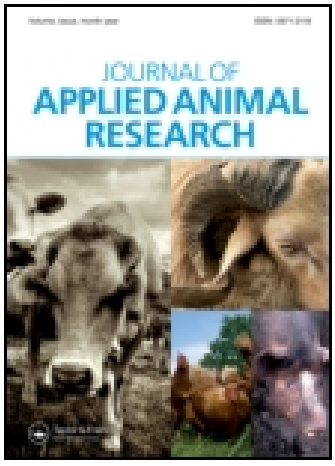

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J.C. Han[a], H.X. Qu[a], J.G. Wang[a], Y.F. Yan[a], J.L. Zhang[a], L. Yang[a], M. Zhang[a] & Y.H. Cheng[b]

[a] Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu, P.R. China

[b] Department of Biotechnology and Animal Science, National Ilan University, I-Lan, Taiwan, Republic of China

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Effects of fermentation products of *Cordyceps militaris* on growth performance and bone mineralization of broiler chicks

J.C. Han[a]*, H.X. Qu[a], J.G. Wang[a], Y.F. Yan[a], J.L. Zhang[a], L. Yang[a], M. Zhang[a] and Y.H. Cheng[b]*

[a]*Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu, P.R. China;* [b]*Department of Biotechnology and Animal Science, National Ilan University, I-Lan, Taiwan, Republic of China*

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The present study aimed to evaluate the effects of fermentation products of *Cordyceps militaris* (FPCM) on growth performance and tibia mineralization of broiler chicks. A total of 240 one-day-old female Ross 308 broilers were allotted into four treatments with six cages of 10 chicks each. Four diets were the control diet supplemented with 0, 1, 2 and 4 g/kg of FPCM, respectively. Compared with the control, supplementation of 1 g/kg FPCM improved body weight gain of broilers from 22 to 42 days of age and from 1 to 42 days of age ($P < 0.05$); 4 g/kg of FPCM enhanced the feed intake of 22- to 42-day-old and 1- to 42-day-old chicks ($P < 0.05$). Addition of 2 g/kg FPCM increased tibia calcium (Ca) content of broilers at 21 and 42 days ($P < 0.01$). FPCM did not affect serum biochemical parameters ($P > 0.05$). These data indicate that FPCM had positive effects on growth performance of broiler chicks and the optimal dietary FPCM level was 1 g/kg in chicks from 1 to 42 days of age.

Keywords: fermentation products of *Cordyceps militaris*; broiler chick; growth performance; bone mineralization

1. Introduction

In the past several decades, antibiotics have been used in poultry diets for promoting growth and control of diseases. However, the use of antibiotics as growth-promoting agents for poultry has been banned in many countries because of antimicrobial resistance and antibiotics residuals in poultry products.

Prebiotics, herbs and plant extracts have gained interest as alternatives to antibiotics. *Cordyceps militaris*, a fungus invading *Lepidoptera* larvae, is used as an herb in China. Their chemical components included cordycepin and polysaccharides (Ohta et al. 2007). Fermentation products of *C. militaris* (FPCM) are produced to improve immune function and growth performance of animals in feed industry. Cordycepin and polysaccharides are also the bioactive components of FPCM. According to the definition of Scholz-Ahrens et al. (2007), cordycepin and polysaccharides in FPCM are known as prebiotics in animal nutrition.

Research has shown that cordycepin or *Cordyceps sinensis* modulated the systemic immune system by upregulating IL-10, IL-1β, IL-6, IL-8 and TNF-α of mice and human (Koh et al. 2002; Zhou et al. 2008). A hot water extract of mycelia from *C. sinensis* significantly increased growth rate of broiler chicks (Koh et al. 2003). These data indicate that cordycepin or *C. sinensis* has a positive effect on immune and growth of animals.

Non-digestible oligosaccharides (fructooligosaccharides [FOS] and inulin) enhanced calcium (Ca) absorption and bone mineralization in laying hens (Chen & Chen 2004), rats (Zafar et al. 2004) and young people (Cashman 2006). However, another research showed that FOS or inulin do not improve the bone growth and skeletal integrity of broiler chicks (Kim et al. 2011; Swiatkiewicz et al. 2011). These data suggest that oligosaccharides may modulate animal mineral metabolism.

The response of broilers to cordycepin or polysaccharides in FPCM has not been examined. Therefore, the present study was to evaluate the effect of FPCM on growth performance and tibia mineralization of broiler chicks.

2. Material and methods

All of the procedures used in the present study were approved by the Animal Care Committee of Shangqiu Normal University.

2.1. *Fermentation products of* C. militaris

C. militaris (BCRC 32219) mycelium was grown on a grain substrate. Cordycepin content in fermentative products after drying and grinding was assessed by high-performance liquid chromatography (HPLC). After filtration, the samples were subjected to cordycepin analysis

*Corresponding authors. Emails: yhcheng@niu.edu.tw; j.c.han@hotmail.com

via SPD-10A HPLC (Shimadzu, Japan) with a pre-packed LiChrospher 100 and RP-18 column (Merck, Darmstadt, Germany). The mobile phase contained a mixture of methanol and 0.02 M KH_2PO_4 (15:85). Elution was performed at a flow rate of 1 mL/min and was determined using a variable wavelength UV detector (10A VP, Shimadzu, Japan) at 254 nm. The cordycepin content of the FPCM obtained in the present experiment was 5.09 mg/g.

2.2. Animals and sampling

On the day of hatching, 240 female Ross 308 broiler chicks were individually weighed and randomly allotted into four treatments with six starter stainless steel cages (70 cm × 70 cm × 30 cm) of 10 broilers each. From 14 to 42 days, the broilers were transferred and reared in growing-finishing stainless steel cages (190 cm × 50 cm × 35 cm). Four diets were the control diet supplemented with 0, 1, 2 and 4 g/kg of FPCM, respectively (Table 1). The broilers were provided with mash feed and water *ad libitum*. The first Newcastle disease virus (NDV) vaccination was conducted via muscular injection on day 7 and the second by water drinking on day 22. The lighting system consisted of 23 h of light from day 0 to 21 and 20 h of light from day 22 to 42. The room temperature was controlled at 33°C from day 0 to 3 and then gradually reduced by 3°C weekly until a final temperature of 24°C was reached.

Table 1. Ingredients and nutrient composition of the control diet.

	Day 1–21	Day 22–42
Ingredients (%)		
Corn	57.20	62.31
Soybean meal	32.00	28.00
Soybean oil	2.68	3.51
Soybean protein powder	3.89	2.61
Limestone	1.37	1.46
Dicalcium phosphate	1.95	1.36
L-Lysine·HCl	0.14	0.14
DL-Methionine	0.14	0.08
Trace mineral premix[a]	0.10	0.10
Vitamin premix[b]	0.03	0.03
Choline chloride	0.20	0.10
Salt	0.30	0.30
Nutrient composition		
Metabolizable energy (kcal/kg)	2976	3073
Analysed crude protein (%)	21.48	19.10
Analysed calcium (%)	1.01	0.92
Analysed total phosphorus (%)	0.65	0.57
Non-phytate phosphorus (%)	0.45	0.35

[a]The trace mineral premix provided the following (per kg of diet): 100 mg iron; 100 mg zinc; 8 mg copper; 120 mg manganese; 0.7 mg iodine; and 0.3 mg selenium.
[b]The vitamin premix provided the following (per kg of diet): 8000 IU vitamin A; 1000 IU vitamin D_3; 20 IU vitamin E; 0.5 mg menadione; 2.0 mg thiamine; 8.0 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 0.01 mg vitamin B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; and 0.18 mg biotin.

The broilers were individually weighed on day 21 and 42. One chick that *weighed close* to the mean weight of the replicate was selected for the collection of blood and tibias. Serum samples (5 mL) were collected into 5 mL anticoagulated syringes through cardiac puncture on day 21 and through the wing vein on day 42. These samples were centrifuged for 10 min at 3000*g* at 20°C. The broilers were killed after collecting the blood samples. The tibias of the individual broilers were excised and frozen at −20°C for further analysis.

2.3. Sample analyses

Ca in diets and tibia were determined by ethylene diamine tetraacetic acid (EDTA) titration. Phosphorus (P) in diets and tibia were determined by photometric methods after reaction with ammonium molybdate and ammonium metavanadate. Crude protein was determined using the Kjeldahl method (PN-1430, Barcelona, Spain). Serum biochemical parameters (creatinine, glucose, cholesterol, triglycerides, alanine aminotransferase and aspartate aminotransferase) were determined with a Shimadzu CL-8000 analyser (Shimadzu Corp., Kyoto, Japan) following the manufacturer's instructions. Antibody titres to NDV were measured based on a haemagglutination inhibition test. The tibia mineralization was measured following the method by Hall et al. (2003).

2.4. Statistical analyses

Replicate means served as the experimental unit for statistical analysis. Data were analysed by the general linear model (GLM) procedure of SAS software (SAS Institute 2002). Orthogonal comparisons were used to determine the linear and quadratic effects of the increasing levels of FPCM. Means were compared by Tukey's test when probability values were significant ($P < 0.05$).

3. Results and discussion

3.1. Growth performance

Compared with the control, FPCM had the trend to linearly improve body weight gain (BWG, $P = 0.07$) in starter boilers (Table 2). Supplementation of 1 g/kg FPCM improved BWG by 3.8% in 22- to 42-day-old broilers ($P = 0.03$). Addition of 1 or 4 g/kg FPCM increased BWG ($P = 0.02$) by 3.2% and 3.0% in 1- to 42-day-old broilers, respectively. A quantity of 4 g/kg of FPCM enhanced feed intake (FI) of 22- to 42-day-old and 1- to 42-day-old chicks ($P < 0.05$). FPCM level had a positive effect on feed efficiency (FE) in starter broilers. However, FE in growing-finishing broilers was

Table 2. Effects of FPCM on growth performance of broiler chicks.

	FPCM (g/kg)					*P* value	
Item	0	1	2	4	SEM	Linear	Quadratic
BWG (g/chick)							
Day 1–21	633	645	660	652	4	0.028	0.185
Day 22–42	1472^{b}	1528^{a}	1489^{ab}	1516^{ab}	8	0.147	0.297
Day 1–42	2105^{b}	2173^{a}	2149^{ab}	2168^{a}	9	0.023	0.125
FI (g/chick)							
Day 1–21	1101	1092	1103	1071	7	0.203	0.386
Day 22–42	3004^{b}	3083^{ab}	3022^{b}	3187^{a}	22	0.005	0.220
Day 1–42	4105^{b}	4175^{ab}	4125^{b}	4258^{a}	19	0.007	0.300
FE (FI/BWG)							
Day 1–21	1.74	1.69	1.67	1.64	0.01	0.018	0.789
Day 22–42	2.04^{ab}	2.02^{b}	2.03^{b}	2.10^{a}	0.01	0.057	0.034
Day 1–42	1.95	1.92	1.92	1.96	0.01	0.603	0.046
Mortality (%)							
Day 1–21	5.0	1.7	3.3	0	0.9	0.104	1.000
Day 22–42	0	0	0	3.3	0.6	0.047	0.130
Day 1–42	5.0	1.7	3.3	3.3	1.2	0.761	0.498

Note: Data are means of six replicate cages consisting of 10 broilers per replicate cage. Means in the same row without common superscript differ significantly ($P < 0.05$).
SEM, pooled standard error of the mean.

negatively affected by FPCM level. Mortality was not affected by FPCM.

C. militaris, an herb to modulate human immune system in China, cannot be used as feed additive in animal production because of its scarcity. Biological fermentation technology promotes its utilization in feed industry and FPCM are produced to improve growth performance of animals in recent years. FPCM have the same bioactive components as *C. militaris* which contain cordycepin and polysaccharides. The effects of FPCM on animal performance were not examined and the present study aimed to evaluate the broiler chick response to FPCM.

Supplementation of 1 or 4 g/kg FPCM improved the growth of broiler chicks in our study. This result was consistent with that of Koh et al. (2003), in which a hot water extract of mycelia from *C. sinensis* significantly increased BWG of broilers. They also found that the hot water extract increased *Lactobacillus* sp. content and decreased *Salmonella* sp. and *E. coli* content in small intestine of broiler chicks (Koh et al. 2003). Their data indicate that the improvement of broiler growth by *C. sinensis* may be come from the control of the ratio of beneficial to pathogenic bacteria in small intestine.

Although limited information about cordycepin or extract of *C. sinensis* was found in animal production, research on a prebiotic in animal feed has been reported. Dietary prebiotics (mannanoligosaccharides, MOS) improved BWG, FI and FE in 1- to 21-day-old broilers fed low-Ca diets (Houshmand et al. 2011b). However, Houshmand et al. (2011a) reported that the addition of MOS did not enhance growth performance or energy utilization in 1- to 42-day-old broilers fed nutrient-adequate diets. Their data suggest that animal response to a prebiotic may be relative to nutrient levels. In our experiment, the broiler chicks were fed diets with adequate nutrients. FPCM slightly increased the growth rate of chicks. Another study also showed the positive effects of a prebiotic (*Saccharomyces cerevisiae* yeast culture) on BWG and FE in 1- to 42-day-old broilers (Salianeh et al. 2011).

3.2. Bone mineralization

The starter broilers fed 2 and 4 g/kg of FPCM obtained higher tibia Ca content than those fed the control diet ($P < 0.01$, Table 3). By contrast, the tibia P content of the broilers fed 4 g/kg of FPCM was lower than that of broilers fed control diet ($P < 0.01$). For growing-finishing broilers, 2 g/kg of FPCM increased tibia Ca content compared with the control ($P < 0.01$). The broilers fed 4 g/kg of FPCM had the lowest values of tibia breaking strength and the content of ash, Ca and P among treatments. There was no significant difference in tibia weight, length, width and ash weight in 42-day-old broilers among treatments. These data indicated that 4 g/kg of FPCM had a negative effect on bone mineralization of growing-finishing broiler chicks.

Studies on non-digestible oligosaccharides elicit a positive response on Ca absorption and retention. Research has shown that FOS increased serum Ca levels as well as tibia ash, Ca and P content in laying hens (Chen & Chen 2004). FOS or a mixture of inulin

Table 3. Effects of FPCM on tibia mineralization of broiler chicks.

	FPCM (g/kg)					*P* value	
Item	0	1	2	4	SEM	Linear	Quadratic
Breaking-strength (N)							
Day 21	115.59	92.53	94.77	112.23	4.04	0.814	0.012
Day 42	302.85^{ab}	368.19^{a}	367.21^{ab}	274.10^{b}	13.96	0.421	0.003
Weight (g)							
Day 21	1.43^{ab}	1.32^{b}	1.37^{ab}	1.53^{a}	0.03	0.115	0.012
Day 42	4.85	5.40	4.80	5.41	0.13	0.350	0.911
Length (cm)							
Day 21	6.28	6.22	6.25	6.39	0.03	0.192	0.121
Day 42	9.34	9.75	9.52	9.69	0.07	0.154	0.364
Width (cm)							
Day 21	0.52^{ab}	0.48^{b}	0.51^{ab}	0.54^{a}	0.01	0.160	0.012
Day 42	0.74	0.74	0.74	0.71	0.02	0.662	0.605
Ash (g)							
Day 21	0.80	0.72	0.76	0.84	0.02	0.198	0.019
Day 42	2.33	2.66	2.54	2.43	0.07	0.729	0.109
Ash (%)							
Day 21	55.74	54.93	55.74	55.01	0.22	0.487	0.931
Day 42	49.47^{a}	49.52^{a}	52.67^{a}	45.02^{b}	0.71	0.020	<0.001
Ca (%)							
Day 21	18.12^{b}	18.19^{b}	19.67^{a}	19.62^{a}	0.20	<0.001	0.822
Day 42	17.83^{b}	17.89^{ab}	19.38^{a}	16.53^{b}	0.28	0.174	0.001
P (%)							
Day 21	10.01^{a}	9.84^{a}	9.79^{ab}	9.34^{b}	0.08	<0.001	0.267
Day 42	8.35^{ab}	8.80^{ab}	9.18^{a}	7.91^{b}	0.15	0.408	0.003

Note: Data are means of six replicate cages consisting of one broiler per replicate. Means in the same row without common superscript differ significantly ($P < 0.05$).
SEM, pooled standard error of the mean.

and FOS enhanced dietary Ca utilization, bone Ca and P content, and bone fracture strength in rats (Zafar et al. 2004; Rodrigues et al. 2012). Similar results were found in human. Consumption of combined short- and long-chain inulin-type fructans increased Ca absorption and enhanced bone mineralization during pubertal growth of human (Abrams et al. 2005). However, Swiatkiewicz et al. (2011) and Kim et al. (2011) reported that FOS or inulin do not improve the bone growth and skeletal integrity of broiler chicks. In the present study, we found the positive effects of FPCM on calcium retention in bone. Whether polysaccharides in FPCM have the similar mechanism to non-digestible oligosaccharides on calcium absorption in intestine and retention in bone of animals should be further clarified.

3.3. Serum parameters

FPCM had no effect ($P > 0.05$) on serum alanine aminotransferase, aspartate aminotransferase, creatinine, glucose, cholesterol and triglycerides as well as NDV titres of broilers in the present study (Table 4).

When mice bearing lymphoma were fed with extract of *C. sinensis*, the phagocytosis of macrophage and the salmonellosis resistance increased and the mice receiving the extract of *C. sinensis* lived longer than those without the extract (Yamaguchi et al. 1990). In this study, after normal broiler chicks were fed FPCM, serum enzyme activities were not significantly changed. These data indicated that the response of animals at a normal physiological condition to *C. sinensis* is not as notable as at a pathological state.

A hot water extract of mycelia from *C. sinensis* increased antibody titres to NDV in broiler chicks on day 35 (Koh et al. 2003). The immune response may be affected by intervals between vaccination and measurement. In the present study, the chicks were vaccinated on day 7 and 22, but NDV titres were determined on day 21 and 42. By contrast, Koh et al. (2003) vaccinated the broilers on day 14 and 28, while they determined NDV titres on day 35.

4. Conclusion

It is concluded that FPCM have positive effects on BWG, FI and tibia Ca content of broiler chicks, although FPCM did not affect serum biochemical parameters. Broilers fed with 1 g/kg of FPCM obtained the greatest

Table 4. Effects of FPCM on serum biochemical parameters and Newcastle disease antibody titres (NDAT) of broiler chicks.

	FPCM (g/kg)					*P* value	
Item	0	1	2	4	SEM	Linear	Quadratic
Alanine aminotransferase (U/L)							
Day 21	6.35	6.04	5.05	5.74	0.38	0.425	0.526
Day 42	4.57	4.73	4.73	4.68	0.13	0.778	0.696
Aspartate aminotransferase (U/L)							
Day 21	250.92	263.88	266.83	276.32	9.13	0.366	0.928
Day 42	447.62	525.32	472.55	377.20	24.84	0.230	0.084
Creatinine (μmol/L)							
Day 21	4.74	4.48	3.20	3.93	0.35	0.251	0.486
Day 42	3.80	4.37	3.75	2.37	0.28	0.040	0.065
Glucose (mmol/L)							
Day 21	11.52	9.69	10.53	10.58	0.51	0.682	0.381
Day 42	8.89	8.34	9.51	9.25	0.23	0.273	0.742
Cholesterol (mmol/L)							
Day 21	3.70	3.35	3.57	3.26	0.09	0.199	0.915
Day 42	3.14	2.68	3.10	3.07	0.09	0.809	0.225
Triglycerides (mmol/L)							
Day 21	0.59	0.60	0.62	0.55	0.02	0.582	0.421
Day 42	0.32	0.29	0.35	0.34	0.01	0.234	0.525
NDAT (log_2)							
Day 21	4.83	4.50	4.17	3.83	0.25	0.164	1.000
Day 42	5.00	5.83	6.00	5.83	0.24	0.229	0.310

Note: Data are means of six replicate cages consisting of one broiler per replicate.
SEM, pooled standard error of the mean.

BWG and the lowest mortality as well as appropriate tibia breaking strength, weight, length, width and ash weight among four treatments. Considering the economic benefits, the optimal FPCM level was 1 g/kg in broilers from 1 to 42 days of age.

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