Unique Second Generation Prebiotic ‘The Scientific Evidence’
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Discoveries in biosciences in recent years have provided evidence that, beyond nutrition, diet may also modulate various bodily functions that are relevant to human health.

These discoveries are shifting nutritional concepts from identifying a ‘balanced’ diet (ensuring an adequate intake of nutrients while avoiding excessive intake of those nutrients that can contribute to disease, e.g. fat and salt) to an ‘optimised’ nutrition. The outcome of ‘optimised’ nutrition is to maximise life expectancy and quality by identifying food ingredients that are able to improve the capacity to resist disease and enhance health when part of a ‘balanced’ diet and lifestyle. The latter provides a concept of functional foods, that link medical and food sciences.

This brochure describes the properties, science and clinical evidence of a colonic functional food ingredient called Bimuno® produced by Clasado.

Bimuno is classed as a food supplement. It is not a medicinal product and is not intended to treat or prevent medical conditions or their symptoms. It is a food supplement (colonic functional food), which is scientifically proven to help maintain digestive, immune and gut travel health.
The human body is host to a large number of commensal bacteria, with most residing in the gut. By far, the most densely populated area of the gut is the large intestine, and its resident microbiota plays a key role in nutrition, proper functioning of the immune system and overall health. 1 This is only possible when the equilibrium within the microbiota, and between the microbiota and the body, is maintained.

The body protects itself against the harmful components of the microbiota via its barrier function, which is largely controlled by the immune system. The beneficial components of the microbiota (good bacteria) also contribute by creating the colonisation resistance against potentially harmful bacteria.

The composition of the microbiota is influenced by various environmental and genetic factors, with dietary residues considered the most important of these. Consequently, dietary modulation of the intestinal microbiota is the main purpose of many current functional foods and it is the basis for the pre-, pro- and symbiotic concepts.

These concepts rely upon enhancing the beneficial components of the intestinal microbiota, namely the bifidobacteria and lactobacilli. While the probiotic concept relies upon the use of live bacteria to modulate the microbiota, the prebiotic concept relies upon the use of non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial groups of bacteria indigenous to the colon. 2,3 The symbiotic concept is a combination of the pre- and probiotic concepts.
Any dietary material that enters the large intestine is a candidate prebiotic. However, current prebiotics are confined to non-digestible oligosaccharides (NDOs). These escape enzymatic digestion in the upper gut, enter the caecum without change to their structure and confer the degree of selective fermentability that is required.

Their fermentation by the colonic microbiota, resulting in the production of short-chain fatty acids (SCFAs), lactic acid and energy, is indicated by the fact that NDOs are not excreted in the faeces.  

Oligosaccharides are sugars consisting of between 2 and 20 saccharide units. Some occur naturally in breast milk and certain foods such as leek, asparagus, garlic, onion, chicory, wheat, oat and soybean. However, these naturally occurring oligosaccharides cannot exert a prebiotic effect in their native state, due to their low concentrations. In order to produce a prebiotic effect these are produced commercially through the hydrolysis of polysaccharides (e.g. dietary fibres and starch) or through catabolic enzymatic reactions from lower molecular weight sugars.

Although, there are many different types of NDOs on the world market, the most thoroughly investigated are inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS), and for these a prebiotic effect has been proven.  

Not all prebiotics have the same effect, and even within the same group (e.g. GOS), they can differ significantly due to differences in their size and structure composition.
Bimuno is a GOS mixture produced using enzymes from the probiotic Bifidobacterium bifidum NCIMB 41171. B. bifidum is an important bacterium since it has been recognised as the main species in healthy breast-fed infants and it is known to be absent in infants with atopy. 6

The source of the enzyme during GOS manufacture determines its final composition and thus functionality. The use of probiotic enzymes therefore, will produce oligosaccharide mixtures that will be more selective towards beneficial bacteria, and this in turn will increase colonisation resistance in the gut. These oligosaccharide mixtures will also carry other beneficial properties, such as direct interaction with pathogens and direct interaction with the host via the effect on the immune system, which will improve the barrier function in the gut.

The use of a probiotic enzyme in the production of Bimuno makes it unique in comparison with other available GOS, which are produced using commercial enzymes widely present in a number of different bacteria (both beneficial and detrimental). As a result, other GOS are not as selective as Bimuno-GOS towards beneficial bifidobacteria in the gut. 7,8

Because of this selectivity towards bifidobacteria and the absence of fructose in the GOS mixture, Bimuno-GOS does not produce gas during fermentation and therefore is not regarded as a FODMAP.

Bimuno-GOS: A unique Gastro-Intestinal Mediator
Overview of Bimuno-GOS Key Clinical and Scientific Studies

- In Healthy Adults, after just 7 days Bimuno-GOS increased the bifidobacterial population significantly higher than other GOS and placebo.

- Compared with 3 leading probiotic brands, Bimuno-GOS resulted in significantly faster and better bifidogenic effect and no GI discomfort in adults.

- In the Elderly, Bimuno-GOS significantly increased bifidobacteria; significantly increased activity of cells involved in killing of pathogens and tumours and anti-inflammatory cytokines, whilst significantly reducing pro-inflammatory cytokines.

- In IBS sufferers, bifidobacteria were significantly increased and a lower daily dose of 1.37g Bimuno-GOS resulted in significant reduction of IBS symptoms such as abdominal pain and bloating.

- Daily intake of 1.37g Bimuno-GOS, after just 7 days, resulted in significant reduction in bloating, flatulence and abdominal pain in adults who suffer from bloating but are otherwise healthy.

- Bimuno-GOS significantly reduced colonisation and pathology associated with salmonellosis in a validated murine model system.

- In Travellers to countries with medium to high risk of diarrhoea, daily Bimuno-GOS consumption resulted in significant reduction in the incidence and duration of diarrhoea compared with placebo.

- In Overweight Adults, Bimuno-GOS significantly increased bifidobacteria and significantly reduced markers of metabolic syndrome and inflammation.
The Prebiotic evaluation of Bimuno-GOS in humans: a randomised, double-blind, crossover, placebo-controlled study

**Objective:** Assess prebiotic potential of Bimuno-GOS against other commercial GOS and placebo.

**Design:** Fifty-nine volunteers took part. Effect of GOS (0 or 2.75g/d) during 7 day treatment periods with a 7 day washout period in between was assessed in 29 volunteers. Then, 30 volunteers were assigned to a sequence of treatments (7 days) differing in the amount of Bimuno-GOS (0, 1.37 or 2.75g/d). Stools were recovered before and after each intervention, and assessed for bacterial numbers using molecular techniques.

**Results:** Significantly higher increase in bifidobacteria after Bimuno-GOS treatment when compared with placebo (P<0.05) and GOS (P<0.05). Significant (P<0.001) dose-response effect between Bimuno-GOS intake and bifidobacterial population increase.

**Conclusion:** Due to the probiotic origin of enzymes used to produce Bimuno-GOS, it resulted in significantly better bifidogenic effect than other commercial GOS.

Prebiotic index (PI) is a quantitative assessment of key changes in major bacterial groups found in the human gut.  

Prebiotic index (PI) and Bifidobacterium population proportion (%)

<table>
<thead>
<tr>
<th>Bimuno-GOS (0g/d)</th>
<th>Bimuno-GOS (1.37g/d)</th>
<th>Bimuno-GOS (2.75g/d)</th>
<th>GOS (0g/d)</th>
<th>GOS (2.75g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prebiotic index</td>
<td>Bifidobacteria</td>
<td>Prebiotic index</td>
<td>Bifidobacteria</td>
<td>Prebiotic index</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>2.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
<td>2</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.5</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Prebiotic index (PI) is a quantitative assessment of key changes in major bacterial groups found in the human gut.
**Objective:** Assess the efficacy of Bimuno-GOS against leading probiotic brands on microbiota and gut function in healthy adults.

**Design:** Volunteers (44) consumed one of 4 commercially available products for 2 weeks. Stool samples were collected at the start, middle (1wk) and the end of the study. Daily records were taken of bowel habits, abdominal pain, bloating and flatulence.

**Results:** Bifidobacteria and lactobacilli were significantly (P<0.01) higher (both at week 1 and 2) following Bimuno-GOS ingestion when compared to the start and all 3 probiotics. Volunteers did not experience any GI discomfort throughout the study with all 4 products. However, at the end of the study, bloating and flatulence were significantly (P<0.05) lower with Bimuno-GOS compared to 2 probiotics, and abdominal pain was significantly (P<0.05) lower compared to 1 probiotic.

**Conclusion:** Daily Bimuno-GOS consumption exerted a more pronounced beneficial effect on microbiota than 3 leading probiotic yogurts and was well tolerated by all volunteers.
Effect of Bimuno-GOS on abdominal bloating and related gut function parameters in humans: a randomised, double-blind, crossover, placebo-controlled study

Abdominal bloating is one of the most common and troublesome GI symptoms. Women tend to report bloating on a monthly basis more often than men (20% vs 11%). Bimuno-GOS helped reduce bloating in IBS patients.

Symptom Scoring:
0 = none
1 = present but tolerated
2 = present interfering but not preventing activities
3 = preventing daily activities.

Objective: Assess Bimuno-GOS potential in reducing bloating and its effect on other gut functions in healthy adults who experience bloating discomfort.

Design: Volunteers (83) consumed placebo or Bimuno-GOS treatments (1.37g) for 2 weeks with a 2-wk washout period in between. Daily records were taken of incidence, duration and severity of bloating, bowel habits, abdominal pain, flatulence, mood and general wellbeing.

Results: After just 1 week of daily intake, Bimuno-GOS significantly (P<0.001) reduced bloating discomfort, flatulence and abdominal pain, compared with start and placebo.

Conclusion: Bimuno-GOS was effective in reducing GI symptoms in healthy adults with a history of bloating.

Bimuno-GOS resulted in significantly (P<0.05) lower scores in all cases compared both to the start and placebo.

Symptom Scoring:
0 = none
1 = present but tolerated
2 = present interfering but not preventing activities
3 = preventing daily activities.
Objective: Investigate the efficacy of Bimuno-GOS in changing microbiota and its effect on the symptomatology of IBS.

Design: Patients (44) with Rome II positive IBS consumed placebo or Bimuno-GOS treatments (1.37g/d or 2.75g/d) for 4 weeks. IBS symptoms were monitored and scored using 7-point Likert scale weekly. Microbiota, stool frequency/form, SGA, anxiety, depression and QOL scores were also monitored.

Results: Both Bimuno-GOS treatments significantly (P<0.05) increased bifidobacteria compared to baseline and placebo. Lower (Bimuno-GOS 1.37g/d) treatment also resulted in significant (P<0.05) reduction of IBS symptoms (abdominal pain, bloating, flatulence, bowel movements).

Conclusion: Bimuno-GOS acted as a prebiotic by stimulating growth of bifidobacteria in IBS sufferers and was effective in reducing their symptoms.

IBS sufferers have lower numbers of bifidobacteria and abnormal fermentation patterns. Bimuno-GOS positively affects fermentation and increases bifidobacteria in healthy humans.
Significantly different from start and placebo ($P<0.05$).

Significantly different from start ($P<0.05$).

Scale of improvement between the end and start of study:

- Flatulence
- Bloating
- Likert scale
- SGA

Bimuno-GOS (1.37g) | Placebo | Placebo

* significantly different from start and placebo ($P<0.05$).
a significantly different from start ($P<0.05$).
The Elderly have increased putrefactive bacteria (e.g. clostridia and enterobacteria) and reduced beneficial bacteria (i.e. bifidobacteria). Ageing also leads to a marked decline in immune function. Consequently, elderly are pre-disposed to infectious and non-infectious diseases.

**Objective:** Assess the effect of Bimuno-GOS on microbiota and immune function in the elderly.

**Design:** Elderly volunteers (44) consumed placebo or Bimuno-GOS treatments (2.75g/d) for 10 weeks with a 4-wk washout period in between. Blood and stools were collected at start, middle (5wk) and end of each period and used to analyse various immune markers and microbiota.

**Results:** Bimuno-GOS significantly (P<0.001) increased beneficial bacteria, especially bifidobacteria, at the expense of harmful groups such as clostridia, both compared to baseline and placebo. Also, phagocytosis (P<0.001), NK cell activity (P<0.001), and anti-inflammatory (IL-10) cytokine (P<0.05) were all significantly increased, and pro-inflammatory cytokines (P<0.05) reduced.

**Conclusion:** Bimuno-GOS is a useful dietary intervention and can help the enhancement of GI health and immune function in elderly persons.

Mean ± SD change at 10 wk from start in the production of cytokines. Bimuno-GOS was significantly different (P<0.05) in all cases.
NK cell activity change from start (ratio of effector to target cells 100:1)

Bimuno-GOS | Placebo
---|---

<table>
<thead>
<tr>
<th></th>
<th>week 5</th>
<th>week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimuno-GOS</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

a significantly different from 5 wk (P<0.001).

b significantly different from placebo (P<0.05).

* significantly different from placebo (P<0.001) and 5 wk (P<0.001).

---

Phagocytic activity

Change in 10 week from start

<table>
<thead>
<tr>
<th></th>
<th>Positive for phagocytosis (%)</th>
<th>Number of bacteria/active cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimuno-GOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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a significantly different from 5 wk (P<0.001).

b significantly different from placebo (P<0.05).

* significantly different from placebo (P<0.001) and 5 wk (P<0.001).
S. Typhimurium infections in humans and animals constitute a major public health and economic burden. Bimuno-GOS was previously shown in-vitro to prevent attachment of EPEC and S. Typhimurium to epithelial cell line.  

**Objective:** Assess Bimuno-GOS potential in reducing infections caused by S. Typhimurium.

**Design:** Infection caused by S. Typhimurium in the presence or absence of Bimuno-GOS, was assessed using a series of models (i.e. adherence and invasion culture assays, murine ligated ileal gut loop model, murine oral challenge model).

**Results:** Bimuno-GOS significantly (P<0.0001) reduced adherence and invasion, prevented colonisation and the associated pathology in the murine ligated ileal gut loops and significantly (P<0.0001) reduced colonisation in all sampled organs following oral challenge.

**Conclusion:** Bimuno-GOS clearly resulted in a protective effect against S. Typhimurium by reducing colonisation and pathology associated with this pathogen.

**Bimuno-GOS**
- No signs of pathology
- No bacteria present
- Brush borders intact

**S. Typhimurium**
- Pathology observed
- Brush borders damaged
- Attachment and invasion

**Bimuno-GOS + S. Typhimurium**
- Protection from pathology
- Brush borders intact
- No attachment or invasion

Studies on the ability of Bimuno-GOS to reduce and protect from *Salmonella enterica* serovar *Typhimurium* infection  

21,22,23
Objective: Assess the effect of Bimuno-GOS on severity and/or incidence of TD.

Design: Volunteers (159), who travelled for minimum of 2 weeks to countries with medium or high risk for diarrhoea, consumed placebo or Bimuno-GOS treatments (2.75g/d) for 1 week prior to and during travel period. Records were taken of bowel habits, abdominal pain, flatulence, bloating and vomiting. In the case of diarrhoea, clinical reports (i.e. duration, symptoms, hospital admission) were completed.

Results: Only 24% of travellers who consumed Bimuno-GOS developed diarrhoea which was significantly (P<0.05) lower than the number observed in the placebo group (39%). Furthermore, duration of diarrhoea and the abdominal pain were both significantly (P<0.05) lower following Bimuno-GOS consumption.

Conclusion: Bimuno-GOS was effective in reducing incidence and duration of TD and abdominal pain.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of diarrhoea</strong></td>
<td>7</td>
</tr>
<tr>
<td>Bimuno-GOS</td>
<td>6</td>
</tr>
<tr>
<td>Placebo</td>
<td>8</td>
</tr>
<tr>
<td><strong>Duration of abdominal pain</strong></td>
<td>4</td>
</tr>
<tr>
<td>Bimuno-GOS</td>
<td>3</td>
</tr>
<tr>
<td>Placebo</td>
<td>5</td>
</tr>
</tbody>
</table>

Effect was significant (P<0.05) between treatments in both cases.
Effect of Bimuno-GOS on markers of metabolic syndrome (Met. Synd.), microbiota and immune function in overweight adults: a randomised double-blind, crossover, placebo-controlled study

Met. Synd. is a set of disorders that increase the risk of developing cardiovascular disease and Type 2 diabetes. The gut microbiota is altered towards a less beneficial composition in overweight adults and this can be accompanied by inflammation.

**Objective:** Assess Bimuno-GOS potential in reducing Met. Synd. markers and its effect on microbiota and immune function in overweight adults.

**Design:** Overweight volunteers (45) consumed placebo or Bimuno-GOS treatments (2.75g/d) for 12 weeks with a 4 week washout period in between. Blood, stools and anthropometric measurements were collected at the start, middle (6 week) and end of each period, and used for various analyses.

**Results:** Bimuno-GOS significantly increased bifidobacteria and sIgA (marker of mucosal immunity). It significantly reduced inflammatory markers; faecal calprotectin and blood CRP. Bimuno-GOS also significantly reduced Met. Synd. markers, namely insulin, cholesterol (TC) and triacylglycerides (TAG).

**Conclusion:** Bimuno-GOS is useful for the enhancement of GI health, immune function and the reduction of Met. Synd. factors in overweight adults.

**Inflammatory Markers**

![Inflammatory Markers Graph]

* P < 0.0001  ** P < 0.0012

**MET. SYND. markers at the end of the study**

<table>
<thead>
<tr>
<th>Intervention/time point</th>
<th>TC</th>
<th>TG</th>
<th>TC/HDL-C</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>6.2 ± 1.3</td>
<td>1.6 ± 0.7</td>
<td>4.5 ± 1.1</td>
<td>64.8 ± 30.6</td>
</tr>
<tr>
<td>week 12</td>
<td>6.2 ± 1.2</td>
<td>1.6 ± 0.7</td>
<td>4.6 ± 1.3</td>
<td>70.1 ± 36.8</td>
</tr>
<tr>
<td>Bimuno-GOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>6.3 ± 1.3</td>
<td>1.6 ± 0.8</td>
<td>4.8 ± 1.3</td>
<td>67.3 ± 30.9</td>
</tr>
<tr>
<td>week 12</td>
<td>5.9 ± 1.1*</td>
<td>1.5 ± 0.6**</td>
<td>4.3 ± 1.1*</td>
<td>58.1 ± 29.7**</td>
</tr>
</tbody>
</table>

* P < 0.0001  ** P < 0.005
References

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