



Strengthening immunity has been an increasingly sought-after feature of supplements, foods and beverages

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In order to know how the eggshell membrane can serve the stimulation of immunity, we have made a bibliographic review of the evidence.

Egg-shell membrane is a novel dietary supplement that has been clinically shown to relieve pain and stiffness in joints. Its mechanism of action is based on the reduction of pro-inflammatory cytokines.

Recently, there has been increasing interest in the use of biomarkers in clinical trials of osteoarthritis in human medicine. Biomarkers can be used as a diagnostic tool for the identification of a disease, an indicator of disease progression, or the monitoring of the clinical response to an intervention. Previous studies have shown that osteoarthritis can alter the serum cytokine profile in humans. These alterations can be used as biomarkers of disease progression. **In particular, serum IL-2 and IL-6 concentrations were significantly elevated in patients with osteoarthritis.** However, it is not clear whether cytokine biomarkers are related to osteoarthritis disease, versus pain associated with it.

IL-2, IL-6 and IL-8 are cytokines that are involved in the inflammatory response. Several studies have shown that serum IL-2 is significantly higher in human patients with osteoarthritis compared to healthy controls. **Synovial levels of certain cytokines, such as IL-6, have been associated with catabolic effects on cartilage due to positive regulation of matrix metalloproteinases.** It is believed that a decrease in these inflammatory cytokines (IL-6) and immunoregulatory cytokines (such as IL-2) may slow down the destruction of cartilage and thus the progression of osteoarthritis.

Eggshell membranes contain a variety of proteins and peptides that aid in the development of the embryo and provide protection. **Many of the peptides and proteins associated with eggshell membranes have antimicrobial, immunomodulatory and adjuvant properties and it is presumed that egg membrane by-products, provided as nutritional supplements, can enhance immunity.** The aim of several studies was to characterize eggshell membranes and study their properties. Processed eggshell particles retain a fibrous structure similar to that observed in the native membrane, and contain collagen and carbohydrate components such as hyaluronic acid and sulfated



glycosaminoglycans, as well as N-glycans, mainly with non-laden structures **that have immunomodulatory properties in monocytes and macrophage-like cells**. More than 300 proteins have been found in eggshell membranes and the abundant ones are lysozyme, ovotransferrin, ovocleidin, clusterin, ovokeratin, ovodefensin and many more. These proteins are not only antimicrobial in nature, but many play a vital role in metabolic and developmental processes.

A study on chickens treated with eggshell membranes showed that **the percentage of leukocytes and lymphocytes increased while the percentage of monocytes decreased. IgM and IgG (Y) levels were elevated and corticosterone levels were reduced**. These results suggest that **eggshell membrane supplements can improve immunity variables without any detrimental effect on other physiological parameters**. Under lipopolysaccharide-induced inflammatory conditions, eggshell membranes reduced the activity of nuclear factor- κ B transcription factor. The expression of the immune regulatory receptors toll-like receptor 4 and ICAM-1, as well as cell surface glycoprotein CD44, all important during the response to inflammation, were negatively regulated by these fractions. Eggshell membranes reduced inflammation by increasing secretion of the anti-inflammatory cytokine IL-10 and reducing secretions of the pro-inflammatory cytokines IL-1 β and IL-6.

The innate immune defense is formed by proteins with antimicrobial and immunomodulatory activities and ensures protection against pathogens. The aim of a study was to identify the proteins of the chicken egg shell membrane that play a role in innate immune defence mechanisms. ESM-Ovocalyxin-36 (OCX-36) is a pattern recognition protein that has antimicrobial activity against *S. aureus* and binds to *E. coli* lipopolysaccharide and *S. aureus* lipoteic acid. It was also found that **OCX-36 has anti-endotoxin properties and is a macrophage immunostimulator to produce NO and TNF- α** .

A new study showed changes in plasma cytokines and levels (from baseline after 7 days of low-dose oral eggshell membrane supplementation) were statistically significant on day 8 for IL- 2, TIMP- 1 and VEGF, on day 21 for IL- 10, and on day 35 for MCP- 1, MCP- 3 and TIMP-1, and at high dose were statistically significant on day 8 for VEGF, on day 21 for MIP- 1 β , MIP- 2 and VEGF, and on day 35 for MCP- 3, MIP- 1 β , MIP- 2 and VEGF.

An in vitro study with eggshell inner membrane has shown that it inhibits pro-inflammatory secretion of cytokines IL-8, while **in vivo (in colitis-induced mice) it reduces interleukin (IL)-6 secretion**. Another study in eggshell-based animals on inflammatory bowel disease showed inflammatory modulation activity, demonstrated by **reducing secretion of NO, IL-6 and TNF- α** .

Another study examined the effects of eggshell inner membrane on interleukin (IL) -2, IL-4, IL-6, IL-10, interferon- γ (IFN- γ) and TNF- α cytokine production by 4-day peripheral blood mononuclear cell (PBMC) cultures exposed to serial dilutions of an in vitro digested aqueous eggshell inner membrane extract.



Effects on cytokine production were also evaluated in the presence of phytohemagglutinin (PHA) and carmine grass mitogen (PWM) where **exposure to eggshell inner membrane resulted in reduced levels of proliferation and statistically significant effects on IL-6, IL-10, IFN- γ , and TNF- α cytokine production. Eggshell inner membrane reduced levels of IL-6, IL-10, IFN- γ , and TNF- α in crops exposed to PHA. In PWM-containing crops, NEM-AQ reduced IL-10 production and at the highest dose tested increased IL-6 and decreased TNF- α cytokine levels.**

In summary, the evidence published so far shows that the internal eggshell membrane may be able to increase immunity specifically by reducing plasma concentrations of IL-6.

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WARNING

This information is intended for professionals and reflects the evidence published so far. The report complies with the provisions of the Spanish Agency for Food Safety and Nutrition (AESAN) in its communication of 26 March 2020 in the sense that "food supplements are foods whose purpose is to supplement the normal diet, to which no properties can be attributed to prevent, treat or cure a human disease, or refer to such properties at all".