www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(6): 163-167

www.thepharmajournal.com
Received: 23-03-2023
Accepted: 26-04-2023

Faheem Abdul Basit
Division of Animal Nutrition, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Ankur Rastogi
Division of Animal Nutrition, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

RK Sharma
Division of Animal Nutrition, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Pratiksha Raghuwanshi
Division of VPB, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Arvind Kumar
Division of LPT, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Puneet Singh
Division of VPB, FVSc & AH, SKUAST-J, R S Pura, Jammu

Zulfqar ul Haq
Division of LPM, FVSc & AH, SKUAST-K, Srinagar, Jammu and Kashmir, India

Ramandeep Kour
Division of Animal Nutrition, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Corresponding Author:
Pratiksha Raghuwanshi
Division of VPB, FVSc & AH, SKUAST-J, R S Pura, Jammu and Kashmir, India

Effect of dietary supplementation of a prebiotic fraction derived from rumen liquor over nutrient utilization in broiler chickens

Faheem Abdul Basit, Ankur Rastogi, RK Sharma, Pratiksha Raghuwanshi, Arvind Kumar, Puneet Singh, Zulfqar ul Haq and Ramandeep Kour

Abstract
The present research work scrutinized the nutrient utilization of broiler chickens as affected by the dietary supplementation of a prebiotic fraction of rumen liquor compared to xylooligosaccharides supplementation. Rumen liquor (RL) of sheep and goats was collected from a slaughterhouse and then processed in the laboratory to extract a soluble fibre fraction. Four hundred eighty broiler chicks were randomly assigned to treatment groups: CO, RL, XOS, and AB. Each group contained six replicates of twenty birds. The CO group was fed a basal diet without supplementation; RL and XOS groups were fed a basal diet supplemented with RL extract or XOS @ 0.05% (w/w), respectively, whereas the AB group was fed a basal diet with antibiotic growth promoter (Enramycin-8) @ 0.0125%. A metabolic trial was conducted during the last week of the experiment on two birds per replicate. The mean daily nutrient intake remained comparable among the experimental groups. The DM and OM metabolizability was similar in RL, XOS and CO groups but was significantly lower in the AB group. The crude fibre metabolizability was higher in XOS and RL groups than in AB and CO groups. There was no significant difference in nitrogen, calcium and phosphorus balance. It is concluded that nutrient utilization is unaffected by prebiotic supplementation, including RL supplementation. Further, no untoward effect of RL supplementation was observed over nutrient utilization in broiler chicks.

Keywords: Broiler, calcium, metabolizability, prebiotic, phosphorus, rumen liquor

1. Introduction
Hindgut microbes and their fermentation end-products have local and systemic influence over the physiological functions of poultry [1]. Modifying the gut microbiome's physiology has emerged as one of the most promising interventional tools to optimize the performance of poultry birds. This is attempted traditionally via dietary supplementation of antibiotics growth promoters and, more recently, through various microbial feed additives or organic compounds. It is now widely accepted that indiscriminate use of these antibiotics in the poultry industry is perhaps one of the factors responsible for the emergence of antibiotic-resistant bacteria [2].
Probiotics, prebiotics, and synbiotics are fast emerging as the most viable alternatives of antibiotic growth promoters (AGPs) [3]. They have effects similar to AGPs without worries of residues or resistance. Prebiotics has been proposed as a preferred alternative to AGP compared to probiotics, as prebiotics stimulate the beneficial bacteria, which have already adapted to the gut environment [4].
Inulin, fructooligosaccharides, galactooligosaccharides, lactulose, polydextrose, isomaltooligosaccharides, xylooligosaccharides (XOS), lactitol, mannitol, maltodextrin, raffinose, sorbitol, resistant starch, oat beta-glucan, flaxseed gum, and mannan oligosaccharide are various established or candidate prebiotics [5].
The microbial ecosystem of the rumen in ruminants consists of many microbial groups acting synergistically, performing bioconversion of feedstuffs that primarily consist of complex polysaccharides of plant origin. A spectrum of microbial and plant enzymes act over these ingredients in the rumen. Haq [6] hypothesized that the presence of polysaccharides and fiber-degrading enzymes in the rumen would lead to the substantial presence of oligosaccharides at any given point of time in rumen liquor. He isolated a rumen liquor fraction that improved the weight gain, feed conversion ratio, and protein efficiency ratio compared to mannan oligosaccharides [7, 8].

The ruminants' diets are, however, rich in structural carbohydrates of plant origin. Therefore, a rumen liquor extract of soluble fiber is likelier to bear compositional and functional similarity to xylooligosaccharides as a prebiotic. Thus, the presented research work scrutinized the utilization of rumen liquor fraction as a feed additive compared to xylooligosaccharides, a proven prebiotic over nutrient utilization of broiler chickens.

2. Materials and Methods

The trial was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, India, in the Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry.

2.1 Rumen liquor (RL) collection and processing

Rumen liquor (RL) of freshly slaughtered sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*) was collected from a Government slaughterhouse. The collection was made in sealable plastic containers of 20 liters capacity. Clean and dry plastic buckets were cooled at the laboratory by keeping them in a deep horizontal freezer (-20 °C) for 15 minutes. RL was transferred to these buckets to arrest microbiological and enzymatic activity using temperature shock. Rumen liquor was then processed as described below as per Haq [9] with slight modifications.

Removal of particulate matter was carried out by filtration through muslin cloth, followed by centrifugation at 12000 rpm for 10 minutes. The supernatant was concentrated to one-fourth volume by rotary vacuum evaporation at 600 mm Hg pressure at 78 °C. Precipitation of soluble fiber fraction was achieved by adding 9 parts of ethanol to 1 part of concentrated and clarified rumen liquor, followed by centrifugation at 3000 rpm for 4 minutes. The supernatant was decanted, and the pellet was dried at 40 °C for 24 hours.

2.2 Experimental birds

Four hundred and eighty unsexed broiler chicks of the same hatch of straight-run Ven Cobb-400 strain were purchased from Venkateshwara Hatcheries Pvt. Ltd., Pune, India. The day-old chicks were randomly and evenly assigned to four treatment groups: CO, RL, XOS, and AB. Each treatment group contained six replicates, each containing twenty birds.

2.3 Housing and management of birds

The chicks were housed in deep litter pens, with one pen for each replicate. Rice husk litter of 5 cm depth was provided on the pen floor. Throughout the study, weekly litter turning with the addition of lime powder was done to ensure dry bedding material at all times. Each replicate received a drinker and a feeder. Each bird had an average floor area of 1 square foot. The temperature of the brooding house was regularly monitored. During the first week, the temperature was set at 32 °C and then reduced by 3 °C weekly until it reached 24 °C, which was maintained till the end of the experiment. The procured chicks were already vaccinated against Marek's disease. All chicks were vaccinated for Ranikhet disease intra-ocularly. IBD vaccine was also given intra-ocularly at 12 days of age, followed by a booster dose of the Ranikhet vaccine on the 24th day.

2.4 Feeds and feeding of birds

According to ICAR [10], three diets were developed: pre-starter, starter, and finisher (Table 1). These diets were based on maize and soybean and served as a mash. Chicks were given a pre-starter diet from 1-14 days of age, a starter diet from 14-21 days, and a finisher diet from 28-42 days age.

Table 1: Ingredient and chemical composition of broiler pre-starter, starter and finisher diet (on % DMB)

Attributes	Pre-starter	Starter	Finisher
Ingredient composition (%)			
Maize	60.50	60.88	67.50
MBM	5.00	5.00	5.00
SBM	31.08	30.37	24.50
SBO	2.00	2.70	2.20
Sodium bicarbonate	0.01	0.01	0.01
Salt	0.25	0.25	0.25
Methionine	0.17	0.13	0.09
Lysine	0.12	0.00	0.00
DCP	0.13	0.00	0.00
LSP	0.59	0.54	0.29
Vitamin supplement	0.05	0.05	0.05
Trace minerals	0.10	0.10	0.10
Chemical composition (%)			
OM	94.66	95.77	95.98
CP	21.83	21.55	19.51
EE	3.19	4.23	5.01
CF	4.08	4.20	4.14
TA	5.34	4.23	4.02
NFE	65.56	65.79	67.32
CA	1.21	1.12	1.00
P	0.86	0.81	0.73
ME (Kcal/kg) calculated	3000	3050	3100

Four different types of each diet were prepared and assigned to dietary treatments as follows:

- CO: Basal diet without prebiotic or antibiotic supplementation
- RL: Basal diet with RL extract @ 0.05% (w/w)
- XOS: Basal diet with 0.05% xylooligosaccharides (XOS, 95% xylooligosaccharides, XI'AN Healthway Biotech Co., China) (w/w)
- AB: Basal diet with antibiotic growth promoter (Enramycin-8) @ 0.0125%

The Enramycin dose was as per the manufacturer's recommendations. The XOS dose was as per Ding [11] and Riberio [12]. RL extract dosage was kept equal to the XOS dose.

2.5 Metabolism trial

A metabolic trial of 4 days was conducted during the last week of the experiment on 2 birds per replicate. During the metabolism trial, birds were kept in wire cages with the provision of collection of excreta replicate-wise. Three days adaptation period in the cages was given to the birds, after which data recording and sample collection were started. Trays coated with polythene sheets were placed beneath the cages for the excreta collection. The excreta was weighed using a digital weighing balance after eliminating feed particles, feathers, and other unwanted components. Daily offered and residual feed were quantitatively recorded and sampled. The excreta was collected at the end of the trial and oven-dried at 60 °C. The oven-dried fed and excreta samples were properly mixed, powdered, and then analyzed for proximate [13], calcium [14] and phosphorus [13] analysis.

2.6 Statistical analysis
The data generated were analyzed using a one-way analysis of variance [15]. Duncan's multiple range test was used to rank the means with significant differences [16].

2.7 Ethical approval
The trial was duly approved by the Institutional Animal Ethics Committee.

3. Results
3.1 Live weight and daily nutrient intake during metabolism trial of broiler chickens
The live weight and nutrient intake during the metabolism trial is shown in Table 2. The mean live weight of experimental birds during the metabolism trial was 1270 g, 1399 g, 1367 g and 1324 g for CO, RL, XOS and AB group birds with no significant difference (p>0.05) between treatment groups.
The mean OM intake of the birds was 123 g, 149 g, 119 g and 128 g for CO, RL, XOS and AB group birds, respectively, with mean daily CP intake was 25 g, 30.3 g, 24.1 g and 26.0 g, respectively.
The mean daily nutrient intake was comparable among experimental broilers, irrespective of the experimental diets.

Table 2: Live weight and daily nutrient intake during metabolism trial of broiler chickens fed unsupplemented or diets supplemented with rumen liquor extract (0.05%), xylooligosaccharides (0.05%) or antibiotic growth promoter (0.0125%)

Attributes	Treatments				SEM	P Value
	CO	RL	XOS	AB		
Live Weight (g)						
Initial	1152	1250	1229	1202	13.38	0.077
Final	1388	1547	1505	1445	27.78	0.167
Average	1270	1399	1367	1324	16.06	0.157
Intake (g/d)						
DM	120	146	116	125	5.18	0.173
OM	123	149	119	128	5.29	0.176
CP	25.0	30.3	24.1	26.0	1.07	0.176
EE	6.41	7.78	6.19	6.70	0.28	0.176
CF	5.30	6.43	5.12	5.53	0.23	0.176
NFE	86.2	104.6	83.2	89.9	3.71	0.176

3.2 Nutrient metabolizability during metabolism trial of broiler chickens
Table 3 shows the nutrient metabolizability during the metabolism trial of broiler chickens.
The per cent dry matter and organic matter metabolizability was statistically similar (p>0.05) in RL, XOS and CO groups but was significantly lower (p<0.05) in AB group birds.
The crude protein, ether-extract and nitrogen-free extract metabolizability (%) were statistically similar in all the groups.
The crude fibre per cent metabolizability was statistically higher (p>0.05) in XOS and RL group birds than in AB and CO group birds, which were comparable.

Table 3: Mean nutrient metabolizability during metabolism trial of broiler chickens fed unsupplemented or diets supplemented with rumen liquor extract (0.05%), xylooligosaccharides (0.05%) or antibiotic growth promoter (0.0125%)

Parameters	Groups				SEM	P Value
	CO	RL	XOS	AB		
Metabolizability (%)						
DM	86.03^{b}	87.64^{b}	82.53^{ab}	78.42^{a}	1.21	0.015
OM	90.60^{b}	92.12^{b}	88.32^{ab}	84.47^{a}	0.98	0.014
CP	91.90	92.66	90.75	91.69	0.77	0.881
EE	86.70	86.77	76.21	72.64	2.91	0.202
CF	18.71^{a}	55.28^{b}	42.33^{b}	11.56^{a}	5.30	0.001
NFE	91.94	91.67	88.37	84.57	1.13	0.050

^{ab}Means bearing different superscripts within a row differ significantly.

3.3 Mean nutrient balance during metabolism trial of broiler chickens
Table 4 shows the mean nutrient balance of broiler chickens during the metabolism trial. There was no significant difference (p>0.05) in nitrogen, calcium and phosphorus balance between different treatment groups during the metabolism trial. The per cent nitrogen retention was 91.90, 92.66, 90.75 and 91.69 in CO, RL, XOS and AB groups, respectively. The mean calcium retention (g/d) was 1.12, 1.40, 1.06 and 1.09 for CO, RL, XOS and AB groups, respectively, whereas mean phosphorus retention (g/d) was 0.71, 0.81, 0.47 and 0.64 for CO, RL, XOS and AB groups, respectively.

Table 4: Mean nutrient balance during metabolism trial of broiler chickens fed unsupplemented or diets supplemented with rumen liquor extract (0.05%), xylooligosaccharides (0.05%) or antibiotic growth promoter (0.0125%)

Parameters	Groups				SEM	P Value
	CO	RL	XOS	AB		
Nitrogen (g/d)						
Intake	4.0	4.85	3.86	4.17	0.17	0.176
Outgo	0.31	0.36	0.35	0.35	0.03	0.951
Retained	3.69	4.49	3.51	3.82	0.17	0.181
Retention (%)	91.90	92.66	90.75	91.69	0.77	0.881
Calcium (g/d)						
Intake	1.28	1.55	1.24	1.33	0.055	0.176
Outgo	0.16	0.16	0.18	0.24	0.014	0.087
Retained	1.12	1.40	1.06	1.09	0.055	0.106
Retention (%)	89.39	89.83	85.82	81.30	1.206	0.063
Phosphorus (g/d)						
Intake	0.93	1.13	0.90	0.97	0.040	0.176
Outgo	0.22	0.33	0.43	0.33	0.045	0.479
Retained	0.71	0.81	0.47	0.64	0.049	0.089
Retention (%)	76.05	71.07	52.42	68.23	10.53	0.268

4. Discussion

We explored the efficacy of a prebiotic fraction of rumen liquor in broiler chickens compared to xylooligosaccharides (XOS). Un-supplemented birds served as the negative control, whereas birds supplemented with antibiotic growth promoter (AGP) served as the positive control.

The mean daily nutrient intake was comparable among experimental broilers, irrespective of the experimental diets.

The per cent dry matter (DM) and organic matter (OM) digestibility were comparable (p>0.05) in the prebiotic supplemented (RL, XOS) and CO group but was significantly lower (p<0.05) in the AB group birds. Crude fiber digestibility varied between prebiotic-supplemented (RL, XOS) groups and control. Our findings are in accordance with Wu *et al.* [17], who reported no effect of a prebiotic (inulin) supplementation (1% and 2% levels) over nutrient digestibility.

This contradicts Kirkpinar *et al.* [18], who reported that crude fiber digestibility was not affected by enzyme prebiotic supplementation. In contrast, Yun *et al.* [19] said that boilers fed prebiotics-based diets had improved dry matter digestibility compared with the control group.

Alzueta *et al.* [20] observed that prebiotics could improve nutrient utilization by modifying the microarchitecture of the intestine.

Lower DM and OM metabolizability in AB group birds contrast the findings of Huang *et al.* [21], who reported that dietary supplementation of antibiotics increased the ileal digestibility of DM.

The mean nutrient balance in broiler chickens during the metabolism trial was comparable for nitrogen, calcium, and phosphorus balance between different dietary groups.

Comparable nitrogen balance among dietary treatments is in contrast with the previous reports [20-25], but in accordance with the findings of Wu *et al.* [17].

Most workers reported higher calcium balance in prebiotics-supplemented birds [11, 17, 22, 23, 26]. This has been attributed to reduced luminal pH in the intestine due to selective fermentation of prebiotics [26] that increases the calcium salts' solubility [27, 28] or increases the content of $1,25(OH)_2 D_3$ in plasma [11]. Although we observed numerically higher values of calcium balance in the RL-supplemented group, no statistical difference was noticed. Thus, no significant effect of prebiotics supplementation over calcium assimilation in our study could be due to the supplementation of a relatively lower concentration of prebiotics in the present study.

Notably, the logic of better mineral availability or modulated $1,25(OH)_2 D_3$ in plasma is not extended to phosphorus bioavailability. This could be probably because most phosphorus in feed is either highly bioavailable (supplemental phosphorus in the mineral mixture) or is in the bound form (phytate). The solubility of phytate phosphorus due to fermentation activity in the lower gut remains an area to be explored. However, comparable phosphorus balance in all the experimental groups, irrespective of the feed additive used, is in accordance with the previous report [23].

5. Conclusion

The present study was conducted to establish the prebiotic efficacy and safety of rumen liquor fraction. Its supplementation has no positive or negative effect on nutrient metabolizability and calcium-phosphorus balances. The result was similar to that observed for positive control, so it is concluded that the nutrient utilization is unaffected by the prebiotic supplementation, including RL supplementation. Further, no untoward effect of RL supplementation was observed over nutrient utilization in broiler chicks.

6. Acknowledgement

The present research work was conducted as a part of SERB, New Delhi funded core research grant project.

7. References

1. Edwards PT, Kashyap PC, Preidis GA. Microbiota on biotics: probiotics, prebiotics, and synbiotics to optimize growth and metabolism. American Journal of Physiology Gastrointestinal and Liver Physiology. 2020;319:G382-G390.
2. Selaledi LA, Hassan ZM, Manyelo TG, Mabelebele M. The current status of the alternative use to antibiotics in poultry production: an African perspective. Antibiotics. 2020;9:1-18.
3. Gaggìa F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology. 2010;141:S15-S28.
4. Alloui MN, Szczurek W, Świątkiewicz S. The usefulness of prebiotics and probiotics in modern poultry nutrition: A review. Annals of Animal Science. 2013;13(1):17-32.
5. Patel S, Goyal A. The current trends and future perspectives of prebiotics research: a review. Biotech. 2012;2(2):115-125.
6. Haq Z. Exploring prebiotic potential of rumen liquor. PhD thesis. SKUAST-Jammu, India, 2017.
7. Haq Z, Rastogi A, Sharma RK, Raghuwanshi P. Effect of rumen liquor extract supplementation as a prebiotic over growth performance, feed intake and cecal lactic acid bacteria and enterobacteria count in broiler chicks. In: Compendium of International conference of SBBS on 'Recent trends in Bioinformatics and Biotechnology for sustainable Development', SKUAST- Jammu. October 12-13, 2017, 202-203.
8. Haq Z, Rastogi A, Sharma RK, Singh M and Raghuwanshi P. *In vitro* assessment of prebiotic activity of a rumen liquor extract. In: Nutritional Challenges for Raising Animal Productivity to Improve Farm Economy. Book of Abstracts of XVII Biennial Animal Nutrition Conference, Junagadh, India, February 1-3, 2018, 172.
9. Haq Z, Rastogi A, Sharma RK, Raghuwanshi P, Kumar S. Isolation of a fraction with prebiotic activity from rumen liquor. Patent application no. 201811022602. Official Journal of the Patent Office (India), 2018;25(2018):22837.
10. ICAR. Nutrient requirement of Animals – Sheep, Goat and Rabbit. ICAR- NIANP, Indian Council of Agriculture Research, India, 2013.
11. Ding XM, Li DD, Bai SP, Wang JP, Zeng QF, Su ZW, *et al*. Effect of dietary xylooligosaccharide on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. Poultry Science. 2018;97(3):874-881.
12. Ribeiro T, Cardoso V, Ferreira LMA, Lordelo MMS, Coelho E, Moreira ASP, *et al*. Xylo-oligosaccharides display a prebiotic activity when used to supplement wheat or corn-based diets for broilers. Poultry Science. 2018;97:4330-4341.

13. AOAC. Official Methods of Analysis of AOAC International, 19th ed. AOAC International, Washington, DC, 2012.
14. Talpatra SK, Ray SC and Sen KC. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in foodstuffs. Indian Journal of Veterinary Sciences and Animal Husbandry. 1940;10:243-258.
15. Snedecor GW, Cochran WG. Statistical methods. 8th Edition, East West Press Private Limited, New Delhi. 1994.
16. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
17. Wu XZ, Wen ZG, Hua JL. Effects of dietary inclusion of Lactobacillus and inulin on growth performance, gut microbiota, nutrient utilization, and immune parameters in broilers. Poultry science. 2019;98(10):4656-4663.
18. Kirkpinar F, Açikgöz Z, Bozkurt M, Ayhan V. Effects of inclusion of poultry by-product meal and enzyme-prebiotic supplementation in grower diets on performance and feed digestibility of broilers. British Poultry Science. 2004;45(2):273-279.
19. Yun W, Lee DH, Choi YI, Kim IH, Cho JH. Effects of supplementation of probiotics and prebiotics on growth performance, nutrient digestibility, organ weight, fecal microbiota, blood profile, and excreta noxious gas emissions in broilers. Journal of Applied Poultry Research. 2017;26(4):584-592.
20. Alzueta C, Rodriguez ML, Ortiz LT, Rebolé A, Trevino J. Effects of inulin on growth performance, nutrient digestibility and metabolizable energy in broiler chickens. British Poultry Science. 2010;51(3):393-398.
21. Huang RL, Yin YL, Wu GY, Zhang YG, Li TJ, Li LL, *et al*. Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poultry science. 2005;84(9):383-1388.
22. Wang XW, Lin X, Zhang L, Du YG, Bai XF, Shu SG. Effect of oligochitosan on broiler performance, small intestine structure and muscle mineral element concentration. Journal of the Chinese Cereals and Oils Association. 2005;20:83-88.
23. Li DD, Ding XM, Zhang KY, Bai SP, Wang JP, Zeng QF, *et al*. Effects of dietary xylooligosaccharide on the performance, egg quality, nutrient digestibility and plasma parameters of laying hens. Animal Feed Science and Technology. 2017;225:20-26.
24. Craig AD, Khattak F, Hastie P, Bedford MR, Olukosi OA. The similarity of the effect of carbohydrase or prebiotic supplementation in broilers aged 21 days, fed mixed cereal diets and challenged with coccidiosis infection. PLOS One. 2020;15(2):e0229281.
25. Zhou J, Wu S, Qi G, Fu Y, Wang W, Zhang H, *et al*. Dietary supplemental xylooligosaccharide modulates nutrient digestibility, intestinal morphology, and gut microbiota in laying hens. Animal Nutrition. 2021;7(1):152-162.
26. Abdelqader A, Al-Fataftah AR, Daş G. Effects of dietary Bacillus subtilis and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. Animal Feed Science and Technology. 2013;179(1-4):103-111.
27. Rémésy C, Levrat MA, Gamet L, Demigné C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. American Journal of Physiology. 1993;264(5):855-862.
28. Roberfroid MB. Chicory fructooligosaccharides and the gastrointestinal tract. Nutrition, 2000;16(7-8):677-679.