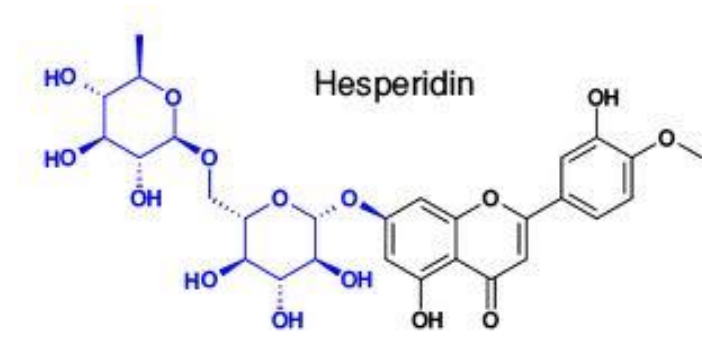
**Key words** : Serum biochemical profile, Immune response, Growing pigs, Fruit by-product extracts

## Introduction

Livestock animals are commonly administrated with antibiotics to prevent disease and metabolic disorders, as well as to improve feed efficiency. However, european countries have recently banned the use of dietary antibiotics due to their bacterial resistance and antibiotic residues in animal products. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics to enhance the health among food-producing animals. Hesperidin has a similar effect to antibiotics *in vivo* and *in vitro* and improves growth performance. Hesperidin, as a member of flavonoids, is an abundant and inexpensive by-product of citrus including sweet orange and lemon. Although application of hesperidin extracted from citrus peel is somewhat novel, it will be plausible to identify the beneficial effects of hesperidin on pig productivity. To this end, we administrated growing pigs with citrus peel-extracted hesperidin and analyzed growth performance, antioxidant activity and immune response.

## Material and Methods

- Experimental Diets : Nutrient Requirement of Pigs (NRC, 1998)
- Animals : 24 barrows [( Landrace x Yorkshire) x Duroc,  $20.4 \pm 0.6$  kg)
- Experimental Design :  
CON, basal diet; Hes-1; basal diet + 0.01% hesperidin; Hes-2; basal diet + 0.02% hesperidin  
\* Immune challenge : LPS(Lipopolysaccharides from *Escherichia coli* 0111:B4)
- Analysis Items :
  - 1) Growth performance
  - 2) Serum biochemical profiles
  - 3) Antioxidant activity (serum SOD activity)
- Statistical Analysis :  
→ SAS (SAS Institute, 2008), Duncan's multiple range test



## Results

**Table 1.** Effect of dietary supplementation of hesperidin on growth performance in growing pigs.

Treatment /Item	CON <sup>1)</sup>	Hes-1 <sup>1)</sup>	Hes-2 <sup>1)</sup>	SEM <sup>2)</sup>	P-value
Initial BW (kg)	19.53	19.63	18.14	1.11	0.61
Final BW (kg)	68.90	69.34	69.37	2.24	0.98
ADG (kg)	0.93	0.94	0.97	0.03	0.71
ADFI (kg)	2.65	2.39	2.42	0.11	0.22
G:F	0.33*	0.40	0.41	0.01	0.02

- <sup>1)</sup> CON (basal diet), Hes-1 (basal diet + hesperidin 0.01%), Hes-2 (basal diet+hesperidin 0.02%).  
<sup>2)</sup> Standard error of means.  
 \*  $P < 0.05$  vs. all other groups within the same row.

**Table 2.** Effects of dietary supplementation hesperidin on serum biochemical profile in growing pigs.

	Treatments			SEM <sup>2)</sup>	P-value	
	CON <sup>1)</sup>	Hes-1 <sup>1)</sup>	Hes-2 <sup>1)</sup>			
Creatinine (mg/L)	0 week	16.41	16.19	0.87	0.08	
	9 week	22.84	18.63	20.29	1.03	0.02
Total Proteins (g/L)	0 week	52.0	53.63	52.75	1.17	0.63
	9 week	60.88	61.25	61.0	1.57	0.99
Albumin (g/L)	0 week	30.13	31.75	30.86	0.99	0.52
	9 week	37.38	37.75	35.0	1.25	0.26
Blood urea nitrogen (mg/L)	0 week	237.5	222.5	215.0	9.29	0.24
	9 week	267.5	281.25	268.75	17.48	0.83

**Table 3.** SOD (Superoxide dismutase) activity in serum of pigs

SOD (unit/mg protein)	Treatment			SEM <sup>2)</sup>	P-value
	CON <sup>1)</sup>	Hes-1 <sup>1)</sup>	Hes-2 <sup>1)</sup>		
	51.20	51.04	63.89*	2.71	0.01

- <sup>1)</sup> CON (basal diet), Hes-1 (basal diet + hesperidin 0.01%), Hes-2 (basal diet + hesperidin 0.02%).  
<sup>2)</sup> Standard error of means.  
 \* $P < 0.05$  vs. all other groups within the same row.

**Table 4.** DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of hesperidin by solvents

Solvents	Treatment		P value
	BHT	Hesperidin	
Methanol	1.06 ± 0.06	0.60 ± 0.02*	0.012
DMSO	245.75 ± 0.16	52.66 ± 3.90*	0.002

- All measurements were done in triplicate, and all values are means ± standard error.  
<sup>1)</sup> IC<sub>50</sub> (mg/ml) : Concentration for scavenging 50% of DPPH radicals.  
 \*Significant difference between treatments ( $P < 0.05$ ).

**Table 5.** Level of WBC, RBC and IgA, IgG and IgM with LPS challenge

Item	Treatment			SEM <sup>2)</sup>	P-value	
	CON <sup>1)</sup>	Hes-0.02% <sup>1)</sup>	Hes-0.04% <sup>1)</sup>			
White blood cell (10 <sup>3</sup> /μl)	Before LPS	17.85	17.21	13.71	2.88	0.52
	After 24h	16.57	16.20	16.68	2.51	0.98
Red blood cell (10 <sup>6</sup> /μl)	Before LPS	6.62	5.79	6.44	0.20	0.06
	After 24h	6.63	6.92	7.15	0.32	0.52
Immunogloblin A (mg/ml)	0 h	1.31	0.99	1.26	0.11	0.11
	3 h	1.38	1.05	1.34	0.09	0.09
	6 h	1.39	1.09	1.31	0.09	0.13
	12 h	1.36	1.02	1.27	0.09	0.11
	24 h	1.32	0.98	1.21	0.11	0.11
	Immunogloblin G (mg/ml)	0 h	7.40	6.49	7.39	1.19
3 h		7.66	6.35	7.80	1.18	0.66
6 h		8.73	7.33	7.96	1.32	0.79
12 h		10.38	8.05	9.52	1.31	0.52
24 h		9.81	8.78	9.11	1.51	0.56
Immunogloblin M (mg/ml)		0 h	1.28	1.58	1.40	0.23
	3 h	1.35	1.62	1.49	0.18	0.64
	6 h	1.42	1.69	1.53	0.16	0.56
	12 h	1.43	1.76	1.61	0.24	0.61
	24 h	1.35	1.60	1.4	0.18	0.69