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Effects of Dietary Chitosan on Hormone Level in Dairy Calves

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Abstract: The objective of the present study was to evaluate the effects of chitosan on serum GH, T3 and T4 in and to determine the appropriate supplemental dosage level of chitosan in dairy calves diet. A 30 days trial of dietary chitosan supplementation was reported. Twenty dairy calves were allotted at random to four groups at 10 days of age. Calves in the control group were fed the basal diet alone and calves in treatment groups I-III were fed the basal diet supplemented with 200, 400, 600 mg/day/h Chitosan (CTS), respectively for 30 days. The trial demonstrated that dietary chitosan supplementation significantly increased serum T3 level ($p<0.05$), when comparing chitosan groups I, II with the control group. Dietary supplementation of chitosan at 400 mg/day/h significantly increased serum T3 ($p<0.05$). Treatments with various levels of chitosan supplementation showed a tendency of increase in serum T4 and GH levels. So, the appropriate supplemental dosage of chitosan in the diets of dairy calves was 400 mg/day/h Chitosan (CTS).

Key words: Chitosan, triiodothyronine, thyroxine, growth hormone, Chinese Holstein dairy calf, China

INTRODUCTION

Chitosan is also called aminopolysaccharide or deacetylchitin. Its chemical name is S (1,4)-2-amino-2-deoxy-D-glucan. It is a natural polysaccharide produced by deacetylation of chitin. Chitosan is abundantly produced in the amount just next to cellulose. It can be found in wide array of lower animals, especially in the exoskeleton of arthropods (insects and crustaceans), cell wall of fungi and algae. It is a pure highly active linear, nontoxic, environmental friendly and biodegradable polysaccharide with good tissue compatibility but without side-effect. Chitosan was initially applied in medicine, (Ramachandran *et al*., 2011) can be concluded that the chitosan microspheres could be considered as a potential biodegradable carrier for controlled drug delivery of ranitidine, food (Chutichudet and Chutichudet, 2011). The results indicated that fruits treated with 3% of chitosan had the least attributes of stomatal conductance, stomata size in terms of stomatal width, stomatal length and stomatal aperture, cosmetics and agriculture field (Aleryani-Raqeeb *et al*., 2008). Chitosan coating had less effect on maintaining firmness (2 folds) but gave better effect in preventing weight loss (5.3%). Chitosan coating treatment markedly slowed the ripening of papaya as shown by their retention of weight loss, delayed changes in their external color and other quality aspects. There are relatively more studies on its applications in humans. Domestic studies of chitosan as a feed supplement for livestock have just started. Yang *et al*. (2007) demonstrated that treatment in drinking water with 0, 0.5, 1.0 and 1.5% microbiological preparation which was prepared with chitosan as substrate increased serum T_3, T_4 levels in chickens without treatment of drug and immunization. Many studies had shown that chitosan had dose-dependent effects on growth performance in broiler chickens, videlicet, the higher concentration eliciting negative effects but the lower concentration eliciting beneficial effects (Razdan and Pettersson, 1996; Shi *et al*., 2005; Alamer and Basiouni, 2005). Tang *et al*. (2005) reported that chitosan could improve growth performance through improving energy, protein and fat metabolism in piglets. In addition, chitosan could also significant increased the concentrations of IGF-I and GH in serum.

Thyroid is an important endocrine organ (Mansourian, 2010, 2011; Salari and Abdollahi, 2011). Its T_3 and T_4 are absolutely required hormone for normal physiology in animals (Issi *et al*., 2011). It regulates metabolism, development, nerve system, cardiovascular system and skin physiology.

The secretion of thyroid hormone is regulated by hypothalamic thyrotropin releasing hormone and pituitary thyroid-stimulating hormone. Large amount of studies have indicated Growth Hormone (GH) is important for growth and development. GH action requires T_3 and T_4 (Wang *et al*., 2006) (Moravej *et al*., 2006). Khazali conducted to evaluate the effect of different energy and protein levels on broiler performance and plasma concentrations of Growth Hormone (GH), Thyroxine

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(T4) and Triiodothyronine (T3). The results of this experiment demonstrate that low-energy intake could increase feed conversion rate and mean plasma concentrations of GH and T3 but could decrease BW and mean plasma concentrations of T4. Different protein levels during starter, grower and finisher periods in broilers may change the plasma concentrations of GH, T4 and T3 but nearly similar protein levels were not changed ($p>0.05$) those hormones (Ghazanfari *et al*., 2010). The result of this experiment indicated that the lowest protein level had the highest growth hormone concentration at 49 days of age. The low energy diet increased growth hormone concentration ($p<0.05$) at 21 days of age in broiler chicken.

Here, the report a study on the effect of chitosan on calf serum GH, T_3, T_4 levels in order to explore the mechanism by which chitosan promotes growth from endocrine point of view.

MATERIALS AND METHODS

Animals: The experiment began on March 2010. Twenty Holstein dairy calves were provide by Great Wall dairy Inc (Zhangjiakou) (http://3813914.71ab.com/prod.asp).

Experiment design: With complete random design, twenty calves of 10 days of age were assigned to four dietary treatments (Control, treatment groups I-III) at random. The trial period was 30 days. Chitosan was supplemented in basal diet at 0, 200, 400, 600 mg day^{-1}, respectively used to treat the four groups.

Management: Animals were managed according to conventional protocol including feed stuff, feed, management and daily arrangement. Each pen holds one animal. Water was provided *ad libitum*. Clear environment was maintained.

Hormone measurement: Blood samples were collected on days 10, 20 and 30 from jugular vein. Samples were centrifuged at 3000 rpm for 15 min (Type 800 centrifuge, Shanghai). Serum was collected and stored at -70°C until test. T_3 and T_4 were measured with a Radioimmuno assay kit produced by Sangbei biomed Inc., (Beijing) (tested by Chinese Agriculture Sciences Academy) (http://www.caas.net.cn/caas/). Serum GH was measured with a Radioimmuno assay kit produced by Shanghai Zhuokang Biotechnology (Measured by Hebei Beifan College Experiment Center) (http://www.hebeinu.edu.cn) according to the protocol.

Statistical analysis: Single factor ANOVA analysis was done with SAS. Results were expressed as mean±SD. Treatments were considered significant difference at $p<0.05$.

RESULTS AND DISCUSSION

Effect of shitosan on calf serum T_3 level: On 10 days of the trial, treatment group I was very significantly different from the control ($p<0.01$) (Fig. 1). Treatment groups II and III were significantly different from control ($p<0.05$). There were no significant difference among treatment groups ($p>0.05$).

On 20 days of the trial, treatment groups I-III were significantly different from the control group ($p<0.05$). There were no significant difference among the treatment groups I-III ($p>0.05$).

On 30 days of the trial, treatment groups I-III were significantly different from the control group ($p<0.05$). There were no significant difference among the treatment groups I-III ($p>0.05$).

Figure 2 shows data from the experiment. Compared with the control, treatment groups I-III had higher serum T_3 level ($p<0.05$). Supplementation with 400 mg chitosan per day per animal showed that most obvious.

Effect of chitosan on calf serum T_4 level: On 10 days of the trial, serum T_4 levels in treatment groups I-III were increased by 2.9, 7.8 and 13.9% compared with the control group, respectively. On 20 days, T_4 levels in treatment groups I-III were increased by 1.4, 3.0 and 7.7%. On 30 days, T_4 levels in treatment groups I-III were increased by 2.7, 4.0 and 23.3%. Overall, it can be seen that chitosan treatment increased serum T_4 level.

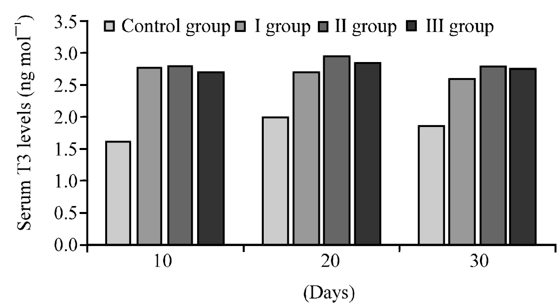

Fig. 1: Effects of chitosan on T3 in dairy calf (ng mL^{-1})

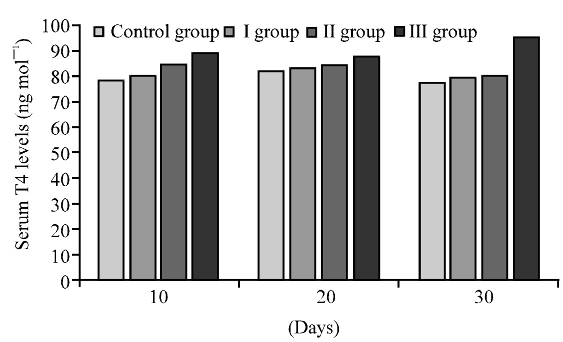

Fig. 2: Effects of chitosan on T4 in dairy calf (ng mL^{-1})

Experimental data indicated that various levels of chitosan supplementation increased serum T_4 level to various extents on days 10, 20, 30 of trial. But it is not statistically significant ($p>0.05$).

Effect of chitosan on calf serum GH level: Figure 3 shows that supplementation of various chitosan increased serum GH level on days 10, 20, 30. Supplementation at 400 mg/head/day increased serum GH level the highest. But this difference was not statistically significant ($p>0.05$).

Thyroid is an important endocrine organ. Its T_3 and T_4 are absolutely required hormone for normal physiology in animals. It regulates metabolism, development, nerve system, cardiovascular system and skin physiology. Many studies have shown that T_3 and T_4 are required for GH action. Deficiency in T_3, T_4 leads to dwarfism. Growth of livestock is regulated by many hormones. Thyroid hormone is the most important hormone. Physiological level of thyroid hormone regulates growth and appetite. Either hypothyroidism or hyperthyroidism causes development retardation. However, if T3 or T4 is supplement timely, growth can resume rapidly. For example, at 1 month of age, chickens that were thyroidectomized at 10 days of age, grew only to 84.5% of normal chicken weight. At 12 months of age, thyroidectomized birds grew only to 64.3% that of normal chicken. The chemistry basis of thyroid hormone promoting growth, development and differentiation is the regulation of protein metabolism. Treatment with protein synthesis inhibitor-actinomycin D promotes metamorphosis of salamander (Gao, 2001). A study in tadpole also proved growth promotion activity of thyroid hormone was protein synthesis dependent. In this study, the tail of a tadpole is removed and put into an independent organ and live for a certain amount of time. When thyroid hormone was added to the culture medium, tail degenerated in a way that was seen in the tadpole. Treatment with actinomycin suppresses thyroid hormone-induced degeneration (Liao, 1992).

Thyroid hormone is not only able to promote growth and development but also stimulated GH secretion and strengthen the effect of GH effect. There is a synergistic effect between thyroid hormone and growth hormone. The effect of thyroid hormone is exerted through stimulating tissue development, secretion, cell size increase, number increase. Its effect is more important for nerve and skeleton system especially during the 1st 4 months. The effect of thyroid hormone on growth and development is dependent on the age of animal. The younger the age, the greater the effect is.

The current experiment showed that treatment groups I-III increased calf serum T_3 compared with the control group ($p<0.05$). Supplementation of chitosan at

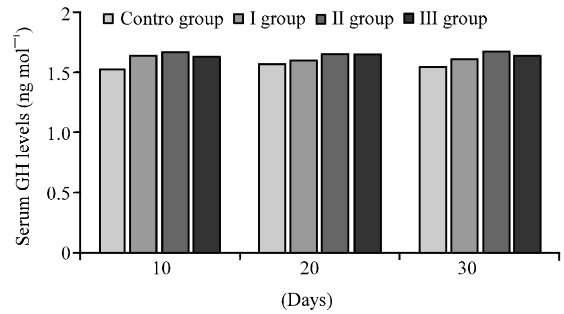

Fig. 3: Effects of chitosan on GH in dairy calves (ng mL^{-1})

400 mg/animal/day increased calf serum T_3 level the most obvious. But the difference was not statistically significant ($p>0.05$). Considering results of the current experiment, it can be deduced that the growth promotion effect may be realized by promoting synthesis and secretion of T_3, T_4.

Brameld *et al*. (1999) reported that serum IGF-I and GH level in piglets are positively related. Tang *et al*. (2005) showed that dietary supplementation of chitosan at 0.025% significantly increased serum IGF-I and liver muscle IGF-I mRNA level. This indicates that chitosan promotes growth through up regulation of GH. Yang *et al*. (2007) discovered that chitosan supplementation can increase serum glucose level, reduce serum lipid level and increase metabolic activity in meat chickens. It improves growth by increasing serum GH level. Zhang *et al*. (2008) showed that serum GH level in piglets is related in a dose-dependent manner to dietary chitosan. Addition of 0.01-0.05% chitosan in diet has a positive effect on promoting serum GH level. These results suggest that dietary supplementation of chitosan can increase piglet's serum GH level, consistent with previous reports. Many studies have shown that GH is important to animal growth and development. This GH-stimulated growth requires T_3 and T_4 (Wang *et al*., 2006). The experiment reported here suggests that chitosan tend to increase serum GH. Considering results from the study reported here, it can be inferred that the growth promoting effect of chitosan may result by stimulating calf GH synthesis and degradation, affecting T_3 and T_4 levels.

CONCLUSION

This study shows that on 10 days of the trial, treatment group I was significantly different from the control group at $p<0.01$; treatment groups II, III were different from the control group at $p<0.05$. On days 20, 30 of the trial, three chitosan groups were different from the control group ($p<0.05$). There were no differences among chitosan groups ($p>0.05$). It can be preliminarily concluded that chitosan significantly stimulates serum T_3 concentration. Supplementation of various levels of chitosan may increase serum T_4 level to certain extent but not statistically significant ($p>0.05$). Supplementation of various levels of chitosan may increase serum GH level to certain extent but not statistically significant ($p>0.05$).

Adding chitosan to diets improved growth performance (Sun *et al*., 2010) the addition of chitosant diets increased levels of serum GH, T3 and T4 References. the appropriate supplemental dosage of chitosan in the diets of dairy calves was 400 mg/day/h Chitosan (CTS).

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