Effects of Dietary Cellulose Levels on Growth, Nitrogen Utilization, Retention Time of Diets in Digestive Tract and Caecal Microflora of Chickens

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ABSTRACT : This study was conducted to examine the effects of dietary cellulose levels on growth, nitrogen utilization, the retention time of diets in the digestive tract, and caecal microflora of 2-month-old Single Comb White Leghorn male chickens fed 3 purified diets that contained 0, 3.5% and 10% cellulose in equal amount of nutrients for 7 days. Body weight gain and nitrogen utilization were significantly higher ($p<0.05$), while total microflora counts in the caecal contents and retention time of the diet in the digestive tract were significantly lower ($p<0.05$) in the group fed 3.5% dietary cellulose compared with the group fed 10% dietary cellulose. Body weight gain, nitrogen utilization and retention time of the diet in the digestive tract decreased significantly while the total microflora count in the caecal contents increased significantly in the group fed 10% dietary cellulose compared to the group fed 0% dietary cellulose ($p<0.05$). Chickens fed 10% dietary cellulose had significantly increased counts of uric acid-degradative bacteria such as *Peptococcaceae and Eubacterium,* including *Peptostreptococcus* ($p<0.05$). The results suggest that cellulose in purified diets is an effective ingredient and the effects on growth, nitrogen utilization, caecal microflora counts and diet retention time in the digestive tract are dependent on the inclusion rate. Positive or negative effects of dietary cellulose are displayed by growth, nitrogen utilization, caecal microflora counts and retention time of the diet in the digestive tract. Positive effects were displayed when the inclusion rate is 3.5% and negative effects were displayed when that is greater than 3.5% of the diet, and the phenomenon is without reference to the age of the chickens. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 6 : 863-866)***

Key Words : Cellulose, Chicken, Growth, Nitrogen Utilization, Retention Time, Caecal Microflora

INTRODUCTION

Cellulose, a major dietary fiber source, is a common constituent in experimental purified diets for many nutritional studies (Anderson and Hill. 1955; Sibbald et al., 1961; Nakahiro and Isshiki, 1975; Nahm and Carlson, 1987; Summers et al., 1990; Muramatsu et al., 1991; Siri et al., 1992; Siri et al., 1994). In these studies the metabolizable energy (ME) of dietary cellulose was estimated to be zero and its physiological and nutritional roles were also ignored. In contrast, a number of reports indicated that dietary cellulose has affected the digestive physiology by increasing DNA weight (Farness and Schneeman, 1982) and thickness (Yamauchi and Isshiki, 1991) of the intestinal wall and the nutritional status by reducing the utilization of dietary protein (Shah et al., 1982).

It has been reported that passage time of digesta was shortened and the fecal excretion was increased in rats (Takehisa et al., 1979) and broilers (Nahm and Carlson, 1987), and that the caecal flora was increased in turkeys (Bedbury and Duke, 1983) by dietary cellulose. These studies indicated that gastrointestinal transit time of digesta might be increase and gastrointestinal flora would be rather hard to stay there, when without undigestible materials in the intestine.

Therefore, cellulose appears to have other effects as an ingredient in purified diets in addition to increasing the gastrointestinal bulk.

Cao et al. (1998a, 1998b) and Cao (2001) reported that 1.5-3.5% dietary cellulose enhanced growth and metabolizable energy retention of 7-15 d old chicks, but that levels of more than 5% suppressed the growth more prominently in chicks fed a lower level of dietary protein. However development of the gastrointestinal enzyme system in the chick body is the fastest in around age of 2 weeks (Nir et al., 1993) compared to other enzyme systems, but the transplantation of the microorganisms in the gastrointestinal tract has not been completed during that growing phase (Mitsuoka, 1978). Therefore, it needs to be validated if the same phenomenon occurs in chicks when intestinal development and transplantation of intestinal microflora have been accomplished, such as that reported by Cao et al. (1998a, 1998b) and Cao (2001). The purpose of the present study was to examine the effects of dietary cellulose levels on nitrogen utilization, retention time of diets in the digestive tract and the cecal flora in 2 month-old chicks fed equal amount of nutrients.

MATERIALS AND METHODS

Animals, experimental feed, feeding and design

Single Comb White Leghorn male chicks (n=45; 2 mo old) of the same body weight were individually caged in

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Table 1. Composition of basal diet

Ingredients (%)	Composition
Isolated soybean protein (CP 82.27%)	19.45
Cornstarch	71.7815
Corn oil	2.00
$NaHCO_3$	1.00
Cystine	0.09
Methionine	0.046
Choline chloride (99.8%)	0.20
Vitamin premix[1]	0.06
Mineral premix[2]	5.36
2, 6-Di-t-butyl-p-cresol	0.0125
Calculated nutrient value	
Protein (%)	16.00
ME (KJ/g)	13.89

[1] Supplied per kilogram of diet: Vitamin A 2,700 IU; vitamin D_3 200 IU; vitamin E 10.0 IU; vitamin K 0.5 mg; thiamin 1.8 mg; riboflavin 3.6 mg; pantothenic acid 10.0 mg; niacin 27.0 mg; pyridoxine 3.0 mg; biotin 0.15 mg; folacin 0.55; vitamin B_{12} 0.009 mg.

[2] Supplied mg per kilogram of diet: Ca 7,000.0; non-phytate P 3,500.0; Mg 600.0; K 3400.0; Na 1,500.0; Cl 1,500.0; Fe 60.0; Cu 4.0; Zn 40.0; Mn 55.0; I 0.35; Se 0.12.

stainless metabolic cages, kept in an air-conditioned environment (23±1°C) during the entire experiment, and were randomly distributed to each dietary treatment group.

The basal diet (table 1) contained isolated soybean protein (CP 82.27%, New Fujiporo-E, Fuji oil Co., Ltd., Ohsaka) and included 16% total protein and 13.89 KJ/g metabolizable energy. Experimental diets were prepared by mixing the basal diet with cellulose powder (mesh 70 μm, 98% alpha-cellulose, HP310, Kohjin Co., Ltd., Toyama) at the rate of 0, 3.63 or 11.12 g of cellulose per 100 g of basal diet, to contain 0, 3.5 and 10% cellulose.

The 10% cellulose diet was fed *ad libitum* to the 10% cellulose group for 7 d. The 0 and 3.5% cellulose groups were fed their respective diets but their intakes were limited to 90% and 93%, respectively, of the dry matter intake of the 10% cellulose group for 7 d. In so doing, the chicks were supplied almost equal amounts of dietary CP and ME in all groups. The actual CP and ME intakes of all groups were 92 and 109%, respectively, of the Japanese Feeding Standard for Poultry (1997). During the entire experiment the chicks had free access to their corresponding diet and to water.

Sample collection and chemical analyses

On the 3rd day after the start of the experiment, chicks were force-fed 5 g of their respective diet with a 0.1% chromic oxide coloring at 8:30 a.m., then a corresponding non-colored diet was fed *ad libitum* in the case of the 10% cellulose diet and restricted in the case of the 0 and 3.5% diets. Time for appearance and disappearance of colored feces in each chick was recorded. Diet retention time was calculated by difference between the appearance and disappearance of colored feces.

During the last 3 d of the experiment, total excreta (feces and urine) were collected every day after spraying with 15 ml of 5% HCl. Wet excreta were stored frozen until later determination of nitrogen (N) content. After thawing, excreta were homogenized and dried in a vacuum oven to a constant weight. The dry samples were analyzed for N content by the Kjeldahl procedure.

At the end of the experiment, the birds were killed with CO_2 suffocation, the cecum was removed, and its contents weighed. Then, the cecal contents were placed in grinding tubes containing 9 ml anaerobic phosphate buffer solution (pH 6.8) in a 4°C, CO_2 environment in a clean room. This liquid (10^{-1} dilution) was continuously diluted 10 times in series. The appropriate diluted liquid (0.5 ml) was dropped onto each medium and spread on 1/3 to 1/4 of the medium area using a sterile Conradi-Stick and was then incubated at 37°C for 2 to 3 d.

The cecal flora was analyzed using three non-selective and eleven selective media as described by Mitsuoka et al. (1976). The identification of bacterial groups was determined using colony and cellular morphologies, Gram-reaction, spore formation and aerobic growth.

The colony count in each medium was calculated after incubation and the logarithmic value was used to determine total bacterial counts per 1 g of cecal contents. The total bacterial counts were determined by totaling each bacterial count.

Statistical analysis

Results were subjected to a one-way analysis of variance and to Duncan's multiple range test using Statistical Soft Excel Stat. 97.

RESULTS AND DISCUSSION

For both body weight gain and nitrogen utilization, there was no significant difference between the 0% and 3.5% dietary cellulose levels but they were significantly lower in the group fed 10% cellulose ($p<0.05$; table 2). This

Table 2. Effect of dietary cellulose levels on body weight gain and nitrogen retention

Dietary cellulose (%)	Body weight gain (g/7d)	N retention (mg/d)	N retention rate (%)	Apparent N digestibility (%)	Retention rate of absorbed N (%)
0.0	58.0±3.3b	1013.0±2.3b	69.6±0.2b	91.1±0.4b	76.3±0.2b
3.5	62.7±2.0b	1007.8±2.4b	69.7±0.4b	91.7±0.5b	76.0±0.4b
10.0	53.3±0.9a	884.4±2.5a	67.7±0.4a	89.3±0.3a	68.0±0.3a

Figures in parentheses indicate number of tested chicks. Means with different superscripts in the column are significantly different ($p<0.05$).

Table 3. Effect of dietary cellulose levels on retention time of diets in digestive tract

Dietary cellulose (%)	Time (minutes)		
	Appearance	Disappearance	Retention
0.0	165.6±3.1b	540.8±3.2b	375.2±1.9b
3.5	153.8±4.1ab	539.6±4.1b	385.8±3.4b
10.0	150.3±2.7a	507.8±4.0a	357.5±2.6a

Figures in parentheses indicate number of tested chicks. Means with different superscripts in the column are significantly different ($p<0.05$).

finding was consistent with previous results reported by Cao et al. (1998a, 1998b). In those studies using 2-wk-old chicks, body weight gain and nitrogen utilization were the highest at 3.5% dietary cellulose but decreased with increasing cellulose level. They concluded that even as the age of the chick increases, a high cellulose diet would negatively influence growth and nitrogen utilization.

Table 3 shows the effect of dietary cellulose levels on the retention time of diets in the intestine. An increase in dietary cellulose levels tends to shorten the appearance time and disappearance time of a dietary marker, and thus indicates a reduced retention time. There were significant differences between 10% and 0% or 3.5% cellulose diets in both the disappearance time and the retention time ($p<0.05$). In rats (Takehisa et al., 1979) and broilers (Nahm and Carlson, 1987) an increase in the amount of dietary cellulose reduced diet passage through the intestine and decreased nitrogen utilization (Shah et al., 1982).

The results suggested that the high intake of dietary cellulose decreased retention time of digesta, and all contents in intestine would be rather hard to stay there. Thus, protein digestion time, nitrogen utilization and body weight gain were decreased.

Table 4 shows the effects of dietary cellulose levels on cecal flora counts. Compared with the 10% cellulose group, total flora counts in the 3.5% cellulose group and *Bifidobacteria*, *Eubacteria* and *Lactobacilli* amounts in the 0 and 3.5% cellulose groups were significantly lower ($p<0.05$). The *Peptococcaceae* count was significantly lower than that of the chicks fed 10 or 0% cellulose ($p<0.05$). Increasing cellulose level decreased the bacilli count, with an obvious difference between the 10 and 0% cellulose levels ($p<0.05$). The dietary cellulose level did not influence counts of *Bacteroidaceae* and lecithinase-positive *Clostridia*, *Enterobacteriaceae*, *Streptococci and Staphylococci*.

Changes in cecal flora counts indicated that increasing dietary cellulose level did not meliorate the cecal micro-environment, but decreasing of anaerobia decreased the total flora counts by feeding 3.5% cellulose for 7 d. *Peptococcaceae and Eubacterium* including *Peptostreptococcus* were superior in the group fed 10% cellulose compared to other groups (table 4), these are uric acid-degradative bacteria found in the chickens' cecum

Table 4. Effect of dietary cellulose levels on caecal counts (log_{10} CFU/g)

Bacterium	Dietary cellulose levels (%)		
	0.0	3.5	10.0
Bifidobacteria	9.01±0.09a	8.99±0.15a	9.30±0.18b
Baceroidaceae	10.04±0.11	9.92±0.06	10.05±0.16
Eubacteria	9.60±0.15a	9.52±0.11a	9.82±0.12b
Peptococcaceae	9.71±0.15b	9.42±0.11a	9.73±0.28b
Clostridia[1]	2.28±1.15	0.63±1.26	1.28±1.28
Lactobacilli	9.53±0.08ab	9.32±0.04a	9.62±0.26b
Enterobacteriaceae	7.17±0.20	6.82±0.38	7.18±0.48
Streptococci	8.03±2.70	8.40±1.78	7.56±1.65
Staphylococci	2.60±2.47	0	1.65±2.11
Bacilli	6.80±0.87b	5.87±1.34ab	4.85±1.05a
Summation	10.39±2.92ab	10.25±2.04a	10.47±2.33b

[1] Lecithinase-positive. Figures in parentheses indicate number of tested chicks. Means with different superscripts in the column are significantly different ($p<0.05$).

(Barnes and Impey, 1972).

In birds, there is cecum-reflux system for urine (Akesster et al., 1967). Urinary nitrogenous flow back into the cecum was converted to ammonia by uric acid-degradative-bacterium in the cecum. This ammonia was then converted to non-essential amino acids in the liver (Karasawa and Maeda, 1994) but the ammonia in the cecum restricted bird growth when it exceeded the requirements for dispensable amino acid synthesis (Bone et al., 1976).

Cao et al. (1998a, 1998b) reported that higher dietary cellulose levels increased excretion of fecal and urinary nitrogen and suggested that the urinary nitrogen flow that was reversed back into the cecum increased as dietary cellulose level increased. Therefore, in the present experiment, the increase in the urinary nitrogen in the group fed 10% cellulose may flow back into the cecum and then contribute to increased caecal microflora counts and increase the ammonia level. It is possible that part of the remaining ammonia in cecum restricted the rate of body weight gain. On the other hand, the reduction of nitrogen excretion and caecal flora counts and effective use for ammonia in cecum may result in the higher body weight gain and nitrogen utilization of the group fed 3.5% cellulose.

IMPLICATIONS

Different cellulose levels in purified diets have caused substantial positive or negative effects for growth, nitrogen utilization and retention time of diets in the alimentary canal of young chicks when the cellulose content is at 3.5% or 10%. An obvious difference in caecal microflora counts occurs when the dietary cellulose level is 3.5% to 10%. Therefore, in a nutritional study that uses cellulose as ingredient of purified diets, it is necessary to take into account the amount, use of, and the dosage responses of dietary cellulose to improve the veracity of the system.

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