*Available online www.jocpr.com*

**Journal of Chemical and Pharmaceutical Research, 2017, 9(2):258-261**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Effect of Different Functional Sugars on Sperm Quality, Reproductive and Immune Organs in Mice

**Jingchun Li, Qi Li, Guosheng Wei, Yinghua Luo, Shengjun Liu and Yanbing Li***

*College of Animal Science & Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang, China*

---

## ABSTRACT

*This study was conducted to investigate the effects of sorbitol, mannitol, fructooligosaccharides (FOS) and raffinose on sperm quality, reproductive and immune organs of mice. 30 male mice were randomly divided into five groups, each group contained 6 mice (each mouse was used as a repeat). The mice of control group were fed a basal diet, and the mice of groups A, B, C and D were fed the experimental diets supplemented with five percent (w/w) of sorbitol, mannitol, FOS and raffinose, respectively. The experimental period was 30 days. The results showed as follows: sorbitol, mannitol, FOS or raffinose supplementation had no significant effects on sperm density and deformity rate (P>0.05). Compared with the control group, the sperm activity in group of sorbitol or raffinose was significantly decreased (P<0.05). The average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), lateral head displacement (ALH) and mobile of average degree (MAD) of sperm in experimental groups were lower than control group. Sorbitol supplementation significantly reduced the VAP, VCL and ALH of sperm (P<0.05). The FOS supplementation decreased the ALH of sperm (P<0.05). Raffinose supplementation significantly reduced VAP, VSL, VCL, ALH and MAD of sperm (P<0.05).The beat cross frequency (BCF) of sperm in group supplemented with mannitol or FOS was higher than control group, but not statistically significant (P >0.05). The linearity (LIN) and straightness (STR) of sperm in each group had no significant difference (P>0.05).There was no difference in organ index (testis, seminal vesicle, spleen and thymus) between each group (P>0.05). These finding showed that basal diet respectively supplemented with 5% sorbitol, 5% mannitol, 5%FOS or 5%raffinose had some negative effect on sperm quality in mice. But, not influence the development of reproductive and immune organs in mice.*

**Keywords:** Mice; Functional sugar; Sperm quality; Reproductive organ; Immune organ

---

## INTRODUCTION

Sorbitol, also known as glucitol, is a sugar alcohol with a sweet taste which the human body metabolizes slowly. Sorbitol has physiological functions of the prevention of dental caries, diuretic, dehydration, loosening bowel, and which can also be used as a nutritive sweetener and drug. However, a large number of sorbitol can lead to diarrhea and digestive disorders by oral or injection [1]. Mannitol, also known as mannite or manna sugar, is a natural six carbon sugar alcohol, which has functions of chemical stability, no moisture absorption, permeability of dehydration and diuretic [2]. In addition, high dose mannitol may lead to kidney damage and other adverse reactions by oral or injection of [3,4]. Fructooligosaccharide (FOS) [5] and raffinose [6] have physiological functions of promoting the proliferation of bifidobacterium, inhibiting the growth of pathogenic fungi colonization and enhancing immunity. It can't be direct digestion and absorption by stomach and small intestine after intake, which was fermentated by intestinal bacterial, to improve the balance of intestinal bacteria group, so they often were used as food and feed additives. The sorbitol, mannitol, FOS, raffinose have been widely used in food industry, medicine, health care products, chemicals, feed and other fields.

In this study, the same level of sorbitol, mannitol, FOS and raffinose were added to mice basal diet, respectively, to investigate that the effects of the four functional sugars on sperm quality reproductive and immune organs in mice, to provide reference for its application in feed, food and other fields.

## MATERIALS AND METHODS

### Experimental materials

Experimental chemicals and reagent included mannitol (*CAS No*.3458-28-4， Hangzhou Wei Sheng Biotechnology Co., Ltd., sorbitol (*CAS No.*50-70-4, North China Pharmaceutical Co., Ltd), Fructo-oligosaccharide (*CAS No.* 223122-07-4, Zhejiang Luzhou biological science and Technology Co., Ltd.） and raffinose (*CAS No*.17629-30-0 Shanghai Mclean biochemical science and Technology Co., Ltd.）. The other chemicals were purchased from Sigma-Aldrich [China (Shanghai)] unless otherwise noted.

### Experimental animal and designs

Thirty Kunming male mice (6-8 weeks old, weight: 28-35 g) were randomly divided into 5 groups (control group, groups A, B, C, D). Each group contained 6 male mice. mice were fed a basal diet in control group; the mice were fed a basal diet supplemented with 5% sorbitol (w/w) in group A; the mice were fed a basal diet supplemented with 5% mannitol (w/w) in group B; the mice were fed a basal diet supplemented with 5% fructose (w/w) in group C; the mice were fed a basal diet supplemented with 5% raffinose (w/w) in group D [7].

### Based diet and Feeding management

Basal diet for mouse was from Changchun Yisheng experimental animal science and technology Co. Ltd. Six mice as a group were housed in a cage, free feeding and drinking water, the bedding was changed once every 4-5 days, to keep the good environment. The experimental period was 30 days.

### Determination of experimental indexes

**Determination of sperm quality:**

All of mice were weighed, executed euthanasia by cervical dislocation method at the end of experiments. The sperm was collected from each epididymal tail of mouse, and then quality parameters of sperm (motility; straight line velocity, VSL; curvilinear velocity, VCL; average path velocity, VAP; amplitude of lateral head displacement, ALH; beat/cross frequency, BCF; linearity, LIN (LIN=VSL/VCL); mobile of average degree, MAD; straightness, STR (STR=VSL/VAP)) were determined using a computer assisted sperm analysis system (CASA, Nanning Song Jing Tianlun Bio-technology Co., Ltd.)

**Determination of organ index:**

Testis, seminal vesicle, prostate, spleen, thymus were collected, weighted and recorded data from each mouse. Then organ index was calculated,

$$\text{Organ index (\%)} = [\text{organ weight (g)} / \text{mouse body weight (kg)}] \times 100$$

### Statistical analysis

Data from each experiment were analyzed by one-way ANOVA using STATVIEW 5.0 software (Abacus Concepts, Inc., Berkeley, CA, USA). If the ANOVA P value was less than 0.05, a Bonferroni/Dunn's HSD test was carried out using the same program. All data were expressed as mean ± SD. Findings were considered significantly different at $P < 0.05$.

## RESULTS

### Effect of different functional sugars on sperm quality

**Effect of different functional sugar on sperm density, vitality, deformity rate:**

There were no significant differences in sperm density, deformity rate in all groups (Table 1) ($P$>0.05).Sperm motility was highest in control group (86.24% ±14.97%) ($P<0.05$), There was a difference in sperm motility between control group and group A (or D) ($P<0.05$), but not group B (or C). There were no significant differences among groups A, B, C ($P$>0.05), but Sperm motility of these groups were significantly higher than D group ($P<0.05$) (Table 1).

**Table 1: Effect of different functional sugar on sperm density, vitality and deformity rate**

| Groups | Sperm density (million/mL) | Motility (%) | Deformity rate (%) |
|---|---|---|---|
| Control | 21.67±11.01 | 86.24±14.97$^{a}$ | 37.00±3.11 |
| Sorbitol (A) | 17.33±11.02 | 57.95±23.12$^{b}$ | 37.12±2.12 |
| Mannitol (B) | 28.00±15.00 | 72.67±2.98$^{ab}$ | 34.40±2.59 |
| FOS (C) | 13.00±6.38 | 65.86±14.02$^{ab}$ | 34.30±1.48 |
| Raffinose (D) | 13.83±7.29 | 30.43±8.42$^{c}$ | 37.78±2.31 |

Data are given as mean ± SD from 6 replicated experiments. Values with different superscripts within column are significantly different ($P<0.05$).

**Effect of different functional sugars on sperm motion parameters:**

From table 2, the VAP and VCL of sperm were highest in control group (*P<0.05*). Control group was higher in VAP and VCL of sperm than groups A and D (*P*<0.05), group C was higher than group D (*P*<0.05), there were no significant differences in other groups (*P*>0.05). The VSL of sperm in control, groups B, C were significantly higher (*P<0.05*) than group D (*P*<0.05), There were no significant differences in other groups (*P*>0.05). BCF of sperm was highest in group B (*P<0.05*), group B was higher in BCF of sperm than groups A and D (*P*<0.05), there were no significant differences in other groups (*P*>0.05). The control group was significantly higher in ALH of sperm than groups A, C, D (*P<0.05*), group B was significantly higher than that of group D (*P<0.05*), there was no significant difference among other groups (*P*>0.05). The control group was significantly higher in MAD of sperm than group D (*P<0.05*), there was no significant difference among other groups (*P*>0.05). There was no significant difference in LIN and STR of sperm among all groups (*P*>0.05).

**Table 2: Effects of different functional sugars on sperm motion parameters**

| | Control | Sorbitol (A) | Mannitol (B) | FOS (C) | Raffinose (D) |
|---|---|---|---|---|---|
| VAP（μm/s） | 285.45±121.57$^{a}$ | 134.05±57.17$^{bc}$ | 185.24±6.95$^{abc}$ | 192.70±80.98$^{ab}$ | 58.08±14.23$^{c}$ |
| VSL（μm/s） | 247.05±29.94$^{a}$ | 181.17±61.20$^{ab}$ | 235.97±25.71$^{a}$ | 221.22±50.81$^{a}$ | 106.15±34.99$^{b}$ |
| VCL（μm/s） | 406.59±173.17$^{a}$ | 190.95±81.43$^{bc}$ | 263.86±9.91$^{abc}$ | 274.48±115.34$^{ab}$ | 82.73±20.27$^{c}$ |
| BCF（frequency /s） | 8.92±3.70$^{ab}$ | 3.29±2.27$^{b}$ | 12.50±8.35$^{a}$ | 9.15±3.39$^{ab}$ | 1.99±0.93$^{b}$ |
| ALH（μm） | 121.15±51.59$^{a}$ | 56.89±24.26$^{bc}$ | 78.62±2.95$^{ab}$ | 65.12±12.48bc | 24.65±6.04$^{c}$ |
| MAD（degree） | 15.26±11.19$^{a}$ | 3.49±2.39$^{ab}$ | 14.17±9.78$^{ab}$ | 10.82±4.19$^{ab}$ | 1.71±0.92$^{b}$ |
| LIN | 0.72±0.26 | 1.05±0.23 | 0.92±0.04 | 1.41±0.95 | 1.46±0.29 |
| STR | 1.02±0.37 | 1.49±0.33 | 1.30±0.06 | 2.00±1.36 | 2.07±0.42 |

Data are given as mean ± SD from 6 replicated experiments. Values with different superscripts within row are significantly different *(P<0.05*)

**Effect of different functional sugars on organ index of mice:**

The results showed that there was no significant difference in organ indexes (testis, seminal vesicle, spleen and thymus ) between groups A, B, C, D and control group (*P>0.05*). Testis index of group C was significantly higher than group B (*P<0.05*), thymus index of group C was significantly higher than group A (*P<0.05*), there was no significant difference among other groups (*P>0.05*) (Table 3).

**Table 3 Effect of different functional sugars on organ index of mice**

| | Control | Sorbitol (A) | Mannitol (B) | FOS (C) | Raffinose (D) |
|---|---|---|---|---|---|
| Testis（%） | 4.13±0.46$^{ab}$ | 4.50±0.34$^{a}$ | 3.61±0.36$^{b}$ | 4.68±0.35$^{a}$ | 4.73±0.37$^{a}$ |
| Seminal vesicle（%） | 7.07±0.26 | 5.94±1.67 | 5.69±0.58 | 6.71±0.65 | 6.75±1.67 |
| Spleen（%） | 3.95±1.12 | 3.59±0.54 | 3.62±0.69 | 4.85±2.09 | 3.82±0.22 |
| Thymus（%） | 2.03±0.69$^{ab}$ | 1.69±1.06$^{b}$ | 1.97±0.42$^{ab}$ | 3.02±0.19$^{a}$ | 2.34±0.05$^{ab}$ |

Data are given as mean±SD from 6 replicated experiments. Values with different superscripts within row are significantly different (*P<0.05*)

## DISCUSSION

In this study, mouse sperm motility were decreased by feeding the a basal diet supplemented with 5% sorbitol, mannitol, FOS, raffinose, respectively. Sperm deformity rate was the highest in a basal diet supplemented with 5% raffinose. Abdul-Salame'resluts showed that sperm density and sperm motility in the epididymis were increased by feeding a rat diet added 5% honey. Meanwhile, Enzyme activities related to the development and maturation of sperm were also increased[8]. Sorbitol and mannitol in the body only a small part were became glycogen in liver and the other part was excreted by kidney, which can make the surrounding tissue dehydration to form a hypertonic blood. Sorbitol is an alcoholic sugar, which can penetrate sperm plasma membrane [9]. Emmens *et al* [10] indicated that the osmotic pressure was higher than that of the sperm. Sperm have a high sensitivity to oxygen free radicals, which are highly sensitive to oxidative damage. FOS and raffinose were not digested and absorbed by stomach and small intestine, but they were fermented to produce acetic acid, propionic acid, butyric acid and short chain fatty acids by intestinal bacterial fermentation, peroxides generated in the process of lipid peroxidation may cause oxidative damage to mice sperm. The VAP, VSL, VCL of sperm were effective indicators of sperm motility, BCF of sperm was the frequency of the sperm head across the average path, and the beat frequency of sperm tail was increased significantly when the sperm motility was weakened or the amplitude of the tail side was decreased; LIN and STR are used to reflect sperm motility [11]. In this study, the addition of functional sugar in the diet decreased the sperm motility parameters in different level. The VAP, VCL, ALH of sperm motion parameters were significantly decreased by feeding a basal diet supplemented with 5% Sorbitol. The VAP, VSL, VCL, ALH LINE, STR of sperm motion parameters were significantly decreased by feeding a basal diet supplemented with 5% raffinose. Effect of mannitol and fructose on sperm motility of male mice is smaller than sorbitol and Raffinose, but The VAP, VSL, VCL, ALH, LINE, STR of sperm motility parameters also showed a downward trend. The results showed that sorbitol, mannitol, FOS and raffinose have

negative effects on sperm quality. Wang's results showed that the growth performance of broilers was decreased with the increase of oligosaccharides in diet [12]; Liu *et al* [13] have confirmed that the diet containing low concentration of raffinose was conducive to the growth of mink. We hypothesized that decrease of sperm motion parameters of male mice may be related to the concentration of functional sugar in the diet. Currently, several researchers were focus on the effect of functional sugar on growth performance, immune function and intestinal microorganism [14,15], and not animal reproductive performance. Thus, studies of different concentration of functional sugars affect animal sperm will be continued in the further.

The main function of the testis is to produce sperm and to secrete testosterone. Seminal vesicle gland secretes seminal fluid, which has an important role in sperm-survival. Spleen is the largest immune organ in the body, which can improve the body immunity through various mechanisms. Thymus is an important lymphoid organ, and its function is closely related with the immune function, which Thymus secretes hormones and hormone like substances. Organ function can be explained by the size of the organ index, which is one of the main biological characteristics. In this study, the four functional sugars have no significant impact on organ index of mice, the results showed that four functional sugars have no significantly impact on development of mouse organs. Mikkelsen *et al* [16] reported that the diets supplemented with oligosaccharides could increase the serum IgA and IgG levels, and enhance the immune function of animals, which was different from the results of this experiment; Abdul-Salam's results showed that 5% honey in rats fed diet had no significantly effect on spleen, kidney, liver, testis, seminal vesicle, which was similar to our results [8,17]. The reason probably is that different animals have different responses to kinds of sugars.

## CONCLUSIONS

This study showed that the basal diet respectively supplemented with 5% sorbitol, 5%mannitol, 5%FOS, 5%raffinose were to certain extent reduced sperm motility and the motion parameters, which have some negative effects on mouse sperm quality. But, not influence the development of reproductive and immune organs in mice. Therefore, application of functional sugar in feed is still to be further studied.

## ACKNOWLEDGEMENTS

This project was supported by National Natural Science Foundation of China (No. 31402136), Natural Science Foundation of Heilongjiang Province of China (No. JJ2017ZR0120), Scientific Research Project of Heilongjiang Provincial Land Reclamation Bureau (No. HNK135-04-06; HNK135-04-02-03), the Doctoral Starting up Foundation of Heilongjiang Bayi Agricultural University (No.B2012-06), and Daqing guiding scientific research project (No. zd-2016-087).

## REFERENCES

[1] Zheng JX. *Chem Ind Press*, **2005**, 114-145.
[2] W Soetaert; PT Vanhooren; EJ Vandamme. *Methods Biotechnol*, **1999**, 10, 261-275.
[3] HX Liu; QB Li. *Pract Pharm Clin Rem*, **2015**, 45-50.
[4] ZY Xu; L Fang; BB Chen. *Chinese J Nephrol*, **2010**, 26 (4), 264-270.
[5] S Xue. *Food Ind*, **2012**, 33 (4), 115-119.
[6] DL Zhou; WG Song; WF Guo; ZK Chen. *Cereal Oil*, **2010**, 12, 39-41.
[7] X Jin; L Xiao; M Xiao; E Sakakuchi. *Nutrition*, **2013**, 29, 325-331.
[8] AG Abdul-Salam; D Nabil; M Rateb. *J Med Food*, **2008**, 11 (4): 799–802.
[9] MM Buhr; P Fiser; JL Bailey; EF Curtis. *J Androl*, **2001**, 22, 961-969.
[10] CW Emmens. *J Physiol*, **2008**, 144(2), 437-458.
[11] YC Shi; XJ Shang; XL Wang; YF Huang. *Natl J Androl*, **2006**, 12(8), 703-705.
[12] ZH Wang; XQ Huang. *Chinese J Anim Sci*, **2012**, 48, 59-61.
[13] BY Liu; GY Li; K Bao; HL Liu; DL Li; D Gu; T Zhang. *Chinese J Anim Nutr*, **2013**, 25, 1123-1130.
[14] J Hua; X He; ZJ Yang; SP Zhang; WY Yang; L Liu. *J B Univ Agr*, **2010**, 25 (2), 1-5
[15] ER Farnworth; HW Modler; JD Jones. *Can Vet J*, **1992**, 72(4), 977-980.
[16] LL Mikkelsen; M Jakobsen; BB Jensen. *Anim Feed Sci Tech*, **2003**, 109(1), 133-150.
[17] SS Nandre, UG Deshpande; SR Patil. *J Chem Pharm Res*, **2015**, 7(6), 328-330.