Effect of Dietary Sorbitol on Growth Performance and Plasma Acute Phase Protein Concentration in Male Broiler Chickens during Immunological Stimulation

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Two experiments were conducted to determine effect of dietary sorbitol on growth performance, plasma $\alpha 1$ acid glycoprotein (AGP) and ceruloplasmin concentrations in male broiler chicks during immunological stimulation due to injections with *Escherichia coli* lipopolysaccharide (LPS) and Sephadex-G50 superfine. In experiment 1, 10 days old chicks were fed a 6% glucose, xylitol, sorbitol or fructose for 8 days. In experiment 2, 7 days old chicks were fed either diet containing 10% glucose or sorbitol supplemented diet for 12 days. During final 6 days of the experimental periods, all chicks in experiment 1 and a half of birds fed each diet in experiment 2 were injected intraperitoneally with *Escherichia coli* LPS (0.5 mg/kg BW) on days 1, 3 and 5 and Sephadex (250 mg/kg BW) on days 2 and 4. Chicks fed the sorbitol diets tended to be better in growth performance than birds fed the glucose or fructose diet in experiment 1. In experiment 2, feeding the sorbitol diet prevented the reductions in body weight gain due to LPS and Sephadex injections. Plasma AGP concentration on day 2 after the immune stimulation in chicks fed the sorbitol diet was lower than that in chicks fed the glucose diet, but that on day 6 did not differ. Plasma ceruloplasmin concentration on days 2 and 6 after the immune stimulation in chicks fed the sorbitol diet was significantly lower than that in chicks fed the glucose diet. These results indicate that dietary sorbitol reduces early inflammatory responses and tended to prevent the reduction of growth due to repeated injections of LPS and Sephadex. In addition, mode of action of sorbitol in preventing growth retardation during immunological stimulation appeared to be different from that of xylitol, which was previously reported in chicks.

Key word : Dietary sorbitol, Immunological stimulation, Inflammatory responses, Performance, Chicks

Introduction

Nutritional regulation of immune-induced decreases in performance would be of great value to the poultry industry. One of the goals for controlling immune system by nutrition is to alleviate decreased performances following immune stimulation without

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changes in the immune responses in domestic animals. Inflammatory responses following immune stimulation were often accompanied by a markedly stimulated tissue glucose use in hosts and this increased peripheral glucose use may support cellular functions responsible for the protection of hosts (Klasing, 1988 ; Klasing and Johnstone, 1991 ; Spolarics 1995). Benson *et al.* (1993) showed that increasing energy density by cornstarch, but not by oil (corn oil), eliminated the growth depressing effect of immunogen and prevented an increase in interleukin (IL)-1 production in chicks when dietary energy level was above 13.4 kJ/kg diet. Takahashi *et al.* (1999 ; 2000) demonstrated that supplementation of xylitol (five-carbon sugar alcohol) had the similar effects on growth preferences as the cornstarch supplementation under condition of immunological stimulation by LPS and Sephadex injections. However, Takahashi *et al.* (1999 ; 2000) suggested that the effect of xylitol was not due to increased energy content in diet as observed in the cornstarch, because xylitol supplementation did not affect production of pro-inflammatory cytokines such as IL-1, 6, and tumor necrosis factor.

Non-glucose monosaccharides, especially sugar alcohols such as sorbitol and xylitol, and fructose have been advocated for use by parenteral infusion for nutritional support in patients suffered from the trauma and severe infections (Levine, 1985). Sorbitol and xylitol have been shown to elicit little or no increases in blood glucose and insulin in human (De Kalbermatten., 1980). Ellwood *et al.* (1999) also showed that sorbitol had similar effect on lipogenic and gluconeogenic enzymes, plasma hormones, lipids, catecholamines in rats as xylitol. Thus, sorbitol may have similar nature as xylitol in many metabolic aspects and serve as a nutrient for modulating metabolic processes during immune stimulation. However, there is little information on effect of sorbitol on growth performance and early inflammatory responses in immune stimulated chicks.

In the present study, we determined if dietary sorbitol have beneficial effects on performances during immune stimulation similarly to dietary xylitol in male broiler chickens.

Materials and Methods

In experiment 1, male broiler chicks (Ross) were housed in battery brooder with electronic heater and fed a commercial broiler starter diet until 10 days of age. Forty chicks were selected from one and half larger population to obtain uniform body weight. They were randomly assigned to 4 groups of 10 chicks, with 5 replicates of 2 chicks in a cage, and were given the test diets supplemented with either 6% glucose, xylitol, sorbitol or fructose for 8 days *ad libitum*. A basal (glucose) diet consisted of 47.87% corn, 30.00% soybean meal, 3.99% isolated soybean protein, 3.64% soybean oil, 10.00% glucose, 1.21% calcium carbonate, 1.78% calcium phosphate, 0.42% sodium chloride, 0.29% DL-methionine, 0.40% vitamin mixture and 0.40% mineral mixture (22% crude protein and 3100 kcal/kg metabolizable energy). Xylitol, fructose and sorbitol in the test diets were replaced by 6% of glucose in the basal diet. During final 6 days of the test diets feeding, chicks were intraperitoneally injected with

Escherichia coli lipopolysaccharide (LPS, 0127B : B8, Difco Laboratories, Detroit, USA) at 0.5 mg/kg BW on days 1, 3 and 5 and with Sephadex-G50 (Pharmacia Biotech AB, Uppsala, Sweden) at 250 mg/kg BW on days 2 and 4. LPS was dissolved in sterilized saline at a concentration of 0.5 mg/ml. Sephadex G-50 superfine was dissolved at 5 g in 100 ml in a sterilized saline (0.9% NaCl).

In experiment 2, forty male broiler chicks (7 days of age) were used. They were randomly assigned to 2 groups of 20 chicks, with 10 replicates of 2 chicks in a cage, and were given the test diets containing either 10% glucose or sorbitol supplemented diet for 12 days *ad libitum*. Composition in the glucose and sorbitol diet was the same as in experiment 1 except for amount of glucose and sorbitol. At 14 days of age immune stimulation by LPS and Sephadex was started. Half of chicks fed the each diet were intraperitoneally injected with *Escherichia coli* lipopolysaccharide (LPS) at 0.5 mg/kg BW on days 1, 3 and 5 and with Sephadex-G50 at 250 mg/kg BW on days 2 and 4. The other half of birds in the each dietary group was served as the control chicks. They were injected with sterilized saline alone in the same manner as in the LPS and Sephadex injected groups. A blood was taken on days 2 and 6 following the immune stimulation.

The method of determination of α -1 acid glycoprotein (AGP) concentrations in plasma were reported previously (Takahashi *et al.*, 1994). Plasma ceruloplasmin was determined by the procedure of Sunderman and Nomoto (1970). The analyses for feed intake, and weight gain to feed intake ratio were based on cage replication. For analyses of the other measurements, individual chicks were considered as experimental unit. A 2 (dietary groups) by 2 (immune stimulation) factorial statistical test was applied to analyze the data using of SAS (SAS Institute, Cary, NC, USA) with mean separation by Duncan's multiple range test in experiment 2. The data of experiment 1 were analyzed using one way ANOVA (SAS Institute, Cary, NC, USA) with mean separation by Duncan's multiple range test. For analyses of body weight, feed intake and body weight to feed intake ratio, the analyses were based on cage replication. For analysis of AGP and ceruloplasmin concentrations in plasma, individual chicks were considered as experimental unit.

Results

Table 1 shows the results of experiment 1. Body weight gain and feed intake during immunological stimulation due to repeated injections of LPS and Sephadex in chicks fed the 6% xylitol diet were significantly greater than those in chicks fed the 10% glucose or fructose diet. Compared with the control chicks fed the glucose diet, feeding the 6% sorbitol diet also improved body weight gain during immunological stimulation. Body weight gain to feed intake ratio did not significantly differ among the dietary treatments, although that in chicks fed the glucose diet tended to be lower than that in chicks fed the other diets.

Table 2 shows growth performance in male broiler chicks fed the sorbitol diet for 7 days before immune stimulation in experiment 2. Growth performance before immune stimulation from 7 to 14 days of age did not significantly differ between chicks

Table 1. Effect of dietary glucose, xylitol, sorbitol and fructose on growth performance following repeated injections of LPS and Sephadex in male broiler chicks (Experiment 1)¹⁾

Diet	Feed intake (g)	Body weight gain (g)	Body weight gain /Feed intake
Glucose	223±10b	127±6b	0.569±0.054
Xylitol	250±7a	150±6a	0.601±0.037
Sorbitol	237±8ab	147±5a	0.618±0.034
Fructose	230±14b	140±6ab	0.610±0.021

Mean±SE (observations=5). a, b : P<0.05.

fed the glucose and sorbitol diet although chicks fed the 10% sorbitol diet tended to be lesser growth performance than chicks fed the 10% glucose diet.

Table 3 shows growth performance in male broiler chicks fed the sorbitol diet during immunological stimulation by repeated injection of LPS and Sephadex in experiment 2. Repeated injections of LPS and Sephadex significantly reduced feed intake, body weight gain and body weight to feed intake ratio. Body weight gain during immunological stimulation was improved by feeding the sorbitol diet compared with the feeding glucose diet. Feed intake and body weight gain to feed intake ratio during immune stimulation were not changed by the sorbitol diet.

Table 4 shows changes in plasma AGP and ceruloplasmin concentrations during immunological stimulation by repeated injection of LPS and Sephadex in experiment 2. Alternating injections of LPS and Sephadex increased plasma ceruloplasmin and AGP concentrations irrespective of days of the injection and dietary treatments. Dietary sorbitol partially prevented increasing plasma ceruloplasmin concentrations due to

Table 2. Effect of dietary sorbitol on growth performance before immunological stimulation
In male broiler chicks (Experiment 2, from 7 to 14 days of age)¹⁾

Diet	Feed intake (g)	Body weight gain (g)	Body weight gain /Feed intake
Glucose	644±19	477±9	0.724±0.014
Sorbitol	626±15	458±9	0.732±0.014

Mean±SE (observations=10).

Table 3. Effect of dietary sorbitol on growth performance after repeated injections of LPS and Sephadex for 6 days in male broiler chicks (Experiment 2)

Diet Treatment	Glucose		Sorbitol		ANOVA		
	Saline	LPS + Sepadex	Saline	LPS + Sepadex	Diet	LPS + Sepadex	Diet X Treatment
Feed intake (g)	459±14a	412±15b	459±20a	397±13b	NS	<0.01	NS
Body weight gain (g)	288±10a	235±6c	283±9a	258±5b	NS	<0.01	<0.1
Body weight gain /Feed intake	0.62±0.03	0.58±0.03	0.63±0.01	0.60±0.03	NS	<0.01	NS

Mean±SE (observations=5). a, b : P<0.05. NS : >0.1.

Table 4. Effect of dietary sorbitol on plasma AGP and ceruloplasmin concentration following repeated injections of LPS and Sephadex for 6 days in male broiler chicks (Experiment 2)

Diet Treatment	Glucose		Sorbitol		ANOVA		
	Saline	LPS + Sepadex	Saline	LPS + Sepadex	Diet	LPS + Sepadex	Diet X Treatment
Ceruloplasmin (mg/l)							
Day 2	25 ± 3c	88 ± 7a	29 ± 6c	69 ± 7b	NS	< 0.01	< 0.1
Day 6	21 ± 3c	61 ± 8a	23 ± 3c	35 ± 4b	< 0.05	< 0.01	< 0.01
AGP (mg/l)							
Day 2	213 ± 17c	708 ± 33a	253 ± 43c	599 ± 40b	NS	< 0.01	< 0.05
Day 6	183 ± 17b	674 ± 51a	217 ± 25b	665 ± 47a	NS	< 0.01	NS

Mean ± SE (observations = 10). a, b : $P < 0.05$. NS : > 0.1 .

repeated injections of LPS and Sephadex on days 2 and 6. Plasma AGP concentration on day 2 after immune stimulation was lower in chicks fed the sorbitol diet than chicks fed the glucose diet, but that on day 6 did not differ.

Discussion

The present experiment showed that dietary sorbitol partly prevented a decrease in body weight gain caused by immunological stimulation due to alternating injections of LPS and Sephadex. This sorbitol effect was similar as effect of xylitol as previously reported (Takahashi *et al.*, 1999 ; 2000). However, mode of action of dietary sorbitol on growth performance during immune stimulation may be different from that of xylitol. AGP and ceruloplasmin are known as positive acute phase proteins in response to immunological stress and would be produced following pro-inflammatory cytokines such as interleukin-1 6, and tumor necrosis factor (Takahashi *et al.*, 1994 and 1998 ; Chamanza *et al.*, 1999). Takahashi *et al.* (1999 ; 2000) observed that dietary xylitol did not affect production of interleukin-1 like activity and acute phase protein concentrations such as AGP and ceruloplasmin in plasma, suggesting that dietary xylitol did not affect production and/or release of pro-inflammatory cytokines. On the other hand, dietary sorbitol significantly prevented to increase in plasma AGP concentration on day 2 and ceruloplasmin concentrations on days 2 and 6 following immunological stimulation in the present study. Thus, effect of dietary sorbitol on growth performance may be associated with reduction of pro-inflammatory cytokines production during immunological stress.

Cook *et al.* (1993) and Miller *et al.* (1994) found that conjugated linoleic acid (CLA), a structural isomer of linoleic acid, was effective in preventing body weight loss following immune stimulation in chicks. Fish oil also improved performance and decreased the inflammatory responses in chicks (Korver and Klasing, 1997 ; Korver *et al.* 1998). Fish oil and CLA isomers are also effective in preventing immune-induced weight loss and anorexia, and may be effective in preventing excess production of pro-inflammatory cytokines and/or prostaglandin E_2 (Li and Watkins, 1998 ; Liu and Belury, Pariza *et al.*, 2000 ; Turek *et al.*, 1998). Takahashi *et al.*, (2001) recently

showed that CLA significantly prevented increasing plasma AGP and ceruloplasmin concentrations due to LPS injection. Thus, alleviating effect of dietary sorbitol on growth performance during immune stimulation appeared to be similar as CLA and fish oil.

In mammals, feeding diets containing sugar alcohols for long terms is often shown to decrease feed intake, which leads to decreased body weight gain (Hamalainen and Makinen, 1985 ; Ellwood *et al.*, 1999). In the present experiment, we observed that feed intake for 7 days before immune stimulation tended to be lower in chicks fed the sorbitol diet than that in chicks fed the glucose diet although the effect is not significant. Since reductions of feed intake and body weight gain are undesirable from the practical viewpoint, for use of sorbitol as a poultry diet we should give attention to its content in diet and periods of use. However, in the stressful conditions, such as vaccination or transportation, short terms supply of sorbitol may be useful.

In conclusions, dietary sorbitol reduced early inflammatory responses and tended to prevent the reduction of growth following immune stimulation due to repeated injections of LPS and Sephadex. However, the effect of sorbitol on growth and inflammatory responses appeared to be different from that of xylitol, which was previously reported in chicks (Takahashi *et al.*, 1999 ; 2000).

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