

Effects of supplemental fructo-oligosaccharide and mannan-oligosaccharide on nutrient digestibilities, volatile fatty acid concentrations, and immune function in horses

Emel GÜRBÜZ^{1,*}, Fatma İNAL¹, Sakine Ülküm ATA¹, Özcan Barış ÇİTİL¹,
Kürşat KAV², Fatih KÜÇÜKKAYA¹

¹Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine,
Selçuk University, 42075, Konya - TURKEY

²Department of Microbiology, Faculty of Veterinary Medicine, Selçuk University, 42075, Konya - TURKEY

Received: 29.02.2008

Abstract: This research was performed to study the effects of fructo-oligosaccharide (FOS) and mannan-oligosaccharide (MOS) on nutrient digestibilities, fecal pH and volatile fatty acids compositions, and immune function in adult horses. Four adult Thoroughbred horses were used in a 4×4 Latin Square design with 20-day treatment periods. A 12-day adaptation phase was followed by 6-day collection of feces and 2-day blood sampling phases. Four different treatments were administered: 1) Control (no supplemental FOS or MOS; 2) 30 g of FOS/d; 3) 30 g of MOS/d; 4) 15 g of FOS+15 g of MOS/d. There were no differences among treatments in the fecal pH and volatile fatty acids compositions ($P > 0.05$). Nutrient digestibilities did not differ among treatments ($P > 0.05$). Also, no differences were found in the IgA, IgG, and IgM levels among the treatments ($P > 0.05$). Fructo-oligosaccharides, MOS, or FOS+MOS supplementation did not affect gut health and immune system in adult horses compared to control.

Key words: Horse, fructo-oligosaccharide, mannan-oligosaccharide, volatile fatty acids, immune function

Atlarda frukto-oligosakkarit ve mannan-oligosakkarit kullanımının besin maddelerinin sindirilme dereceleri, uçucu yağ asitleri ile immun sistem üzerine etkileri

Özet: Bu araştırma, atların yemlerine katılan frukto-oligosakkarit (FOS) ve mannan-oligosakkaritin (MOS) besin maddelerinin sindirilme dereceleri, dışkı pH'sı ve uçucu yağ asitleri kompozisyonu ile immun sistem üzerine etkilerini incelemek amacıyla yapılmıştır. Araştırma 4×4 Latin kare deneme düzenine göre yürütülmüş ve değerlendirilmiştir. Dört dönem halinde yürütülen araştırmanın, her bir dönemi 12 günü adaptasyon, 6 günü dışkı ve 2 günü kan numunesi toplama olmak üzere 20'şer gün, toplam 80 gün sürdürülmüştür. Her dönemde dört gruptan biri kontrol olarak tutulmuş, diğer gruplar ise, rasyona 30 g/gün FOS ilave edilen grup, 30 g/gün MOS ilave edilen grup ve 15 g/gün FOS + 15 g/gün MOS ilave edilen grup şeklinde oluşturulmuştur. Dışkı pH'sı ve uçucu yağ asitleri kompozisyonları arasındaki farklılıklar önemli bulunmamıştır ($P > 0,05$). Gruplara göre besin maddelerinin sindirilme dereceleri arasında farklılıklar önemli bulunmamıştır ($P > 0,05$). Serum IgA, IgG ve IgM konsantrasyonlarında da önemli bir değişiklik oluşmamıştır ($P > 0,05$). Ergin atların rasyonlarına FOS, MOS veya FOS + MOS ilavesinin bağırsak sağlığı ve immun sistem üzerinde etkisi bulunmamıştır.

Anahtar sözcükler: At, frukto-oligosakkarit, mannan-oligosakkarit, uçucu yağ asitleri, immun sistem

* E-mail: vet_em@yahoo.com

Introduction

Fructo-oligosaccharides (FOS) have been recommended as a prebiotic compound. Commonly FOS are extracted chicory root in the form of inulin or synthesized from sucrose through transfructosylation by an enzyme from fungus, such as *Aspergillus niger* or *Aurebasidium pullulans* (1).

Fructo-oligosaccharide is believed to bypass the upper gastrointestinal tract of human and monogastric animals without being hydrolyzed or absorbed, and is used in the colon as substrate for potentially beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli* (2). The growth of these bacteria has been shown to increase the production of short-chain fatty acids and organic acids, which lowers pH in the large intestine and inhibits the growth of pathogenic bacteria, and improves host health (3).

Mannan-oligosaccharides (MOS) contain mannan-based oligosaccharides derived from yeast cell wall. Dietary supplementation of MOS has enhanced performance, improved immune function, and inhibited colonization of the digestive tract. Mannan-oligosaccharides attach to the lectins of pathogenic microorganisms thus preventing the bacteria from attaching to intestinal epithelial cells in a number of livestock species (4). While some studies have reported improvements of performance or immune function, such as weight gain, feed conversion, lymphocyte transformation, and Ig concentrations (5), other studies have shown no effects of MOS on immune function (6,7).

The objective of the present study was to compare the effects of FOS, MOS, or FOS+MOS on nutrient digestibilities, fecal pH and volatile fatty acids concentrations, and immune function in adult horses.

Materials and methods

Experimental design and animals: This research was performed by the 4×4 Latin Square design. Four clinically healthy Thoroughbred males (6-12 years old) with an average body weight of 471.25 ± 1.28 kg were used. The horses were housed individually in stalls with a layer of wooden shavings as bedding. Each period continued for 20 days, and consisted of 12 days for adaptation, 6 days for collection of feces, and 2 days for blood sampling.

Diets and feeding: Four different treatments were administered: 1) Control (no supplemental FOS or MOS); 2) 30 g of FOS/d; 3) 30 g of MOS/d; 4) 15 g of FOS+15 g of MOS/d. The FOS supplement was obtained from Beghin Meiji (Marckolsheim, France). The MOS supplement was obtained from Alltech (Nicholasville, KY). The FOS and MOS were in dry powder and were mixed to concentrate before morning feeding. The composition of concentrate is shown in Table 1. Horses were fed concentrate at 0.6% of their BW on an as-fed basis daily according to NRC requirements (9). Alfalfa hay, which was used as forage, was also given at 1.2% of their BW.

Sample collection: During the adaptation period, horses were exercised every day for 30-60 min. Horses were not exercised during the sample collection period. Single fecal samples were collected from each horse immediately after defecation for the measurement of digestibility of nutrients, volatile fatty acids, and pH. Apparent total tract digestibilities of nutrients were determined by an indicator method, in which acid insoluble ash was used as the indicator. Fecal pH was determined immediately after defecation by thoroughly mixing equal amounts of feces and double-deionized water and submerging the pH probe (Hanna Instrument 8314) in the mixture (10).

On day 19-20, blood samples (10 mL) were collected via jugular puncture into nonheparinized tubes for determining serum Ig concentrations.

Feeds and feces analyses: Feeds and feces were analyzed for dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF) (11). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) levels were measured in the Ankom Fiber Analyzer according to the Goering and Van Soest method (12).

Volatile fatty acid analyses: Previously frozen fecal samples were thawed and 2 g samples were taken. The concentrations of acetate, propionate, butyrate, valerate, isovalerate and isobutyrate concentrations were determined via gas chromatography (13) [GC 15-A gas chromatograph (Kyoto, Japan) equipped with glass column (1.83 m × 2 mm i.d.) and 10% SP-1200/1% H₃PO₄ on 80/100 Chromosorb W AW (Supelco, Bellefonte, PA)]. Nitrogen was the carrier

Table 1. Composition and nutrient content of the diet.

Ingredient	as-fed basis(%)
Dried sugar beet pulp	26.00
Oat	24.00
Barley	23.45
Corn	18.00
Soy bean meal	5.00
Dicalcium phosphate	1.50
Limestone	1.10
Vitamin-mineral premix ^a	0.20
Sodium Chloride	0.75
Analyzed composition	
Dry matter (%)	93.87
Ash (%)	5.37
DE ^b (Mcal/kg)	2.87
Crude protein (%)	10.95
Ether extract (%)	2.24
Crude fiber (%)	14.02
NDF (%)	38.71
ADF (%)	11.33

^a Per kg: vit A 10,000,000 IU, vit D3 200,000 IU, vit E 20,000 mg, vit K3 12,000 mg, vit B1 6700 mg, vit B2 3300 mg, nicotinamide 5200 mg, vit B6 5000 mg, vit B12 3300 mg, folic acid 1400mg, D-Biotin 40 mg, cholin 67,000 mg, vit C 34,000 mg, MnSO₄ 6700 mg, FeSO₄ 8000 mg, ZnO 12 000 mg, CuSO₄ 4000 mg, Co 67 mg, Se 10 mg, Na 1140 mg, L-Lysine 70,000 mg, DL-Methionine 35,000 mg

^b DE Mcal/kg; $2118 + 12.18(\text{CP}) - 9.37(\text{ADF}) - 3.83(\text{NDF} - \text{ADF}) + 47.18(\text{EE}) + 20.35(100 - \text{CP} - \text{EE} - \text{NDF} - \text{ash}) - 26.3(\text{ash})$ (8).

gas with a flow rate of 40 mL/min. Air and hydrogen gas were used for detector with a flow rate of 300 mL/min and 30 mL/min. Oven temperature was 125 °C.

Immunological analyses: After the blood was collected in nonheparinized tubes, samples were centrifuged at 2000 ×g for 20 min at 4 °C and the serum was collected. Serum IgA, IgG, and IgM concentrations were determined using ELISA test kits (Bethyl Laboratories, UK).

Statistical analyses: All response variables were analyzed according to the General Linear model in SPSS (14). The experimental design was a 4×4 Latin Square design. Four sequences of diets were used (ABDC, BCAD, CDBA, and DACB), in which A was the control, B was the MOS treatment, C was the FOS treatment, and D was the FOS+MOS treatment. The model used for all dietary variables had fixed effects: diet, period, and horse.

Results

There were no differences among treatments in terms of the total tract digestibilities of DM, organic matter (OM), CP, EE, CF, NDF, and ADF ($P > 0.05$) (Table 2).

Supplementation of FOS and MOS did not affect fecal pH, acetate, propionate, butyrate, valerate, isobutyrate, or isovalerate concentrations compared to control ($P > 0.05$) (Table 3).

In the present research, FOS and MOS given alone or together to horses did not affect the fecal pH or VFA concentrations. The numerical increases in acetate ($P = 0.149$), propionate ($P = 0.191$), and butyrate ($P = 0.340$) concentrations were only observed when FOS or MOS were fed alone, but not when FOS and MOS were fed together.

Table 2. Nutrient digestibilities in horses with FOS or/and MOS^a supplementation.

Item	Control	FOS	MOS	FOS + MOS	SEM ^b	P-Value
Dry matter (%)	77.07	75.28	74.10	73.07	0.64	0.095
Organic matter (%)	77.72	76.01	74.69	73.66	0.64	0.087
Crude protein (%)	75.68	73.29	72.14	71.33	1.78	0.308
Ether extract (%)	52.75	49.89	47.21	48.36	1.56	0.458
Crude fiber (%)	63.37	60.75	62.11	55.87	1.16	0.063
NDF (%)	60.40	57.19	55.63	53.41	1.14	0.092
ADF (%)	42.38	39.14	36.47	32.26	1.58	0.073

^aValues are means, n = 4.

^bPooled Standard error of the mean.

No differences in serum IgA, IgG, and IgM concentrations were observed among treatments ($P > 0.05$) (Table 4).

Discussion

In this study, feeding adult horses daily with 30 g FOS, 30 g MOS, or 15 g FOS+15 g MOS did not affect horse body weights. This may have resulted because the animals were at their mature weight and they were not under performance stress.

Feeding FOS, MOS, or FOS+MOS in horses decreased the total tract digestibilities of DM, OM, CP, EE, CF, NDF, and ADF, but these decreases were not statistically significant. Some studies in other species also found that FOS and/or MOS supplementation did not affect the total tract digestibilities of DM, OM, CP, ash, and EE (15-17). However, other studies with dogs and cats found that

FOS supplementation decreased total tract digestibilities of DM, OM, and CP (15,18).

The fecal pH and VFA concentrations found in the present study were similar to those reported by Hussein et al. (Table 3) (19). The VFA concentration in feces is used as an indicator for the fermentation in hindgut. In yearling horses fed 8 or 24 g FOS daily, there was a decrease in fecal pH but an increase in VFA concentrations due to short chain fatty acids and lactic acids in the intestine (9).

In a similar study, the fecal pH in dogs was not affected by FOS supplementation, but MOS supplementation increased the fecal pH (17). In other studies with rats and pigs (20,21), FOS supplementation did not affect the fecal pH, which supports the findings in the present study.

Changes in fecal pH or VFA concentrations may not have been observed because FOS and MOS function in the hindgut and most of the short chain

Table 3. Fecal pH and volatile fatty acids concentrations of horses with FOS or/and MOS^a supplementation.

Item	Control	FOS	MOS	FOS + MOS	SEM ^c	P-Value
pH	6.92	6.96	6.88	7.00	0.020	0.226
Total VFA ^b (mg/g)	1.74	1.80	1.89	1.50		
Acetate	1.204	1.277	1.343	1.111	0.070	0.149
Propionate	0.286	0.304	0.321	0.232	0.030	0.191
Butyrate	0.088	0.088	0.108	0.059	0.020	0.340
Valerate	0.009	0.008	0.011	0.003	0.002	0.741
Isovalerate	0.058	0.062	0.064	0.040	0.008	0.785
Isobutyrate	0.056	0.060	0.053	0.054	0.005	0.955

^aValues are means, n = 4.

^bData are on a wet basis.

^cPooled Standard error of the mean.

Table 4. Serum immunoglobulin concentrations in horses with FOS or/and MOS^a supplementation.

Item	Control	FOS	MOS	FOS + MOS	SEM ^b	P-Value
IgA (g/L)	2.210	2.061	2.054	2.065	0.080	0.904
IgM (g/L)	0.779	0.701	0.689	0.690	0.031	0.739
IgG (g/L)	16.446	13.951	16.308	16.759	0.917	0.729

^aValues are means, n = 4.

^bPooled Standard error of the mean

fatty acids are absorbed in the colon. Campbell et al. (20) reported that fecal pH was not affected by oligosaccharides supplementation, while cecal pH decreased.

In another study with various animal species, age, diet, and microbial population in the intestine, FOS and/or MOS supplementation affected the fecal pH and/or VFA concentrations. In one study with pigs, MOS decreased the total VFA concentrations while FOS had no effect (22). In other studies with dogs and pigs, FOS and/or MOS did not affect the amount of short chain fatty acids (17,23). Other studies, however, reported an increase in fecal propionate or short chain fatty acids in dogs fed FOS, while there was no change in fecal short chain fatty acids in female dogs fed 1, 2 or 3 g/day of FOS (3,18).

Serum IgA, IgM, and IgG concentrations are shown in Table 4. FOS, MOS, and FOS+MOS supplementation did not affect the serum Ig levels. FOS and MOS supplementation may not have been effective because the horses used in the present study were mature and were not under any type of performance stress. Feed additives are more effective in animals under stress conditions. Spearman (24) reported that the level of serum Ig in mares or their foals did not change by MOS supplementation.

In studies with cows, dogs or pigs, the addition of MOS also did not affect the level of serum Ig (7,8,25,26). In other studies, however, the addition of MOS increased the level of serum Ig in dogs and pigs (17,27).

In another study with mature horses, the levels of serum IgG, IgA, and IgM were reported between 10-20.19 g/L, 0.6-3.5 g/L, and 0.65-2 g/L, respectively (24,28). In the present study, the levels of serum IgG, IgA, and IgM in horses fed control diet were 16.45, 2.21, and 0.78 g/L, respectively.

In conclusion, FOS, MOS, or FOS+MOS supplementation at the doses used had no effect on fecal pH and VFA, nutrient digestibilities, or the immune system in adult horses. The use of FOS or MOS may be most beneficial in young horses or horses under performance stress.

Acknowledgement

This study was supported by the Scientific and Technological Research Council of Turkey (TOVAG-105 O 145).

References

1. Sangeetha, P.T., Ramesh, M.N., Prapulla, S.G.: Production of fructo-oligosaccharides by fructosyl transferase from *Aspergillus oryzae* CFR 202 and *Aureobasidium pullulans* CFR 77. *Process Biochem.*, 2004; 39: 755-760.
2. Perrin, S., Warchol, M., Grill, J.P., Schneider, E.: Fermentations of fructo-oligosaccharides and their components by *Bifidobacterium infantis* ATCC 15697 on batch culture in semi-synthetic medium. *J. Appl. Microbiol.*, 2001; 90: 859-865.
3. Flickinger, E.A., Van Loo, J., Fahey, G.C. Jr.: Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: a review. *Crit. Rev. Food Sci. Nutr.*, 2003; 43: 19-60.
4. Franklin, S.T., Newman, M.C., Newman, K.E., Meek, K.I.: Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. *J. Dairy Sci.*, 2005; 88: 766-775.
5. Flemming, J.S., Freitas, J.R.S., Fontoura, P., Montanhini Neto, R., Arruda, J.S.: Use of mannanoligosaccharides in broiler feeding. *Rev. Bras. Cienc. Avic.*, 2004; 6: 159-161.
6. Davis, M.E., Maxwell, C.V., Kegley, E.B., de Rodas, B.Z., Friesen, K.G., Hellwig, D.H., Dvorak, R.A.: Efficacy of mannan oligosaccharide (Bio-Mos) addition at two levels of supplemental copper on performance and immunocompetence of early weaned pigs. *J. Anim. Sci.*, 1999; 77 (Suppl. 1): 63 (Abs.).
7. Davis, M.E., Brown, D.C., Maxwell, C.V., Johnson, Z.B., Dvorak, R.A.: Efficacy of mannan oligosaccharide (Bio-Mos) as a complete or partial replacement for zinc oxide in the diets of weanling pigs. *J. Anim. Sci.*, 2001; 79 (Suppl. 2): 78.
8. Harris, P.A.: Comparison of the digestible energy (DE) and net energy (NE) systems for the horse. In: Pagan, J.D., Ed. *Advances in Equine Nutrition*. Nottingham University Press, United Kingdom. 2001; 199-215.
9. NRC-National Research Council: *Nutrient Requirements for Horses*, 5th edn., National Academy Press, Washington, DC. 1989.
10. Berg, E.L., Fu, C.J., Porter, J.H., Kerley, M.S.: Fructooligosaccharide supplementation in the yearling horse: effects on fecal pH, microbial content and volatile fatty acid concentrations. *J. Anim. Sci.*, 2005; 83: 1549-1553.

11. AOAC: Official Methods of Analysis. 14th edn. Assoc. Offic. Anal. Chem., Gaithersburg, MD. 1984; 152-159.
12. Goering, H.K., Van Soest, P.J.: Forage Fiber Analyses (Apparatus, Reagents and Some Applications). US Department of Agriculture Handbook, No.379 ARS-USDA, Washington, DC. 1970.
13. Otto, E.R., Yokoyama, M., Hengemuehle, S., von Bermuth, R.D., van Kempen, T., Trotter, N.L.: Ammonia, volatile fatty acids, phenolics, and odor offensiveness in manure from growing pigs fed diets reduced in protein concentration. *J. Anim. Sci.*, 2003; 81: 1754-1763.
14. SPSS: SPSS / PC + V.2.0. Base manual for the IBM PC/XT/AT and PS/2, Marjia and Morusis, 14 Inc. 444 N. Michigan Avenue, Chicago, IL, 60611. 1998.
15. Diez, M., Hornick, J.L., Baldwin, P., Istasse, L.: Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrient digestibility and plasma metabolite concentrations in healthy beagles. *Am. J. Vet. Res.*, 1997; 58: 1238-1242.
16. Houdijk, J.G.M., Bosch, M.W., Tamminga, S., Verstegen, W.A., Berenpas, E.B., Knoop, H.: Apparent ileal and total-tract nutrient digestion by pigs as affected by dietary non-digestible oligosaccharides. *J. Anim. Sci.*, 1999; 77: 148-158.
17. Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Healy, H.P., Dawson, K.A., Merchen, N.R., Fahey, G.C. Jr.: Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. *J. Nutr.*, 2002; 132: 980-989.
18. Propst, E.L., Flickinger, E.A., Bauer, L.L., Merchen, N.R., Fahey, G.C. Jr.: A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. *J. Anim. Sci.*, 2003; 81: 3057-3066.
19. Hussein, H.S., Vogedes, L.A., Fernandez, G.C.J., Franken, R.L.: Effects of cereal grain supplementation on apparent digestibility of nutrients and concentrations of fermentation end-products in the feces and serum of horses consuming alfalfa cubes. *J. Anim. Sci.*, 2004; 82: 1986-1996.
20. Campbell, J.M., Fahey, G.C. Jr., Wolf, B.W.: Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH, and microflora in rats. *J. Nutr.*, 1997; 127: 130-136.
21. Mikkelsen, L.L., Jakobsen, M., Jensen, B.B.: Effects of dietary oligosaccharides on microbial diversity and fructo-oligosaccharide degrading bacteria in faeces of piglets post-weaning. *Anim. Feed Sci. Technol.*, 2003; 109: 133-150.
22. Smiricky-Tjardes, M.R., Flickinger, E.A., Grieshop, C.M., Bauer, L.L., Murphy, M.R., Fahey, G.C. Jr.: In vitro fermentation characteristics of selected oligosaccharides by swine fecal microflora. *J. Anim. Sci.*, 2003; 81: 2505-2514.
23. Howard, M.D., Liu, H., Spencer, J.D., Kerley, M.S., Allee, G.L.: Incorporation of short-chain fructooligosaccharides and tylosin into diets of early weaned pigs. *J. Anim. Sci.*, 1999; 77 (Suppl. 1): 63 (Abs.).
24. Spearman, K.R.: Effect of mannan oligosaccharide (MOS) supplementation on the immune status of mares and their foals. MSc Thesis, University of Florida, 2004.
25. Franklin, S.T., Newman, K.E., Newman, M.C.: Evaluation of mannan oligosaccharide on the immune status of dairy cows and their calves. *J. Anim. Sci.*, 2002; 80 (Suppl): 192.
26. Grieshop, C.M., Flickinger, E.A., Bruce, K.J., Patil, A.R., Czarnecki-Maulden, G.L., Fahey, G.C. Jr.: Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. *Arch. Anim. Nutr.*, 2004; 58: 483-493.
27. Davis, M.E., Maxwell, C.V., Brown, D.C., de Rodas, B.Z., Johnson, Z.B., Kegley, E.B., Hellwig, D.H., Dvorak, R.A.: Effect of dietary mannan oligosaccharide and (or) pharmacological additions of copper sulfate on growth performance and immunocompetence of weanling and growing/finishing pigs. *J. Anim. Sci.*, 2002; 80: 2887-2894.
28. Raymond, S.L., Smith, T.K., Swamy, H.V.L.N.: Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J. Anim. Sci.*, 2003; 81: 2123-2130.