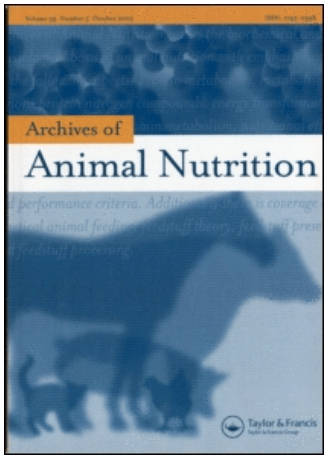

Archives of Animal Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453455

Effects of fermentation products of **Ganoderma lucidum** on growth performance and immunocompetence in weanling pigs

Su-Der Chen [a]; Meng-Chen Hsieh [a]; Ming-Tang Chiou [b]; Yu-Shen Lai [a]; Yeong-Hsiang Cheng [a]
[a] Institute of Biotechnology, National I-Lan University, I-Lan, Taiwan
[b] Department of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan

Online Publication Date: 01 February 2008
To cite this Article: Chen, Su-Der, Hsieh, Meng-Chen, Chiou, Ming-Tang, Lai, Yu-Shen and Cheng, Yeong-Hsiang (2008) 'Effects of fermentation products of **Ganoderma lucidum** on growth performance and immunocompetence in weanling pigs', Archives of Animal Nutrition, 62:1, 22 - 32
To link to this article: DOI: 10.1080/17450390701780201
URL: http://dx.doi.org/10.1080/17450390701780201

Archives of Animal Nutrition
Vol. 62, No. 1, February 2008, 22–32

Effects of fermentation products of *Ganoderma lucidum* on growth performance and immunocompetence in weanling pigs

Su-Der Chen[a], Meng-Chen Hsieh[a], Ming-Tang Chiou[b], Yu-Shen Lai[a] and Yeong-Hsiang Cheng[a]*

[a]*Institute of Biotechnology, National I-Lan University, I-Lan, Taiwan;* [b]*Department of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan*

(*Received 19 June 2007; accepted 19 October 2007*)

The purpose of this study was to test fermentation, for its products of a Chinese medicinal mushroom, *Ganoderma lucidum*, cultured by submerged fermentation for its effect on growth performance and immunocompetence in weanling piglets. In Experiment 1, 72 weanling piglets were allotted to one of four treatments receiving these fermentation products (GLF, expressed as amount of β-glucans) at 0 (control), 50, 100, and 150 mg/kg feed for 4 weeks. The results showed that at a supplementation level of 50 mg/kg feed, GLF caused the best growth performance, the highest pseudorabies antibody titre, and a decrease of blood glucose level. It was also demonstrated that GLF up-regulated the cell-mediated immune response related cytokines (IL-2, IFN-γ, and TNF-α) expression in different lymphoid tissues. After challenging with porcine circovirus (PCV) type 2 (Experiment 2), a supplementation with 50 mg GLF per kg feed also inhibited PCV-2 virus amplification, and ameliorated lymphocyte depletion in different lymphoid tissues. Conclusively, feed supplemented with GLF at 50 mg/kg could be beneficial to counteract the physiological stress in weanling piglets.

Keywords: *Ganoderma lucidum*; Chinese herb; growth; immunocompetence; pigs

1. Introduction

Ganoderma lucidum (Lingzhi in Chinese, Reishi in Japanese) has been used in China to prevent and treat various human diseases for a long time. Lingzhi was classified as a drug of ''high grade'', that is a medicinal herb without toxicity in the Shen Nong's Materia Medica (Shen Nong Ben Cao Jing), which was published in the second century BC by Li Shi-Jen, a well-known ancient Chinese medicinal scientist. The efficacy and medical uses of Lingzhi was also described in the classic Compendium of Material (Ben Cao Gang Mu) in the sixteenth century.

Polysaccharides are believed to be one of the efficacious components of *G. lucidum* (Lin 2005). A number of reports have demonstrated that *G. lucidum* polysaccharides modulate immune function both *in vivo* and *in vitro* by promoting the function of antigen-presenting cells, mononuclear phagocyte system, humoral immunity, and cellular immunity (Adachi et al. 1994; Mao et al. 1999; Lai et al. 2004; Lin 2005).

β-glucan (1,3-β-D-glucan), is a heterogenous group of glucose polymers present as structural elements in the cell walls of yeasts, and other fungi including fermentation

*Corresponding author. Email: yhcheng@niu.edu.tw

ISSN 1745-039X print/ISSN 1477-2817 online

DOI: 10.1080/17450390701780201
http://www.informaworld.com

products of *G. lucidum*. The β-glucans have been reported to inhibit tumour formation (Ohno et al. 1995), enhance defence against bacterial challenge (Onderdonk et al. 1992; Babineau et al. 1994), and increase growth performance (Schoenherr et al. 1994) in pigs. Glucans from a variety of yeast cell walls have been shown to stimulate both specific and non-specific immune responses (Dritz et al. 1995). *In vitro* studies have shown that β-glucans influence macrophage morphology (Burgaleta et al. 1978), increase nitric oxide production (Ohno et al. 1996), and stimulate the release of cytokines such as TNF-α, IL-6, IL-1, and IL-2 via macrophages (Adachi et al. 1994; Chen et al. 2003). Glucans can also inhibit the inflammatory cytokine responses, thereby allowing nutrients to be partitioned toward the growth demand (Klasing et al. 1987).

Porcine circovirus type 2 (PCV-2) is an important viral infectious disease in pigs, which induces lymphocytes apoptosis, lymphocyte depletion, over-expression of interleukin-10, and consequently cause T cells immune suppression and secondary infection (Darwich et al. 2003). Although β-glucans have not been widely used in animal production, Liu and Li (1999) reported that lentinan (a type of glucan) has effects on enhancing interleukin-2 (IL-2) production in broilers infected with Marek's virus. This result suggests that lentinan may be useful for treating against Marek's disease and other viral diseases because of its immunostimulating activities. In our broiler experiments, β-glucan at dosages of 250 and 500 mg/kg feed could increase the chemotaxis and phagocytosis capability of macrophages (Cheng et al. 2004). In the current study, β-glucan present in fermentation products from *G. lucidum* was tested as a feed supplement to evaluate its efficacy on growth performance and immunocompetence in weanling piglets suffering from physiological stress during weaning.

2. Materials and methods

2.1. *Fermentation of* G. lucidum *and estimation of β-glucans*

The mycelium of *G. lucidum* (CCRC 36421) was grown by submerged fermentation at 28°C for 14 days. After drying and grinding, the content of polysaccharides and β-glucan in the fermentation product was assessed by fluorescence microassay. Briefly, the ethanol insoluble portion was re-suspended in 1 N NaOH. To 0.3 ml of the curdlan standard solution and pre-treated sample solution 30 μl of 6 N NaOH was added, then mixed well and heated at 80°C for 30 min. Then the solutions were cooled immediately on ice for 2 min and aniline blue was added to react at 50°C for 30 min. Finally, samples were cooled down to room temperature for 30 min and then the fluorescence intensity was measured at an excitation wavelength of 398 nm and an emission wavelength of 502 nm by using a fluorescent spectrometer (Ko and Lin 2004). The concentration of polysaccharides and β-glucans in the fermentation products of *G. lucidum* (GLF) was 19.08 ± 3.23% and 7.25 ± 0.03%, respectively. In the present study, GLF was incorporated into feeds on the basis of its β-glucan content and was calculated on mg/kg basis.

2.2. *Animals and experimental design*

Experiment 1. A total of 72 weanling piglets at three weeks of age were randomly allocated to four different treatment groups. The four treatments ($n = 6$ per group, three replicates) were supplemented with 0 (control), 50, 100 and 150 mg GLF per kg feed, respectively. Water and feed was provided *ad libitum* throughout the entire experiment. The experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the National I-Lan University. Body weights were determined at weaning and

after two and four weeks of experimental feeding. Feed intake was calculated per pen every week. To evaluate the effects of GLF supplements on specific immune response, all animals were vaccinated against pseudorabies on day 1 after weaning.

Experiment 2, consisted of two treatment groups, a control and a group given 50 mg GLF per kg feed (6 pigs per treatment). Pigs of both groups were all challenged with PCV-2 by intranasal and intramuscular route at three weeks of age. Specific pathogen free piglets previously identified as PCV-2 negative by PCR, were inoculated intranasally with $10^{5.2}$ $TCID_{50}$ PCV-2, and $10^{4.5}$ $TCID_{50}$ PCV-2 by intramuscular injection at the first day of experiment. The survival rate and cytokines interleukin (IL)-1, IL-2, IL-4, IL-10, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) in three major lymphoid tissues (spleen, inguinal and mandible lymph node), and PCV-2 nucleic acid were determined by RT-PCR and PCR, respectively.

2.3. Antibody titres, blood biochemistry and haptoglobin

Pigs were vaccinated subcutaneously with one dose of pseudorabies vaccine at weaning and received a booster two weeks later. Blood samples were taken on day 14 and 28 from four randomly selected pigs of each treatment.

The pseudorabies antibody titres were measured with ELISA (IDEXX, MAINE, USA) and the data were expressed as log2 titre basis. For blood biochemistry, blood from three pigs per treatment was collected and centrifuged at 2654 *g* for 10 min. The sera were collected for the determination of total protein, albumin, globulin, γ-glutamyltransferase (GGT, EC 2.3.2.2), aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2), by automatic clinical chemistry analyser (Roche, Cobus-Mira-Plus, Roche Diagnostic System Inc. USA). Haptoglobin was determined according to Makimura and Suzuki (1982) with minor modifications.

2.4. Alveolar macrophages functions

Alveolar macrophages were collected from the pigs by bronchi alveolar lavage with phosphate-buffered saline. The function of alveolar macrophages chemotaxis was measured by the Boyden chamber technique. A cell culture containing a polyethylene terephthalate membrane with a pore size of 3 μm served as the upper chamber and was placed in one well of a 24-well cell culture plate (Costar, Cambridge, Mass., USA), which served as the lower chamber. A total of 800 μl of DMEM (Dulbecco's modification of Eagle's medium) with 10, 1, or 0.1% of pooled normal serum from conventionally housed pigs as the chemo-attractant was added in the lower chamber; DMEM without normal serum was used as a negative control. Alveolar macrophages (10^6/ml) were incubated at 37°C for 30 min in DMEM without phenol red. After incubation, 200 μl of the macrophage suspension was added to the upper chamber and the macrophages were allowed to migrate for 1–2 h at 37°C in a 5% CO_2 atmosphere. After removal of the cell culture insert, 200 μl of MTT (3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide) solution was added to the lower chamber. After 4 h of MTT reduction by macrophages, the cells were lysed by adding 1 ml of 20% (wt/vol) sodium dodecyl sulfate – 50% dimethyl formamide (pH 4.7). The absorbance of each well was read at 595 nm wavelength using spectrophotometer. Chemotaxis was expressed as a chemotactic index, which was obtained by dividing the value for chemoattracted macrophages by the value for randomly migrated macrophages in the negative (normal saline) control.

For the phagocytic assay, the alveolar macrophage coverslip cultures were incubated with 2 ml of *Candida albicans* suspension (1×10^7/ml) in a complete medium. After 60 min of incubation, the coverslips were washed with sterile saline, fixed in methanol, and then stained with May-Grunwald-Giemsa stain. The percentages of phagocytoxic macrophages and average number of internalised *Candida albicans* per phagocytic macrophage were determined by scoring stained coverslip cultures under microscope at 1000× magnification. A total of 200 macrophages were scored per coverslip from three pigs of each treatment.

2.5. Total RNA and RT-PCR

Total RNA from spleen tissue was extracted by Ultraspec and reverse transcription was performed. Various cytokine oligonucleotides were applied to amplify specific amplicons of the reverse transcripted cDNA followed previous described (Darwich et al. 2003) with minor modification. The RT-PCR products was loaded in 1% agarose gel for electrophoresis analysis, the signal of agarose gel with EtBr stain was analysed by real-time image capture for quantitative analysis with a control of β-actin. The cytokine gene expressed as relative intensities of β-actin.

2.6. Virus inoculation and identification

The PCV-2 strain was isolated from an outbreak in a pig farm in Taiwan. The virus was isolated from lymphoid nodes and mixed with 10% of emulsified solution, then, after centrifugation and micro-filtration, was inoculated into a PCV-1-free PK-15 cell line. For additional culture of 3 days, the supernatant was collected for virulence titre test. PCV-2 in lymphoid tissues (spleen, inguinal and mandible lymphonode) were detected by multiplex PCR method as Pallares et al. (2002) described. Briefly, primer 1C478 (PCV-1): 5′CCG CGG GCT GGC TGA ACT T3′; primer 2C16R (PCV-2): 5′ACC CCC GCC ACC GCT ACC3′; a common primer 1C1108 (PCV common): 5′CTC GGC TAT GCG CTC CAA AAT G3′, for detecting of PCV-1 (652 bp amplicon) and PCV-2 (1154 bp amplicon) was used, respectively.

2.7. Statistical analysis

All data were analysed using the General Linear Models procedure of SAS (SAS 1989). The results were expressed as the percentage subjected to logarithmic transformation prior to analysis of variance. The significance of the differences between individual means was determined by Duncan's new multiple range test.

3. Results and discussion

3.1. Growth performance

The growth performance of piglets weaned at three weeks of age is shown in Figure 1. As shown in Figure 1A, 50 mg GLF/kg feed caused the best average daily gain (ADG) after 2 and 4 weeks of supplementation. However, increasing the dose of GLF failed to show this positive effect and after 4 weeks feeding the ADG dropped to the control level. There were no effects on feed intake when diets were supplemented with 50 or 100 mg GLF/kg (Figure 1B), but the highest dose of 150 mg GLF/kg significantly inhibited feed intake (Figure 1B). This may be related to the bitter ingredients in GLF, such as secondary

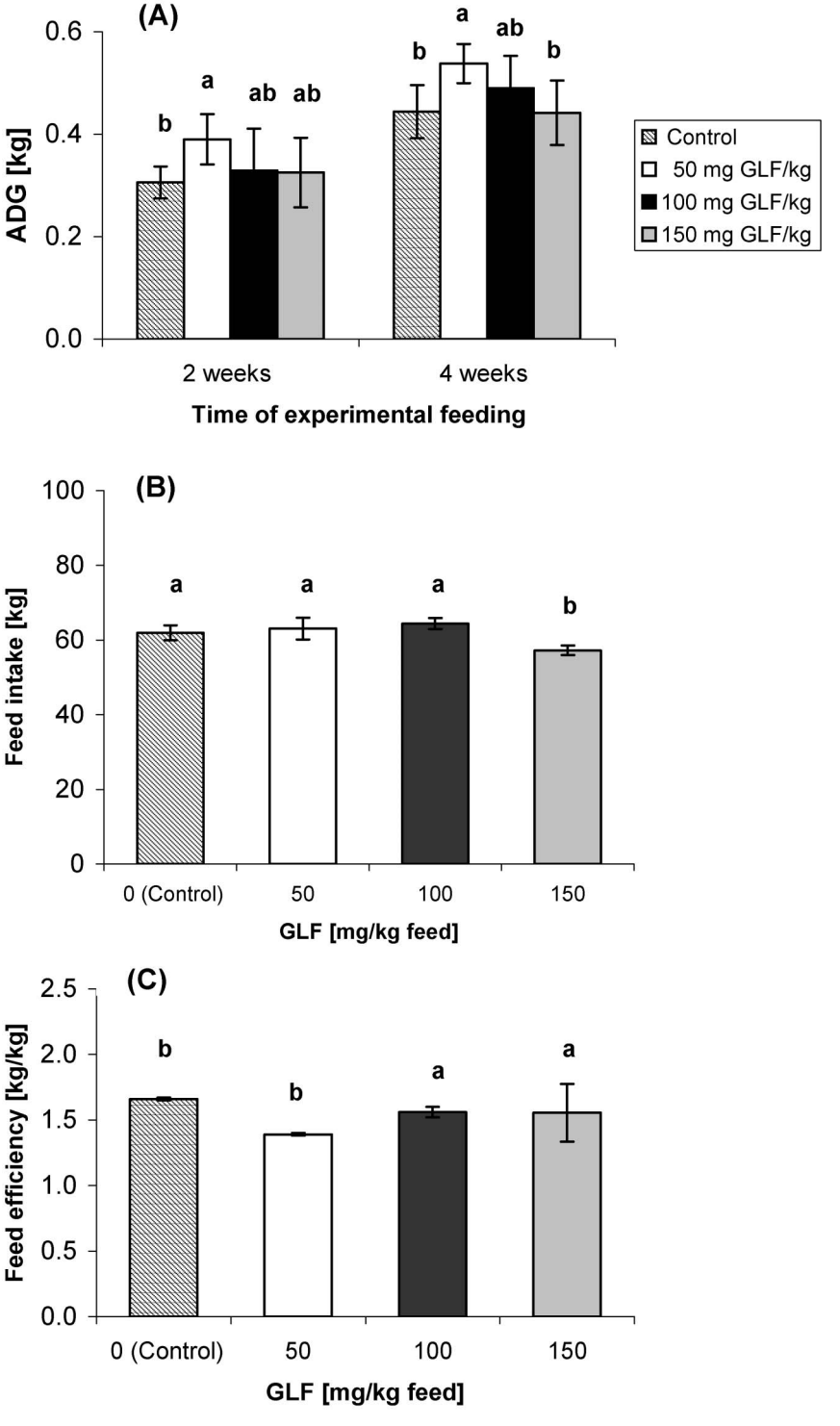

Figure 1. Effects of fermentation products of *Ganoderma lucidum* (GLF) on average daily gain (A), feed intake (B) and feed efficiency (C) of weanling pigs after 4 weeks experimental feeding (Experiment 1). Means not sharing the same superscript differ significantly at $p < 0.05$.

metabolites, as for instance triterpenoids. The effects of GLF supplementation on feed efficiency are summarised in Figure 1C. The results showed that 50 mg GLF/kg improved the feed efficiency significantly, which was again not observed with higher levels of GLF. Therefore, 50 mg GLF/kg was applied in the second experiment described below. Thus, our current results showed that the zootechnical performance could be significantly increased by 50 mg GLF/kg. However, no further improvement or even a negative effect on feed intake occurred by increasing levels up to 150 mg GLF/kg.

Beneficial effects of GLF have been previously documented in animals, such as mice, rats, rabbit and even human beings. However, there is hardly any evidence to prove that GLF has a growth promotion activity in pigs or other domestic animals except horses (Lai et al. 2004). A similar study showed that β-glucan from yeast can improve ADG and feed intake at a supplementation level of 250 mg/kg (Dritz et al. 1995). However, with doses up to 1000 mg/kg, feed intake and ADG were significantly decreased compared to the control group. Hiss and Sauerwein (2003) have demonstrated that β-glucan at 300 mg/kg increased feed intake in weaned pigs. Our results showed that the growth performance was not proportional to the supplemented level of GLF. Bitter components, e.g. ganodermic acid and terpenoids (Nishitoba et al. 1985) may have caused the poor performance at high levels of supplementation.

3.2. *Evaluation of immune functions*

The variable effects of different dosages of GLF on the functions of macrophages are summarised in Table 1. The results show that the addition of 50–150 mg GLF/kg significantly enhanced the chemotaxis index, however, the chemotaxis index was not dose dependent. Furthermore, there were no significant effects on the percentage of phagocytic macrophages and phagocytosis ability per macrophage.

To investigate the antibody response under the influence of GLF, piglets were vaccinated with a pseudorabies vaccine. The results of antibody production are summarised in Table 2. They show that GLF did not have any beneficial effects on primary antibody titres against pseudorabies antigen (day 14 of experiment). However, the secondary antibodies (day 28 of experiment) were significantly increased in GLF-treated animals when compared with the control group. The immuno-modulatory activity of *G. lucidum* is well established in mice as an experimental model, where *G. lucidum* can activate humoral immunity as well as the cell-mediated immune response (Lai et al. 2004; Lin and Zhang 2004; Lin 2005).

Table 1. Effects of fermentation products of *Ganoderma lucidum* (GLF) on the functional profile of alveolar macrophages in weanling pigs (Experiment 1) (Means $\pm$ SD).

	GLF [mg/kg feed]			
	0 (Control)	50	100	150
Chemotaxis* [%]	3.05 ± 0.42^{b}	3.24 ± 0.81^{a}	4.42 ± 0.69^{a}	4.20 ± 0.43^{a}
Phagocytotic [%]	27.6 ± 3.10	25.8 ± 2.40	25.6 ± 4.30	25.2 ± 3.30
Number of *C. albicans*$^{\#}$	1.81 ± 0.58	1.51 ± 0.21	1.17 ± 0.17	2.02 ± 0.59

*Chemotaxis was expressed as a chemotactic index, which was obtained by dividing the value for chemo-attracted macrophages by the value of randomly migrated macrophages in the negative control; $^{\#}$Average phagocytised number of *C. albicans* per 200 macrophages was scored from three pigs of each treatment. Means not sharing the same superscript differ significantly at $p < 0.05$.

3.3. Blood biochemistry and haptoglobin

Selected markers of blood biochemistry of weaners after ingestion of GLF are listed in Table 3. There was no significant difference in the tested parameters, except for blood glucose levels, which were significantly decreased. In a previous report it was already revealed that GLF possesses hypoglycemic potential by increasing the plasma insulin level in mice (Hikino et al. 1985), suggesting that the regulating mechanism is through its insulin-releasing activity via facilitating the calcium inflow to the pancreatic β-cells (Zhang and Lin 2004).

Hapotoglobin, an acute phase protein in plasma, is often chosen as a biomarker of primary inflammation (Dritz et al. 1995; Hiss and Sauerwein 2003). As demonstrated in Figure 2, GLF supplemented animals had significantly elevated hapotoglobulin concentrations up to 2-fold compared to the control group. Hiss and Sauerwein (2003) reported that feeding various levels of dietary β-1,3-glucan to weanling piglets caused, an inverse relationship between haptoglobin concentration and ADG. Similar findings were obtained in the current study, which revealed that after feeding 150 mg GLF/kg, the concentration of haptoglobin was significantly higher than at 50 mg GLF/kg. At the same time lower ADG and feed efficiencies were observed. Nevertheless, a chronic inflammation elicits the mediator of prostaglandin E2 synthesis from polyunsaturated fatty acids in cell membrane, and consequently, is expected to inhibit feed intake in animals (Cheng et al. 2004).

Table 2. Effects of fermentation products of *Ganoderma lucidum* (GLF) on pseudorabies antibody titres in vaccinated weanling pigs (Experiment 1) ($n = 4$)*.

	GLF [mg/kg feed]			
	0 (Control)	50	100	150
Time of blood sampling				
Day 14 of experiment	0.62 ± 0.08	0.58 ± 0.10	0.57 ± 0.10	0.63 ± 0.11
Day 28 of experiment	6.19 ± 0.86[b]	6.24 ± 0.34[a]	7.12 ± 0.71[a]	7.17 ± 0.34[a]

*Challenged with pseudorabies inactivated vaccine at day 7 and 14 after experiment started. Antibody titres were subjected to log2 transformation, Serum was collected from thoracic vena cava. Means not sharing the same superscript differ significantly at $p < 0.05$.

Table 3. Effects of fermentation products of *Ganoderma lucidum* (GLF) on blood biochemistry determined in weanling pigs (Experiment 1).

	GLF [mg/kg feed]			
	0 (Control)	50	100	150
Creatinine [mg/l]	1.00 ± 0.11	0.97 ± 0.09	0.67 ± 0.32	0.70 ± 0.24
AST* [U/l]	72.0 ± 27.4	58.5 ± 21.4	64.2 ± 31.9	95.0 ± 78.5
ALT* [U/l]	53.2 ± 14.4	70.7 ± 14.0	52.5 ± 10.7	62.7 ± 18.89
Glucose [mg/100 ml]	131.0 ± 8.85[a]	112.0 ± 11.1[b]	114.7 ± 8.18[b]	109.0 ± 8.18[b]
Cholesterol [mg/100 ml]	90.2 ± 7.27	82.0 ± 8.75	82.2 ± 6.18	93.7 ± 19.4
Triglycerides [mg/100 ml]	46.0 ± 12.75	46.7 ± 11.5	36.7 ± 9.2	54.5 ± 16.8

*AST, aspartate transaminase; ALT, alanine transaminase; (Means not sharing the same superscript differ significantly at $p < 0.05$).

3.4. PCV-2 challenge and cytokine profile

In our second experiment, the gene expression profiles for selected cytokines in spleen were investigated and the results are shown in Figure 3. When GLF was incorporated into diets,

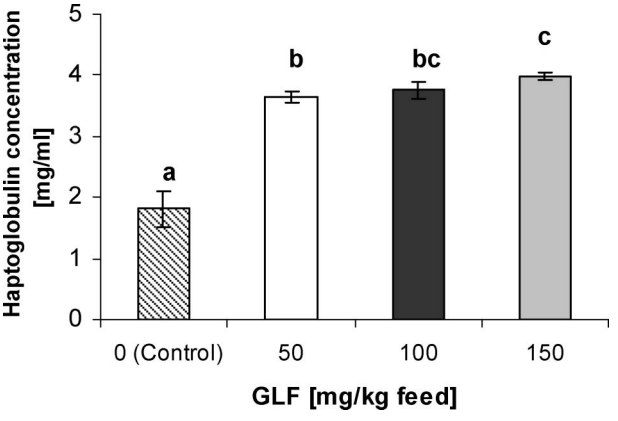

Figure 2. Effect of fermentation products of *Ganoderma lucidum* (GLF) on serum haptoglobin levels in weanling pigs (Experiment 1). Means not sharing the same superscript differ significantly at $p < 0.05$.

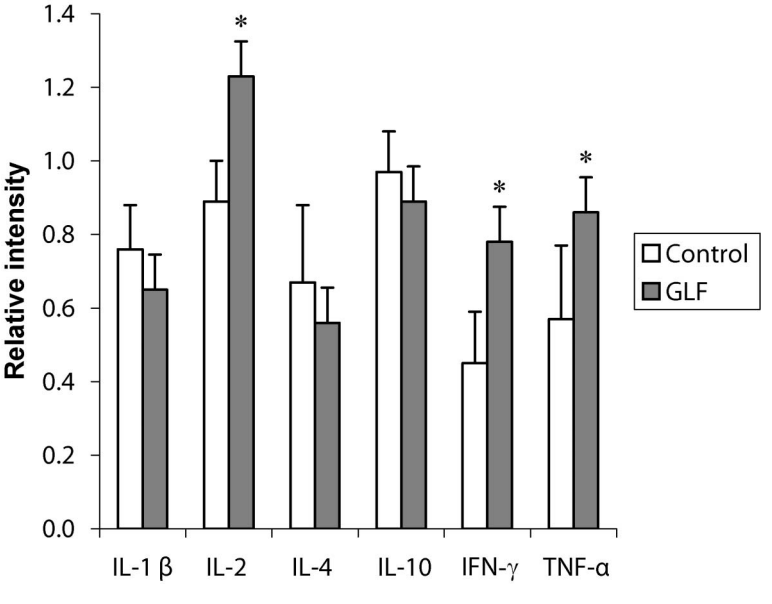

Figure 3. Effects of selected cytokine expression in spleen. Comparison between control group and group receiving fermentation products of *Ganoderma lucidum* (GLF) at 50 mg/kg. (Experiment 2) (IL = interleukin; IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α). The values are expressed as mean $\pm$ SD of three independent determinations. *Means differ significantly between control and GLF ($p < 0.05$).

the cytokines of IL-2, INF-γ and TNF-α mRNA expression relative to β-actin were significantly up-regulated. These results demonstrated that GLF could induce cytokines of the Th1 subsets and modulate the cell-mediated immune response. This latter effect should become more apparent in a challenge experiment using porcine circovirus. The significantly increased of INF-γ and TNF-α expression may enhance the anti-virus ability of the host, and humoral immune response network are also modified by an increase of IL-2 level. Cytokines play an important role in the network of immune responses and account for communication, activation, maturation and differentiation among immune cells. Taken together, this study suggests that GLF can modify gene expression of cytokines and might induce disease resistance in animals.

In Figure 4, the results of a challenge experiment with PCV-2 via intranasal inoculation at a dosage of $10^{5.2}$ $TCID_{50}$ are shown. Two pigs in the challenge group (not GLF supplemented) showed obviously a respiratory syndrome and typical signs of hypersensitivity dermatitis, which were induced by viremia. By PCR the PCV-2 nucleic acid of the virus was detected in different lymphoid tissues including inguinal, mandible and spleen (Figure 4). Interestingly, no virus specific nucleic acid was detected in GLF groups and the histopathological examination showed only mild pathologic lesions. Moreover, in the control animal lymphocyte depletion, necrosis and haemorrhage in lymphoid tissues were observed in microscopic examination (data not shown).

Previous studies have demonstrated that crude water-extracted polysaccharides from fresh fruiting bodies of *G. lucidum* can potentiate the production of cytokines including IL-1β, IL-6, IFN-γ and TNF-α in human macrophages (Han et al. 1998). The crude water-extract of *G. lucidum* also induced the expression of cytokines including IL-10 and TNF-α, IL-1β, IL-6 and IL-2 mRNA in human peripheral blood mononuclear cells (Mao et al. 1999). However, we found that GLF can stimulate IL-2, IFN-γ and TNF-α production in weanling pigs moderately. The immunomodulatory effects of GLF on the expression of cytokines can show two ways of modulation of Th1 and Th2. However, over-expression of

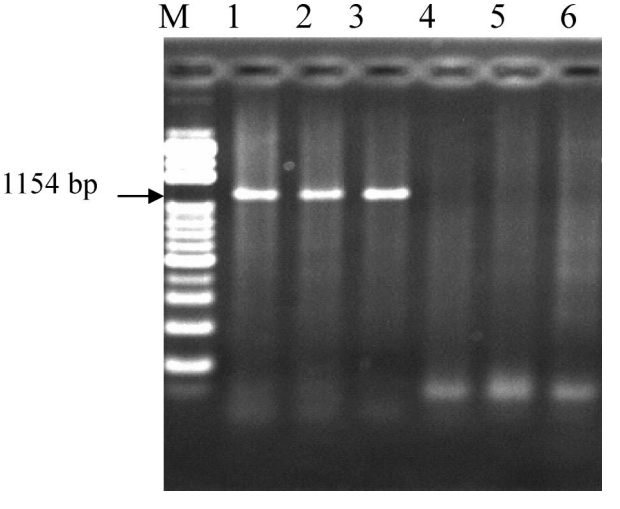

Figure 4. Detection of PCV-2 nucleic acid in lymphoid tissue of weanling pigs after challenge with PCV-2 virus and feeding without or with fermentation products of *Ganoderma lucidum* (GLF) at 50 mg/kg for 4 weeks. M; 100 bp ~ 1400 bp DNA marker; lane 1–3, spleen, inguinal and mandible lymph node without GLF; lane 4–6, spleen, inguinal and mandible lymph node with 50 mg GLF/kg.

the pro-inflammatory cytokines could have detrimental effects on the growth performance and health status (Spurlock 1997). Furthermore, the increase of cytokine secretion results in nutrient repartitioning from growth towards immune response (Johnson 1997), which is a disadvantage from an economic point of view.

4. Conclusion

Our study suggests that supplementation of fermentation products of *G. lucidum* at 50 mg/kg, can improve growth performance and produces an increase on chemotaxis ability of alveolar macrophages and elevated secondary antibody titres when challenged with pseudorabies vaccine. Furthermore, no side-effects are caused by GLF at tested dosages with respect to the majority of biochemical measurements except for a weak hypoglycaemic response. However, our present results demonstrate that significantly higher haptoglobulin levels are detected after GLF supplementation, implicating GLF would induce an inflammatory signal to initiate the immune network in piglets. Therefore, *G. lucidum* has the potential as an immune modulator at appropriate supplementation levels and feeding duration. In particular, the alterations in gene expression of cytokines and the results of the PCV-2 challenge experiment suggest that a potent antiviral activity may be due to the modulation of the cytokine profile and enhancing the virus elimination by the addition of GLF in feeds.

Acknowledgements

This study was supported by Council of Agriculture Taiwan, under the project of enhancement of pigs rearing percentage grand 94AS- 4.1.1– AD-U1. The authors thank Prof. C. F. Weng for his critical reading and suggestions to this manuscript.

References

Adachi Y, Okazaki M, Ohno N, Yadomae T. 1994. Enhancement of cytokine production by macrophages stimulated with (1,3)-β-D-glucan, grifolan (GRN), isolated from *Grifola frondosa*. Biol Pharm Bull. 17:1554–1560.

Babineau TJ, Marcello P, Swails A, Kenler B, Bistrian C, Forse RA. 1994. Randomised phase I/II trial of a macrophage-specific immunomodulator (PGG, Glucan) in high-risk surgical patients. Ann Surg. 220:601–609.

Burgaleta C, Territo MC, Quan SG, Golde DW. 1978. Glucan-activated macrophages: Functional characteristics and surface morphology. J Reticuloendothel Soc. 23:195–204.

Chen HL, Li DF, Chang BY, Gong LM, Piao XS, Yi GF, Zhang JX. 2003. Effects of lentinan on broiler splenocyte proliferation, interleukin-2 production, and signal transduction. Poultry Sci. 82:760–766.

Cheng YH, Lee DN, Wen CM, Weng CF. 2004. Effects of β-glucan supplementation on lymphocyte proliferation, macrophage chemotaxis and specific immune responses in broilers. Asian Austral J Anim Sci. 17:1145–1149.

Darwich L, Pié S, Rovira A, Segalés J, Domingo M, Oswald IP, Mateu E. 2003. Cytokine mRNA expression profiles in lymphoid tissues of pigs naturally affected by postweaning multisystemic wasting syndrome. J Gen Virol. 84:2117–2125.

Dritz SS, Shi J, Kielian TL, Goodband RD, Nelssen JL, Tokach MD, Chengappa MM, Smith JE, Blecha F. 1995. Influence of dietary β-glucan on growth performance, nonspecific immunity, and resistance to *Streptococcus suis* infection in weanling pigs. J Anim Sci. 73:3341–3350.

Han MD, Lee ES, Kim Y, Lee JW, Jeong H, Yoon KH. 1998. Production of nitric oxide in RAW264.7 macrophages with ganoderan, the β-glucan of *Ganoderma lucidum*. Korean J. Mycol. 26:246–255.

Hikino H, Konno C, Mirin Y, Hayashi T. 1985. Isolation and hypoglycemic activity of *Ganoderma*s A and B, glycans of *Ganoderma lucidum* fruit bodies. Planta Med. 4:339–340.

Hiss S, Sauerwein H. 2003. Influence of dietary ss-glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentration in pigs. J Anim Physiol Anim Nutr. 87:2–11.

Johnson RW. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J Anim Sci. 75:1244–1255.

Klasing KC, Laurin DE, Peng RK, Fry DM. 1987. Immunological mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. J Nutr. 117:1629–1637.

Ko YT, Lin YL. 2004. 1,3-β-Glucan quantification by a fluorescence microassay and analysis of its distribution in foods. J Agr Food Chem. 52:3313–3318.

Lai SW, Lin JH, Lai SS, Wu YL. 2004. Influence of *Ganoderma lucidum* on blood biochemistry and immunocompetence in horses. Am J Chinese Med. 32:931–940.

Lin SB, Zhang HN. 2004. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanism. Acta Pharm Sinic. 25:1387–1395.

Lin ZB. 2005. Cellular and molecular mechanisms of immunomodulation by *Ganoderma lucidum*. J Pharm Sci. 99:144–153.

Liu YJ, Li QZ. 1999. Effect of Lentinan and astragalan on IL-2 inductive activity and lymphocyte proliferation in chicks infected with vMDV. Chinese J Vet Med. 25:3–5.

Makimura S, Suzuki N. 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. Nippon Juigaku Zasshi. 44:15–21.

Mao T, van De Water J, Keem CL, Stern JS, Hackman R, Gershwin ME. 1999. Two mushrooms, *Grifola frondosa* and *Ganoderma lucidum*, can stimulate cytokine gene expression and proliferation in human T lymphocytes. Int J Immunother. 15:13–22.

Nishitoba T, Sato H, Sakamura S. 1985. New terpenoids from *Ganoderma lucidum* and their bitterness. Agric Biol Chem. 49:1547–1549.

Ohno N, Miura NN, Chiba N, Adachi Y, Yadomae T. 1995. Comparison of the immunopharmacological activities of triple and single-helical schizophyllan in mice. Biol Pharm Bull. 18:1242–1247.

Ohno N, Egawa Y, Hashimoto T, Adachi Y, Yadomae T. 1996. Effect of beta-glucans on the nitric oxide synthesis by peritoneal macrophage in mice. Biol Pharm Bull. 19:608–612.

Onderdonk AB, Cisneros RL, Hinkson P, Ostroff G. 1992. Anti-infective effect of poly-beta 1-6-glucotriosyl-beta 1-3-glucopyranose glucan *in vivo*. Infect Immun. 60:1642–1647.

Pallares FJ, Halbur PG, Opriessnig T, Sorden SD, Villar D, Janke BH, Yaeger MJ, Larson DJ, Schwartz KJ, Yoon KJ, Hoffman LJ. 2002. Porcine circovirus type 2 (PCV-2) coinfections in US field cases of postweaning multisystemic wasting syndrome (PMWS). J Vet Diagn Invest. 14:515–519.

SAS. 1989. SAS/STAT User's guide. Release 6.03 Edt. Cary (NC): SAS Institute Inc. 1028 pp.

Schoenherr WD, Pollmann DS, Coalson JA. 1994. Titration of MacroGrad-S on growth performance of nursery pigs. J Anim Sci. 72(Suppl. 2):57.

Spurlock ME. 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J Anim Sci. 75:1773–1783.

Zhang HM, Lin ZB. 2004. Hypoglycemic effect of *Ganoderma lucidum* polysaccharides. Acta Pharm Sinic. 25:191–195.