

Effects of probiotics on Gluten-Induced Changes in Gene Expression in *Drosophila melanogaster*

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Abstract

Celiac disease is an autoimmune intestinal disorder affecting approximately 1% of the global population and is characterised by chronic inflammation of the small intestine following gluten exposure. Probiotic dietary supplements have been proposed as potential modulators of disease activity through enhanced gluten metabolism or alteration of the gut microenvironment. This study investigated transcriptional responses to gluten and probiotic supplementation in *Drosophila melanogaster* larvae as a model for examining strain-specific microbial effects on stress-related pathways. Larvae were raised on diets containing gluten alone or in combination with selected bacterial strains (*Corynebacterium variabile*, *Corynebacterium casei*, *Microbacterium gubbeenense*, or a blend of *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus bulgaricus*). Total RNA was extracted from homogenised soft tissue, reverse transcribed to cDNA, and analysed by PCR to assess differential expression of genes associated with immune signalling (*Rel*), DNA repair (*mre11*, *XRCC1*), metabolic stress (*SlgA*), and developmental regulation (*egr*). Gluten supplementation was associated with directional changes in all five genes examined, including downregulation of *egr* and upregulation of *Rel*, *mre11*, *XRCC1*, and *SlgA*. Although not all differences reached statistical significance, the overall pattern was consistent with modest immune engagement, activation of oxidative stress-associated repair pathways, and suppression of growth-related signalling. Probiotic supplementation modified these responses in a strain-dependent manner rather than uniformly restoring baseline expression. Comparison with human orthologues revealed both parallels and divergence, highlighting species-specific differences in immune and stress regulation. These findings suggest that gluten exposure in larval *Drosophila melanogaster* induces coordinated transcriptional rebalancing influenced by the presence of gut microbiota.

Keywords: Celiac disease; probiotics; oxidative stress; DNA repair; *Drosophila melanogaster*

Popular science summary

There is growing evidence that probiotic supplements can be of great help to people who suffer from inflammation caused by gluten intolerance. Certain bacterial strains like *C. casei* and *L. plantarium* appear to be able to help the digestive system break down gluten proteins that it cannot effectively manage on its own.

By extracting RNA from test animals, researchers can measure the changes in the number of copies of transcripts created from genes related to inflammation, the immune system, or “normal” states of being under different conditions. RNA gets back-translated into DNA, after which it is used in a technique called “polymerase chain reaction” to create thousands (or millions) of identical copies of the original molecule, much like minting new coins from a template. Later, the number of copies created can be counted - the more copies you can find, the more active the gene is at the moment.

This experiment tried to test this theory by feeding larva of fruit flies different diets and afterwards checking to see how the expression of genes related to inflammation changed in the individuals. The larvae were divided into groups; one group received normal foods, another got the same foods but with an extra helping of gluten, and still other groups were fed gluten plus one or more probiotic supplements.

The experiment found that in general, gene behavior became more or less active in ways that were predicted when gluten was added to the diet - genes related to immune function became more active, and genes related to normal day-to-day activities became quieter. When probiotics became a factor, the outcomes started to become less straightforward. Some probiotics reversed the effects of inflammation; in a few cases they made it worse. Very often the probiotics appeared to act as a shield, offering what looked like a protective influence against the effects of gluten.

The genes investigated all had human versions which for the most part were very similar in structure and function, so-called “conserved genes”. This means that anything learned from experimenting on the animals can (in theory) be directly applied to human medicine.

In many cases, the human versions of the genes behaved oppositely than their fly counterparts, possibly because human systems have evolved to be more complex, or possibly because the RNA tested came from worms and not adult creatures. The conclusion was that every combination of gene and probiotic had to be looked at as a unique phenomenon; that each probiotic had individual characteristics that made it function differently in each situation.

Understanding how bacteria in the gut are going to react to the presence of other specific, beneficial organisms could make it possible to custom-tailor a treatment plan for celiac patients, cutting costs for the individual and improving quality of life. In that way this research is a step towards “personalised medicine”, or a plan of action unique to *you*. Understanding the framework behind how probiotics work on your unique genetic profile is a step on the pathway to understanding how they will work on *your* body, making treatment of a condition much more efficient and comfortable.

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1 Introduction

1.1 The nature of gluten intolerance and celiac disease

Celiac disease (CD) is an autoimmune enteropathy characterised by chronic inflammation throughout the small intestine, with particular involvement of the duodenum (Kivrakoglu *et al.*, 2025). The disease affects genetically predisposed individuals who carry the alleles for HLA-DQ2 or HLA-DQ8 (Keppeler *et al.*, 2024; Kivrakoglu *et al.*, 2025; McCreery *et al.*, 2025) and is the predominant form of immune-mediated enteropathy across the globe (Auricchio *et al.*, 2022). Celiac disease partially manifests as an array of intestinal complaints - abdominal pain, chronic diarrhea, bloating, fatigue and nutrient malabsorption (Gupta *et al.*, 2025). Aside from gastrointestinal issues, CD has been associated with increased blood pressure and vascular inflammation, extraintestinal autoimmunity, intestinal T-cell lymphoma and an enhanced risk of renal disease (Keppeler *et al.*, 2024). A number of metabolic functions become measurably disrupted in patients suffering from CD - oxidative stress regulation, nucleotide synthesis, DNA repair, energy production and amino acid metabolism are all examples of processes which are negatively impacted (McCreery *et al.*, 2025).

Worldwide prevalence for celiac disease is circa 1%, while in Europe the rate of occurrence is slightly less, with a 2025 estimate placing the frequency at 0.8% (Gupta *et al.*, 2025; Kivrakoglu *et al.*, 2025).

The principal factor initiating symptoms is gliadin, a protein component of wheat gluten which is rich in proline and glutamine (Bellomo *et al.*, 2025; Sabanci *et al.*, 2025; Said *et al.*, 2025), although the ingestion of the rye and barley protein counterparts secalin and hordein, respectively, also have inflammatory consequences (Sabanci *et al.*, 2025; Said *et al.*, 2025). Polypeptides which are particularly rich in proline and glutamine, such as gliadin, are exceptionally difficult for digestive enzymes to break down into their constituent parts (McCreery *et al.*, 2025; Said *et al.*, 2025). Other significant identified disease triggers include dysbiosis of gut microbiota, defined by Chibbar & Dieleman (2019) as “...the loss of balance between pathogenic and protective microbes of the intestinal tract”, viral infections (Gupta *et al.*, 2025), and various environmental factors such as early-life gastrointestinal infections, antibiotic or proton pump inhibitor use, or possibly maternal iron supplementation (Bellomo *et al.*, 2025; Gupta *et al.*, 2025).

1.2 The pathogenesis of celiac disease

The molecular interactions behind the origin of inflammation can be summarised as follows. Upon the introduction of gluten, α -gliadin protein undergoes degradation into its constituent peptides including A-gliadin 33-mer and p31-43 (Bellomo *et al.*, 2025; Gupta *et al.*, 2025). These in turn induce the production of zonulin, a protein which regulates the permeability of tight junctions in the digestive tract (Rossi *et al.*, 2023). As permeability is increased, peptides enter the lamina

propria by way of binding to secretory IgA immunoglobulins (de Sousa Moraes *et al.*, 2014; Olshan *et al.*, 2020; Verdu & Schuppan, 2021). Once within the lamina propria, the gliadin peptides are deamidated by tissue transglutaminase 2 (TG2), an enzyme expressed during inflammation. This deamidation facilitates binding to HLA-DQ2 or -DQ8 epitopes expressed on antigen presenting cells, who in turn present these peptides to CD4⁺ Th1 cells. Recognition of antigen by helper cells induces an increase in inflammatory cytokines such as interferon- γ (IFN- γ), interleukin-15 (IL-15), interleukin-12 (IL-12) and tumor necrosis factor-alpha (TNF- α) (Gupta *et al.*, 2025). Inflammation of the intestinal mucosa follows, ultimately resulting in crypt hyperplasia and villous atrophy (Caio *et al.*, 2020; Pecora *et al.*, 2020; Wu *et al.*, 2021). The immune response from T-cells and IFN- γ results in destruction of intestinal mucosa, although it is not known if the damage induced by a reaction to gluten is due to a loss of tolerance or a failure to establish tolerance (Auricchio *et al.*, 2022; Sabanci *et al.*, 2025).

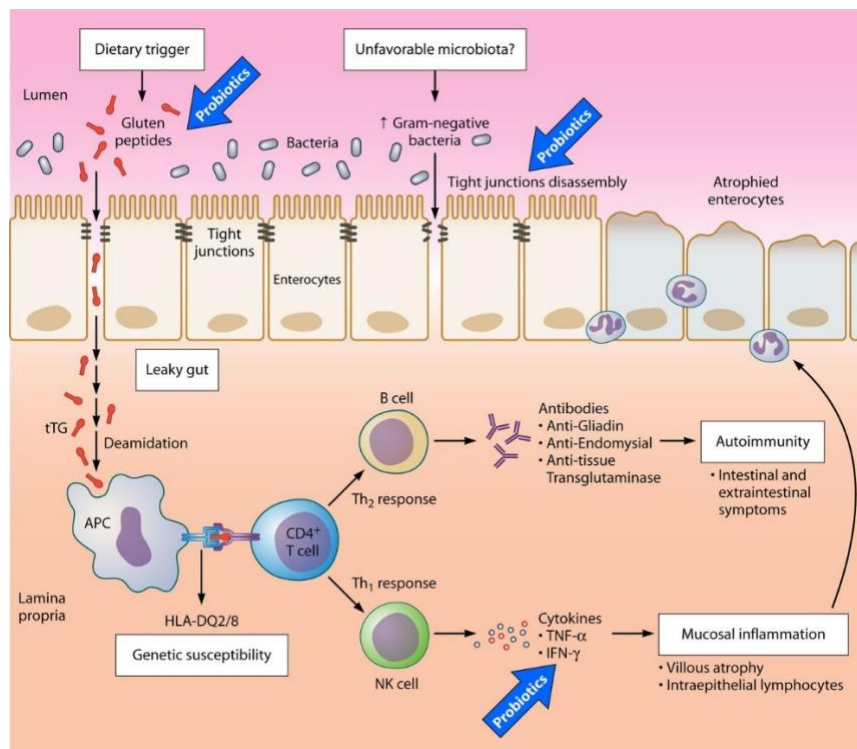


Figure 1. The pathogenesis of celiac disease. Degradation of α -gliadin induces production of zonulin. With increased permeability, peptides enter the lamina propria via IgA antiglobulins, and deamidation by tTG and presentation to CD4⁺ T immune cells by antigen-presenting cells, (haplotypes DQ2 and DQ8) follows. Th1 and Th2 immune responses are triggered, resulting in autoimmunity, mucosal inflammation, and the growth of unfavorable microbiota. Arrows indicate where probiotics could have effect (From de Sousa Moraes *et al.*, 2014)

1.3 The role of diet and probiotics

Currently, the only widely accepted treatment for celiac disease is dietary exclusion (Bellomo *et al.*, 2025). However, adopting gluten-free dietary habits is not necessarily an ideal solution, as some research suggests that strict adherence to such regimens can cause micronutrient deficiencies (Gupta *et al.*, 2025).

As an alternative, much research is currently underway regarding the effects of probiotic dietary supplements on the prevention or reversal of CD symptoms in humans. Probiotic strains of particular interest to this study and their potential effects are briefly explained below.

1.3.1 Probiotics with potential benefits for intestinal health

Upon the ingestion of gluten by individuals with celiac disease, the immune system initiates an inflammatory response. This inflammatory process damages the villi, which are microscopic, finger-like projections lining the intestinal wall and crucial for nutrient absorption. The resultant damage leads to villous atrophy, characterised by the flattening or destruction of the villi, which impairs the intestine's ability to absorb essential nutrients, including carbohydrates, fats, proteins, vitamins, and minerals. This malabsorption can lead to various health issues stemming from nutrient deficiencies. Probiotics may contribute towards reversing these effects by supporting the recuperation of the intestinal mucosa. This is achieved through attenuating inflammation, reinforcing the integrity of the gut barrier, and reestablishing a harmonious balance of gut microbiota. Through these mechanisms probiotics can facilitate the restoration of the villi and enhance overall gut health (Khorzoghi *et al.*, 2023; Rossi *et al.*, 2023).

1.3.1.2 Corynebacterium variabile. Corynebacterium Casei. Microbacterium gubbeenense.

To date, the specific mechanisms of action of these three probiotic strains remain poorly understood. Generally, probiotics with established health benefits have been shown to exert effects such as the production of short-chain fatty acids (SCFAs) like butyrate, regulation of inflammation, and support of the immune system. They are also known to contribute to the maintenance of gut barrier integrity, thereby preventing the entry of harmful substances into the bloodstream, and additionally produce antimicrobial compounds that inhibit the colonization of pathogenic bacterial strains. While these three strains exhibit promising potential in these areas, the current body of evidence is insufficient to conclusively demonstrate their efficacy (Rao & Samak, 2013; Trebichavský & Splíchal, 2006).

1.3.1.3 Lactobacillus plantarum, Lactobacillus paracasei, and Lactobacillus bulgaricus.

The combination of these three probiotic actors influences gut function through helping to maintain a healthy balance of gut microbiota; these species compete with pathogenic bacteria for limited resources and adhesion sites. In a similar fashion as the above-mentioned species, this trio promotes the production of tight-junction proteins, enhancing gut barrier function and thereby reducing systemic inflammation. This probiotic combination simultaneously stimulates the production of anti-inflammatory cytokines while decreasing pro-inflammatory cytokine production. Bacteriocin production, antioxidant effects and improved glucose and lipid metabolism are other documented benefits. These probiotics can influence the activities of the enteric nervous system either through production of neurotransmitters and neuropeptides

(such as serotonin and gamma-aminobutyric acid) or alternatively through conversion of precursors to neurotransmitters such as tryptophan. *L. plantarum* is additionally known to produce conjugated linoleic acids with anti-inflammatory properties (Bellomo *et al.*, 2025; Iorizzo *et al.*, 2024; Shah *et al.*, 2024)

1.4 Summary of important genes and orthologues

The effects of the aforementioned probiotics on gluten-induced inflammation can be quantitatively studied by measuring the differential expression of genes associated with either inflammatory or non-inflammatory conditions. Presented below is a small selection of human genes of interest related to stress or inflammation, along with their closest *Drosophila melanogaster* orthologues as identified through ensembl.org and flybase.org databases. Studying the expression of genes in *D. melanogaster* involves fewer ethical complications than conducting experiments directly on humans. This approach enables researchers to control environmental conditions more stringently and is both time-efficient and cost-effective. The presence of conserved orthologous genes related to inflammation between *D. melanogaster* and humans facilitates the possibility of identifying similarly structured pathways and processes, while minimizing potential adverse effects on human subjects.

1.4.1 EDA (*D. melanogaster* orthologue *egr*)

Ectodysplasin A (*EDA*) encodes a protein of the tumor necrosis factor (TNF) family which is involved in the development of ectodermal tissues such as teeth and hair (Pantalacci *et al.*, 2008). In *Drosophila*, *egr* is involved in cell differentiation and the triggering of apoptosis, especially in regard to tissue remodelling and patterning. Additionally, *egr* regulates several genes related to innate immunity (Zhang *et al.*, 2024).

1.4.2 MRE11 (*D. melanogaster* orthologue *mre11*)

Encodes a key protein component of the MRN complex, involved in sensing and repairing double strand DNA breaks as well as protecting telomeres through preventing them from being recognised as damaged DNA. This complex is important for maintaining genomic stability and is highly conserved across species (Tarapara & Shaw, 2025). In *Drosophila*, *mre11* serves the same functions as in humans (Ciapponi *et al.*, 2004).

1.4.3 XRCC1 (*D. melanogaster* orthologue *XRCC1*)

Human gene *XRCC1* codes for a 663 amino acid molecular scaffold protein which is known to have several functions related to different types of single-strand DNA repair (Caldecott, 2019). Likewise in *Drosophila* *XRCC1* is a part of the base excision repair pathway which removes and replaces damaged bases in single-strand DNA breaks (Dashnamoorthy *et al.*, 2009).

1.4.4 *PRODH* (*D. melanogaster* orthologue *SlgA*)

PRODH regulates the production of proline dehydrogenase, which converts proline to pyrroline-5-carboxylate, which can then be further metabolized to glutamate. Proline dehydrogenase is integral to several cellular processes including energy production, regulation of redox homeostasis and programmed cell death. Proline dehydrogenase catalyses the oxidation of proline to glutamate within the mitochondria (Natarajan & Becker, 2012; Servet *et al.*, 2012). Proline can act as a source of reserve amino acids and energy and is thought to have a signalling role in the response to “microenvironmental nutrient stresses” (Servet *et al.*, 2012). *Drosophila* orthologue *SlgA*, not directly involved in immune function, has the primary function of encoding a proline oxidase (Hayward *et al.*, 1993).

1.4.5 *NFKB1* (*D. melanogaster* orthologue *Rel*)

NFKB1 is a member of the NF- κ B transcription factor family which regulates cellular inflammatory activities, immune response and cell survival in response to pro-inflammatory cytokines or the activation of innate or T-cell receptors (Lougaris *et al.*, 2016; Zingarelli, 2005). In *Drosophila* species, *Rel* is involved in immune response through activating the Toll pathway and activating transcription of antimicrobial peptide genes in response to fungal or Gram-positive bacterial pathogens (Ganesan *et al.*, 2011; De Gregorio *et al.*, 2002).

1.5 Aim

The overarching aim of this research is to investigate the impact of gluten intake and probiotic supplements on the expression of inflammatory and growth-related genes in *D. melanogaster* larvae.

The specific aims of this research are to:

1. Demonstrate the connection between gluten intake and inflammatory gene expression by testing the hypothesis that *D. melanogaster* larvae provided with high-gluten diets will upregulate the expression of inflammatory or stress genes compared to normal controls.
2. Evaluate the effect of probiotic supplements on inflammatory gene expression by testing the hypothesis that *D. melanogaster* larvae receiving gluten with probiotic supplements will display either only slightly elevated expression or potentially a downregulation of inflammatory or stress genes.
3. Assess the impact of gluten intake on growth and cell proliferation genes by testing the hypothesis that genes associated with growth and cell proliferation will show downregulation with a heavy gluten diet versus controls.
4. Determine the effect of probiotic supplements on growth and cell proliferation genes by testing the hypothesis that genes associated with growth and cell proliferation will show lesser degrees of downregulation with the addition of dietary probiotics to a high-gluten diet.

2 Methods and materials

2.1 Biological materials

Drosophila melanogaster strain Da-Gal4 was caged overnight. Eggs were collected and divided between plates with different additions to standard food. Five days after eclosion the larvae were weighed, collected in tubes and stored at -80°C. This process was done previously as part of a different project.

The initial step in the experimental procedure consisted of choosing representative specimens for each of the six categories to be investigated. As a baseline, *D. melanogaster* larvae with no special treatment were used as experimental controls. These juveniles were raised on a diet consisting of yeast, mashed potato powder, sugar, apple juice and water. The second category included larvae which were fed a diet similar to the control organisms, but with the addition of 5g gluten per 60g base food. The remaining four groups all received the same dietary treatment as the Gluten category, but with the addition of one or more probiotic supplements. Group three received 9mg *Corynebacterium casei* per 4g base food, group four 8mg *Corynebacterium variable* per 4g food, group five 2.3mg *Microbacterium gubbeenense* per 4g food and the sixth group (Active) a combination of 3.4mg *Lactobacillus plantarum*, 3.3mg *Lactobacillus paracasei* and 6.8mg *Lactobacillus bulgaricus* per 4g base food. This analysis utilized cDNA, synthesised from total RNA extracted from the homogenized soft tissue of *Drosophila* larvae. Each dietary group included six individual larvae, which were pooled into three biological samples consisting of two larvae each for RNA extraction.

2.2 RNA and cDNA preparation

To gather raw material for RNA extraction three samples, each composed of two individual larvae, were randomly selected in each of the previously mentioned categories, resulting in a total of 18 experimental samples. The extraction procedure was identical for all groups. Two larvae were placed into a 1.5ml tube along with a 5mm stainless steel ball and 350µl RLT buffer from RNeasy mini kit (Qiagen) for cell lysis and RNA stabilisation. Samples were homogenised with a Qiagen TissueLyser for one minute at a setting of 50Hz. The homogenate was incubated on ice for 20 minutes, then centrifuged for 4 minutes at 14,100g. 310µl of supernatant was then combined with an equal volume of 70% ethanol in a new 1.5ml tube. 550µl of this mixture was placed in a Qiagen mini-spin column and centrifuged for 1 minute. After discarding the contents of the collection tube 700µl of RW1 buffer was added to the column to remove residual salts and impurities. The column was again centrifuged for 1 minute, and the contents of the collection tube discarded. 500µl of RPE buffer was added (to remove impurities and salts) followed by one minute of centrifugation, after which a second cycle of 500µl RPE buffer was carried out, this time with two minutes of centrifugation. Subsequent to this step 30µl of RNase-free water was pipetted into the spin column followed by two minutes of room-temperature incubation. A final 1 minute of centrifugation was employed to release the extracted RNA into the collection tube. RNA concentration and

purity were determined spectrophotometrically at 260nm using a Denovix DS-11 spectrophotometer.

cDNA was synthesised using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, product number 4368814) according to the manufacturer's protocol. Reverse transcription reactions were prepared in a total volume of 20µl. The reaction master mix consisted of 1µl reverse transcriptase, 2µl 10× RT buffer, 2µl random primers, and 0.8µl dNTP mix. This master mix was combined with RNA and nuclease-free water to yield a final RNA input of 1.45µg per reaction.

Reverse transcription was performed using a Biometra TProfessional Basic Gradient thermocycler under the following conditions: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min, followed by a hold at 4°C. The resulting cDNA was diluted 1:50 with nuclease-free water prior to qPCR analysis. A volume of 2.25µl diluted cDNA was used in each qPCR reaction, corresponding to approximately 3.3ng of input RNA.

2.3 Quantitative PCR

Each PCR plate contained five genes of interest and six unique cDNA samples as well as a negative control containing no probe for each sample. Individual wells contained 2.25µl cDNA, 0.25µl TaqMan gene expression assay probe (FAM) (Applied Biosystems), and 2.50µl Fast advanced master mix (Applied Biosystems, product number 4444557). The contents of negative control wells for all samples consisted of 2.50µl Fast advanced master mix (Applied Biosystems) combined with 2.50µl cDNA.

For the qPCR procedure which followed, a 5µl reaction volume per well protocol was chosen. Reactions were carried out on a Thermo Scientific PikoReal 96 Real-Time PCR System (Thermo Fisher Scientific) in 96-well PCR plates. qPCR reactions were performed using the following cycling conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 amplification cycles of 95°C for 15s and 60°C for 1 min. Each biological sample was analysed in technical triplicate. Twenty-two target gene sequences, including the reference genes *alphaTub6+* and *Rpl32*, were amplified and quantified using TaqMan probes (Applied Biosystems) in a real-time PCR assay.

The genes investigated in this study were: *Drsl2*, *GLS*, *Pi3K21B*, *XRCC1*, *dlg1*, *Parp*, *CASK*, *Mettl3*, *Fen1*, *CycA*, *Mettl14*, *Pi3K92E*, *metro*, *CG32368* and *lig3*. In addition to these fifteen genes, the following five and their human orthologues were chosen for in depth analysis: *egr/EDA*, *XRCC1/XRCC1*, *SlgA/PRODH*, *mre11/MRE11* and *Rel/NFKB1*. Assay identification numbers for probes used to measure expression levels are shown below in Table 1. Raw data from the full complement of genes investigated can be found in Appendix 2.

Table 1. Genes included in this study along with specific probes used to measure expression.

GENE NAME	ASSAY ID
<i>AlphaTub6+</i>	Dm02362441_s1
<i>Rpl32</i>	Dm02151827_g1
<i>Drsl2</i>	Dm01832532_s1
<i>GLS</i>	Dm01793294_g1
<i>Pi3K21B</i>	Dm01842882_m1
<i>XRCC1</i>	Dm01795840_g1
<i>dlg1</i>	Dm01799281_g1
<i>Parp1</i>	Dm03419822_m1
<i>CASK</i>	Dm02136251_g1
<i>Mettl3</i>	Dm02143923_g1
<i>Fen1</i>	Dm01821494_g1
<i>CycA</i>	Dm01822597_m1
<i>Mettl14</i>	Dm01809600_g1
<i>Pi3K92E</i>	Dm02142679_g1
<i>metro</i>	Dm01819480_g1
<i>CG32368</i>	Dm01835862_s1
<i>lig3</i>	Dm02139557_g1
<i>egr</i>	Dm01794373_m1
<i>PGRP_SD</i>	Dm01840723_s1
<i>mre11</i>	Dm01817703_g1
<i>Rel</i>	Dm02134843_g1
<i>SlgA</i>	Dm01799725_m1

2.4 Statistical analysis

Statistical analysis of qPCR results involved several key steps: calculating the relative expression of target genes under experimental versus control conditions and determining the statistical significance of expression changes for all pairwise combinations of target genes. After statistical analysis, a comparison was made regarding the changes in fold expression of target genes with alterations in their closest human orthologues in the context of inflammatory conditions. Statistical analyses were performed using GraphPad Prism (version 11.0.0; GraphPad Software, USA).

2.4.1 Comparison of fold expression

The $2^{-\Delta\Delta Ct}$ method is a quantitative real-time PCR technique employed to assess relative gene expression levels. It facilitates comparisons of gene expression across various samples by normalising data against a reference gene, thereby illustrating changes in expression relative to a control group. Changes in cycle threshold (Ct) values obtained from qPCR results for each gene of interest were normalised against the geometric mean of the Ct values for the *alphaTub6+* and *Rpl32* reference genes. The relative expression of genes in control larvae compared to those subjected to specialised diets was calculated using the aforementioned method.

2.4.2 ANOVA and Tukey HSD tests

The ANOVA (Analysis of Variance) method is employed to determine whether there are statistically significant differences among the means of three or more groups. It helps determine if at least one group mean is different from the others, highlighting variations across multiple datasets. The Tukey HSD test can be applied after an ANOVA to identify which specific groups differ from one another.

Gene expression levels between *D. melanogaster* dietary conditions were analysed using an ANOVA statistical test to identify any genes that showed a significant response to changes in diet. $2^{-\Delta\Delta Ct}$ values were utilized to calculate the ratios of variance within and between experimental groups and controls, and to further ascertain the statistical significance of those observations for each individual gene of interest. In cases where a statistically significant difference in expression (defined as $p < 0.05$) was detected, a Tukey HSD test was applied ($\alpha = 0.05$) to determine between which pairs of samples significance existed. Statistical analyses were performed using three biological replicates per dietary condition ($n = 3$).

2.4.3 Comparison with human orthologues

The five *Drosophila* genes which showed the greatest variation in level of expression after exposure to gluten were investigated and compared with their closest human orthologues to determine if similar patterns of up- or down-regulation of expression under inflammatory conditions caused by gluten ingestion existed in both species. Data regarding fold changes for human orthologues was taken from van der Graaf *et al.* (2021) RNA-sequencing study entitled “RNA sequencing derived from intestinal biopsies from healthy controls and celiac disease patients” (GEO accession

GSE146190). This dataset was based upon the GRCh38.p13 reference genome assembly.

3 Results

3.1 Comparative Gene Expression Analysis Across Experimental Diets and Inflammatory Conditions

While the differential expression of 20 genes was measured during this study, analysis and discussion is restricted to *egr*, *mre11*, *XRCC1*, *Rel* and *PRODH*. To evaluate the impact of dietary treatments on host physiological responses, genes associated with immune signalling, DNA repair, and metabolic stress were analysed. Information about relative expression levels for all other genes investigated and their human orthologues can be found in Appendix 2.

Results from qPCR analysis of relative gene expression levels for these five genes across all experimental diets as derived by the $2^{-\Delta\Delta Ct}$ method as well as changes in fold expression for human orthologues under inflammatory conditions as reported by van der Graaf *et al.* (2021) are shown in Figures 2 through 6 below. The 2021 study included 12,948 individuals with histology proven celiac disease and 14,826 healthy controls, using expression quantitative trait loci (eQTL) from whole blood. The authors note that while whole blood is an appropriate tissue type for celiac disease investigations, DNA segments from this source material will not be representative of all tissue types. An ANOVA analysis was performed for all diets within each experimental gene to determine whether any significant differences in expression were shown by the data, after which Tukey's HSD test was applied to indicate which specific diets were associated with statistically significant differences in expression in comparison to Control and gluten categories.

3.1.1 Immune signalling genes

3.1.1.1 Results of probiotic supplementation on *Rel* expression

As shown in Figure 2, the mean fold change of the control group for the *Rel* gene was 1.00 ± 0.093 SD. The *C. casei* treatment produced the highest mean expression level, with a fold change of 3.43 ± 1.715 SD, although variability between replicates was substantial. The *C. variabile* group showed a mean fold change of 1.11 ± 0.471 SD, while the *M. gubbeenense* group exhibited reduced expression relative to the control with a mean fold change of 0.750 ± 0.357 SD. The Active probiotic blend produced a mean fold change of 1.31 ± 0.816 SD. The Gluten treatment group displayed a mean fold change of 1.94 ± 2.165 SD, again with considerable variation among replicates.

Human orthologue *NFKB1* displayed a fold change of 0.915 (\log_2 fold change = -0.128 ± 0.239 SE, $p = 0.816$), indicating no statistically significant difference in expression relative to the reference condition.

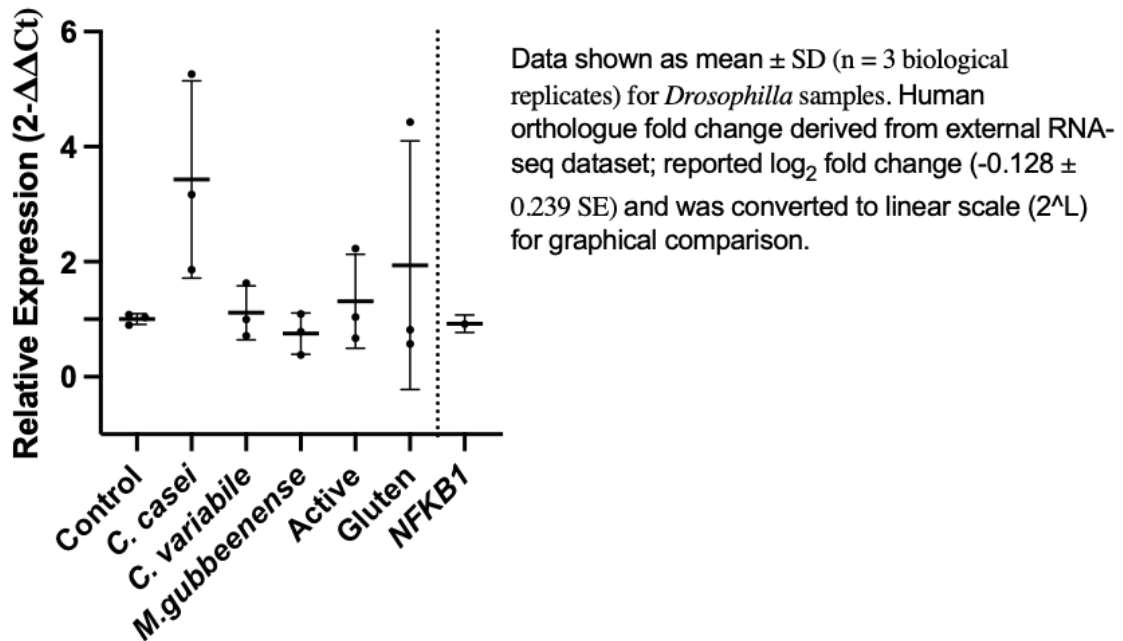


Figure 2. Relative expression of *Rel* in *Drosophila melanogaster* larvae under different dietary treatments with comparison to human orthologue *NFKB1*. Gene expression was measured using qPCR and calculated as fold change using the $2^{-\Delta\Delta Ct}$ method relative to control conditions. Individual points represent biological replicates (n = 3 per treatment). Horizontal lines indicate mean expression; error bars represent standard deviation. Human orthologue values were derived from RNA-seq data reported as \log_2 fold change and converted to fold change for comparison. A fold change of 1 indicates no change in expression relative to controls.

Expression of the *Rel* gene showed no clear pattern of change across the dietary treatments relative to the control group. One-way ANOVA indicated that diet did not significantly affect *Rel* expression ($F(5,12) = 2.03$, $p = 0.1464$). Consistent with this result, post-hoc analysis using Tukey's HSD test did not identify any significant pairwise differences between treatments. These findings are summarised in Table 2.

Table 2. Tukey HSD pairwise comparisons of *Rel* gene expression among dietary treatments in *D. melanogaster*. Values represent differences in mean fold change between the first and second groups listed with adjusted *p*-values for multiple comparisons.

Comparisons	Mean difference	95% CI	Adjusted <i>p</i> -value
Control vs. <i>C. casei</i>	-2.43	-5.72 to 0.862	0.2049
Control vs. <i>C. variabile</i>	-0.110	-3.40 to 3.18	>0.9999
Control vs. <i>M. gubbeenense</i>	0.253	-3.04 to 3.54	0.9998
Control vs. Active	-0.310	-3.60 to 2.98	0.9995
Control vs. Gluten	-0.937	-4.23 to 2.35	0.9231
<i>C. casei</i> vs. Gluten	1.49	-1.80 to 4.78	0.6583
<i>C. variabile</i> vs. Gluten	-0.827	-4.12 to 2.46	0.9528
<i>M. gubbeenense</i> vs. Gluten	-1.19	-4.48 to 2.10	0.8216
Active vs. Gluten	-0.627	-3.92 to 2.66	0.9854

3.1.1.2 Results of probiotic supplementation on *egr* expression

Expression of the *egr* gene across dietary treatments is illustrated in Figure 3. The control group exhibited a mean fold change of 1.02 (SD \pm 0.265). Reduced expression relative to the control was observed in several treatment groups, including *C. casei* with a mean fold change of 0.463 (SD \pm 0.104) and *C. variabile* with 0.483 (SD \pm 0.107). The *M. gubbeenense* condition produced the lowest mean fold change at 0.350 (SD \pm 0.165). The Active treatment group showed a mean fold change of 0.637 (SD \pm 0.118), while the Gluten treatment yielded 0.720 (SD \pm 0.135). For comparison, human orthologue *EDA* exhibited a fold change of 0.778 (log₂ fold change -0.3625 , *p* = 0.755).

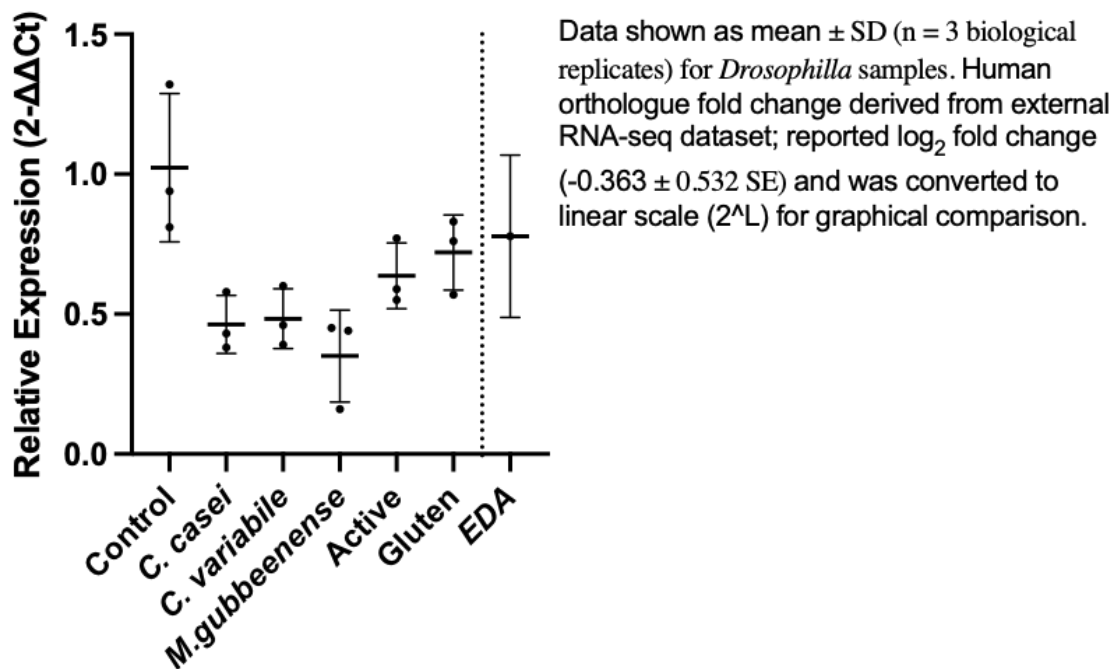


Figure 3. Relative expression of *egr* in *Drosophila melanogaster* larvae under different dietary treatments with comparison to human orthologue *EDA*. Gene expression was measured using qPCR and calculated as fold change using the $2^{-\Delta\Delta C_t}$ method relative to control conditions. Individual points represent biological replicates (n = 3 per treatment). Horizontal lines indicate mean expression; error bars represent standard deviation. Human orthologue values were derived from RNA-seq data reported as \log_2 fold change and converted to fold change for comparison. A fold change of 1 indicates no change in expression relative to controls.

In *D. melanogaster*, the expression of the *egr* gene was generally reduced across dietary treatments relative to the control group. ANOVA analysis indicated a significant effect of diet on gene expression ($F(5,12) = 6.87$, $p = 0.0046$). Post-hoc analysis using Tukey's HSD test identified significant reductions in expression for the *C. casei*, *C. variabile*, and *M. gubbeenense* treatments relative to the control group (Table 2). The largest decrease was observed for *M. gubbeenense* (mean difference = 0.673, $p = 0.0031$), followed by *C. casei* (0.560, $p = 0.0128$) and *C. variabile* (0.540, $p = 0.0166$).

Table 3. Tukey HSD post-hoc comparisons for *egr* expression across dietary treatments in *D. melanogaster*. Values represent pairwise differences in mean fold change relative to the control group.

Comparisons	Mean difference	95.% CI	Adjusted <i>p</i> -value
Control vs. <i>C. casei</i>	0.56	0.11 to 1.0	0.0128
Control vs. <i>C. variable</i>	0.54	0.086 to 0.99	0.0166
Control vs. <i>M. gubbeenense</i>	0.67	0.22 to 1.1	0.0031
Control vs. Active	0.39	-0.067 to 0.84	0.1161
Control vs. Gluten	0.30	-0.15 to 0.76	0.3011
<i>C. casei</i> vs. Gluten	-0.26	-0.71 to 0.20	0.4721
<i>C. variable</i> vs. Gluten	-0.24	-0.69 to 0.22	0.5573
<i>M. gubbeenense</i> vs. Gluten	-0.37	-0.82 to 0.084	0.1420
Active vs. Gluten	-0.083	-0.54 to 0.37	0.9936

3.1.2 DNA repair genes

3.1.2.1 Results of probiotic supplementation on *mre11* expression

Expression of the *mre11* gene across dietary treatments is shown in Figure 4. The control group exhibited a mean fold change of 1.01 (SD \pm 0.144). Reduced expression relative to the control was observed in several treatment groups, including *C. casei* (0.640 \pm 0.184 SD) and *C. variable* (0.663 \pm 0.127 SD). The *M. gubbeenense* category produced a mean fold change of 0.787 \pm 0.101 SD, while the Active treatment group showed 0.713 \pm 0.130 SD. In contrast, the Gluten treatment yielded a mean fold change of 1.04 \pm 0.256 SD, comparable to the control group. For comparison, human orthologue *MRE11* displayed a fold change of 0.749 (log₂ fold change -0.417, *p* = 0.309).

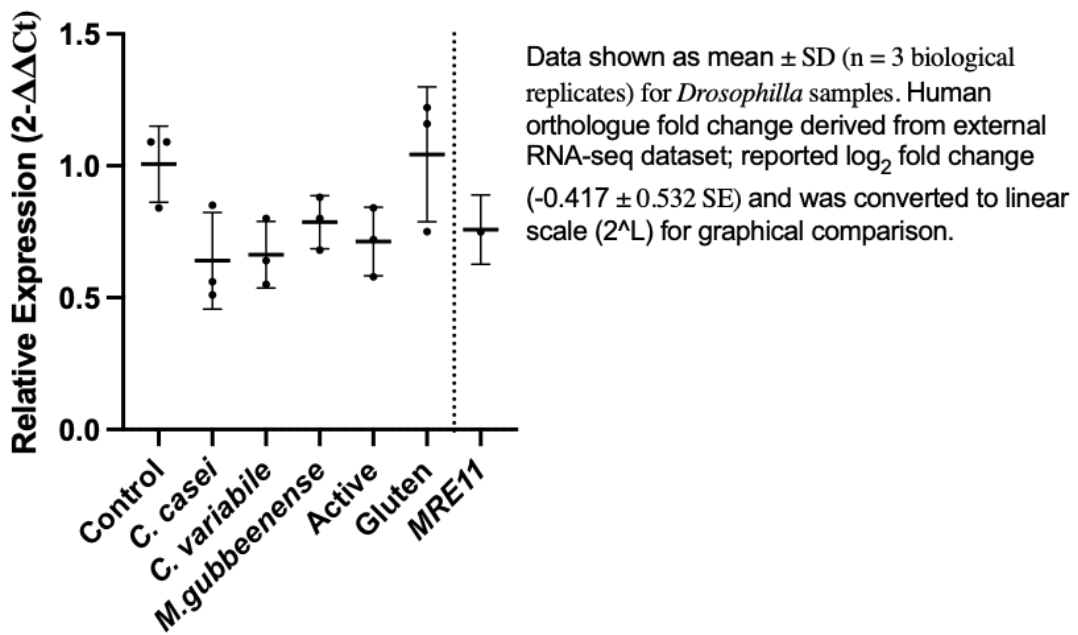


Figure 4. Relative expression of *mre11* in *Drosophila melanogaster* larvae under different dietary treatments with comparison to human orthologue *MRE11*. Gene expression was measured using qPCR and calculated as fold change using the $2^{-\Delta\Delta C_t}$ method relative to control conditions. Individual points represent biological replicates (n = 3 per treatment). Horizontal lines indicate mean expression; error bars represent standard deviation. Human orthologue values were derived from RNA-seq data reported as \log_2 fold change and converted to fold change for comparison. A fold change of 1 indicates no change in expression relative to controls.

Expression of the *mre11* gene was reduced across most dietary treatments relative to the control group, with the exception of the Gluten diet, which showed a slight increase in expression. A one-way ANOVA indicated a significant overall effect of diet on gene expression ($F(5,12) = 3.39$, $p = 0.0387$). However, post-hoc analysis using Tukey's HSD test did not identify any statistically significant pairwise differences between the control group and the experimental diets, nor between the experimental diets and the Gluten treatment. Pairwise comparisons are summarised in Table 4.

Table 4. Tukey HSD post-hoc pairwise comparisons of *mre11* expression across dietary treatments in *D. melanogaster*. Values represent differences in mean fold change between treatment groups, with adjusted *p*-values reported for multiple comparisons.

Comparisons	Mean difference	95% CI	Adjusted <i>p</i> -value
Control vs. <i>C. casei</i>	0.367	-0.0855 to 0.819	0.1408
Control vs. <i>C. variable</i>	0.343	-0.109 to 0.795	0.1839
Control vs. <i>M. gubbeenense</i>	0.220	-0.232 to 0.672	0.5937
Control vs. Active	0.293	-0.159 to 0.745	0.3135
Control vs. Gluten	-0.0367	-0.489 to 0.415	0.9997
<i>C. casei</i> vs. Gluten	-0.403	-0.855 to 0.0488	0.0911
<i>C. variable</i> vs. Gluten	-0.380	-0.832 to 0.0721	0.1204
<i>M. gubbeenense</i> vs. Gluten	-0.257	-0.709 to 0.195	0.4427
Active vs. Gluten	-0.330	-0.782 to 0.122	0.2133

3.1.2.2 Results of probiotic supplementation on *XRCC1* expression

As shown in Figure 5, the mean fold change of the control group for the *XRCC1* gene was 1.02 ± 0.237 SD. The *C. casei* treatment produced a mean fold change of 0.803 ± 0.075 SD, while the *C. variable* group showed a mean value of 0.850 ± 0.047 SD. Expression in the *M. gubbeenense* group was lower, with a mean fold change of 0.573 ± 0.187 SD. The Active probiotic blend produced a mean fold change of 0.790 ± 0.192 SD. In contrast, the Gluten group exhibited increased expression relative to the control, with a mean fold change of 1.25 ± 0.201 SD.

Human orthologue *XRCC1* displayed a fold change of 0.994 (\log_2 fold change = -0.0085 ± 0.286 SE, $p = 0.992$), indicating no detectable difference in expression relative to the reference group.

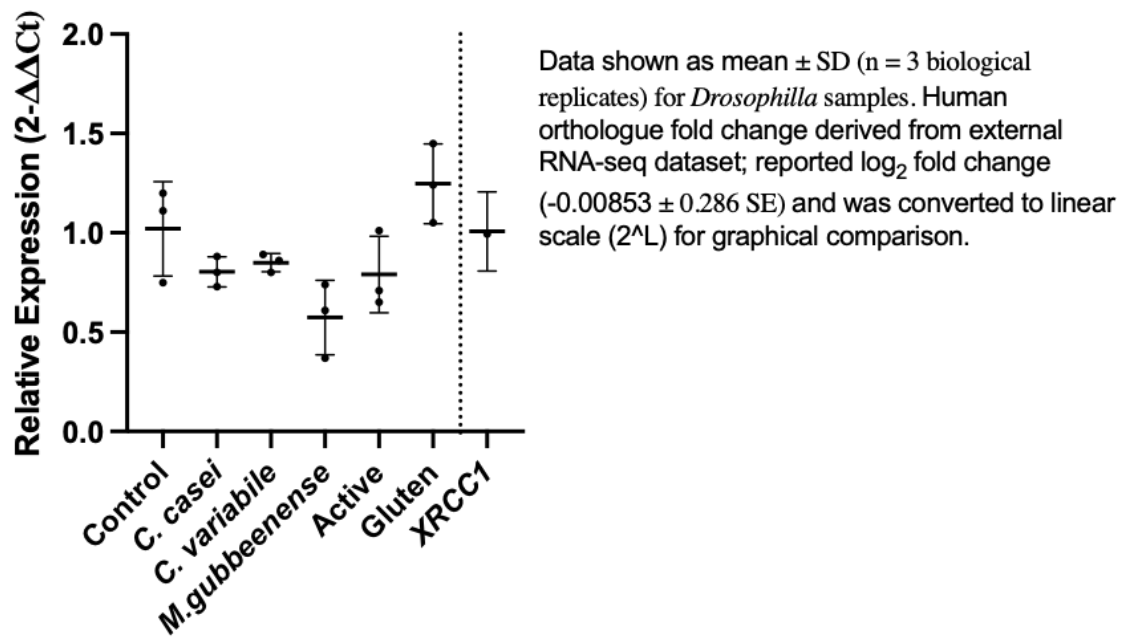


Figure 5. Relative expression of *XRCC1* in *Drosophila melanogaster* larvae under different dietary treatments with comparison to human orthologue *XRCC1*. Gene expression was measured using qPCR and calculated as fold change using the $2^{-\Delta\Delta Ct}$ method relative to control conditions. Individual points represent biological replicates (n = 3 per treatment). Horizontal lines indicate mean expression; error bars represent standard deviation. Human orthologue values were derived from RNA-seq data reported as \log_2 fold change and converted to fold change for comparison. A fold change of 1 indicates no change in expression relative to controls.

Expression of the *XRCC1* gene generally decreased across several dietary treatments relative to the control group. Reduced expression was observed for the *C. casei*, *C. variabile*, *M. gubbeenense*, and Active diets, whereas the Gluten diet showed increased expression compared with the control. A one-way ANOVA revealed a significant effect of diet on gene expression ($F(5,12) = 5.35$, $p = 0.0081$). Post-hoc comparisons using Tukey's HSD test identified a significant difference between the Gluten and *M. gubbeenense* treatments. No other pairwise comparisons were statistically significant. Detailed results of the multiple comparison analysis are presented in Table 5.

Table 5. Results of Tukey's HSD multiple comparison test for *XRCC1* expression across dietary treatments in *D. melanogaster*. Mean differences, 95% confidence intervals, and adjusted p-values are shown for each pairwise comparison.

Comparisons	Mean difference	95% CI	Adjusted p-value
Control vs. <i>C. casei</i>	0.217	-0.254 to 0.688	0.6448
Control vs. <i>C. variabile</i>	0.170	-0.301 to 0.641	0.8230
Control vs. <i>M. gubbeenense</i>	0.447	-0.0242 to 0.918	0.0667
Control vs. Active	0.230	-0.241 to 0.701	0.5901
Control vs. Gluten	-0.227	-0.698 to 0.244	0.6038
<i>C. casei</i> vs. Gluten	-0.443	-0.914 to 0.0276	0.0694
<i>C. variabile</i> vs. Gluten	-0.397	-0.868 to 0.0742	0.1192
<i>M. gubbeenense</i> vs. Gluten	-0.673	-1.14 to -0.202	0.0045
Active vs. Gluten	-0.457	-0.928 to 0.0142	0.0592

3.1.3 Metabolic response gene

3.1.3.1 Results of probiotic supplementation on *SlgA* expression

As shown in Figure 6, the mean fold change of the control group for the *SlgA* gene was 1.01 ± 0.178 SD. The *C. casei* treatment produced a mean fold change of 0.837 ± 0.241 SD, while *C. variabile* yielded a value of 0.940 ± 0.132 SD. Expression was lowest in the *M. gubbeenense* group, which showed a mean fold change of 0.603 ± 0.199 SD relative to the control. In contrast, the Active probiotic blend resulted in a modest increase in expression, with a mean fold change of 1.24 ± 0.121 SD. The Gluten treatment group displayed the highest expression among the *Drosophila* treatments, with a mean fold change of 1.47 ± 0.123 SD.

Human orthologue *PRODH* exhibited a fold change of 5.01 (\log_2 fold change = 2.324 ± 0.375 SE, $p = 7.92 \times 10^{-8}$), indicating a statistically significant increase in expression relative to the reference group.

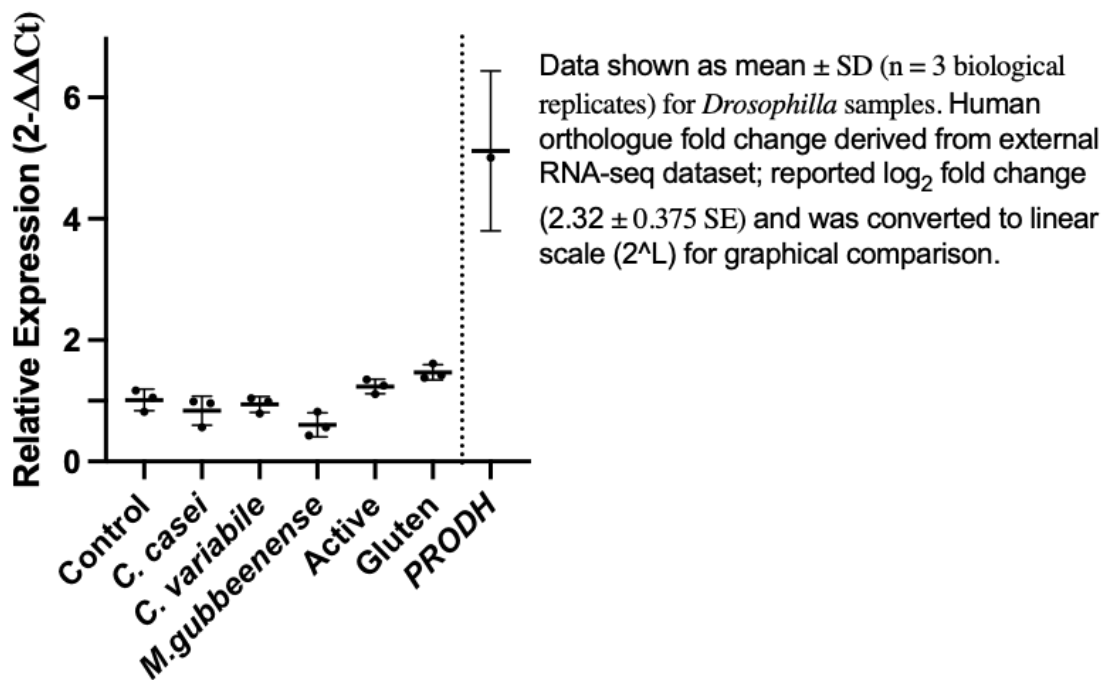


Figure 6. Relative expression of *SlgA* in *Drosophila melanogaster* larvae under different dietary treatments with comparison to human orthologue *PRODH*. Gene expression was measured using qPCR and calculated as fold change using the $2^{-\Delta\Delta Ct}$ method relative to control conditions. Individual points represent biological replicates (n = 3 per treatment). Horizontal lines indicate mean expression; error bars represent standard deviation. Human orthologue values were derived from RNA-seq data reported as \log_2 fold change and converted to fold change for comparison. A fold change of 1 indicates no change in expression relative to controls.

Expression of the *SlgA* gene varied across dietary treatments relative to the control group. Reduced expression was observed for the *C. casei*, *C. variabile*, and *M. gubbeenense* diets, whereas the Active and Gluten diets showed increased expression compared with the control. A one-way ANOVA indicated a significant effect of diet on gene expression ($F(5,12) = 9.48$, $p = 0.0008$). Post-hoc analysis using Tukey's HSD test did not identify significant differences between the control group and the experimental diets. However, significant reductions in expression were observed for the *C. casei*, *C. variabile*, and *M. gubbeenense* treatments when compared with the Gluten diet. Pairwise comparison results are summarised in Table 6.

Table 6. Post-hoc pairwise comparisons of *SlgA* expression across dietary treatments in *D. melanogaster* using Tukey's HSD test. Mean differences, 95% confidence intervals, and adjusted p-values are presented.

Comparisons	Mean difference	95% CI	Adjusted <i>p</i> -value
Control vs. <i>C. casei</i>	0.177	-0.293 to 0.646	0.7981
Control vs. <i>C. variable</i>	0.0733	-0.396 to 0.543	0.9940
Control vs. <i>M. gubbeenense</i>	0.410	-0.0595 to 0.879	0.1009
Control vs. Active	-0.223	-0.693 to 0.246	0.6147
Control vs. Gluten	-0.457	-0.926 to 0.0128	0.0583
<i>C. casei</i> vs. Gluten	-0.633	-1.10 to -0.164	0.0070
<i>C. variable</i> vs. Gluten	-0.530	-0.999 to -0.0605	0.0241
<i>M. gubbeenense</i> vs. Gluten	-0.867	-1.34 to -0.397	0.0005
Active vs. Gluten	-0.233	-0.703 to 0.236	0.5736

4 Discussion

4.1 Transcriptional effects of gluten exposure

Gluten supplementation produced measurable changes in the expression of all five genes examined. Consistent with the original hypothesis, genes associated with immune signalling and cellular stress responses (*mre11*, *XRCC1*, *Rel*, and *SlgA*) exhibited directional trends toward upregulation, whereas the growth-associated gene *egr* showed a downward trend. This pattern supports the premise that gluten exposure in *Drosophila melanogaster* larvae induces a coordinated shift away from developmental processes toward stress adaptation and immune regulation.

4.2 *egr* and developmental reprioritisation under stress

A downward trend in *egr* expression (fold change 0.720) was observed following gluten exposure, although this did not reach statistical significance. *egr*, the *Drosophila* homolog of tumor necrosis factor (TNF), plays roles in apoptosis, tissue homeostasis, and developmental signalling (Zhang *et al.*, 2024). While the reduction observed in the gluten-only group cannot be viewed as conclusive, the consistent directionality suggests that gluten exposure may influence pathways involved in growth and differentiation.

Probiotic supplementation resulted in further suppression of *egr* expression across all treatment groups. Notably, *C. casei* (0.463), *C. variabile* (0.483), and *M. gubbeenense* (0.350) produced statistically significant downregulation relative to controls, while the active blend demonstrated a more moderate decrease (0.637). The uniform direction of change across probiotic treatments indicates that the microbial environment has a measurable influence on developmental signalling pathways. The probiotic treatments consistently reduced *egr* expression relative to the control diet, suggesting that microbial supplementation may suppress host inflammatory signalling pathways. Among the tested strains, *M. gubbeenense* produced the largest reduction in expression, indicating that this bacterium may exert a stronger immunomodulatory effect than the other strains examined.

When considered alongside modulation of immune (*Rel*) and DNA repair (*mre11*, *XRCC1*) genes, suppression of *egr* expression may reflect a systemic resource redistribution under stress conditions. Under conditions of immune activation or metabolic stress, organisms often reallocate resources away from growth-related processes toward defence and maintenance mechanisms (Colombani & Andersen, 2023). In this framework, reduced *egr* expression is consistent with adaptive stress reprogramming rather than isolated pathway inhibition. The more pronounced suppression observed in probiotic groups suggests that microbial signalling may further reinforce this reprioritisation, potentially amplifying host defensive or homeostatic responses.

In humans, TNF signalling is closely linked to inflammatory processes and tissue remodelling (Pantalacci *et al.*, 2008); however, its additional roles in development and apoptosis make direct comparison with *Drosophila* difficult. Furthermore, the lack of adaptive immunity and differences in immune function in *Drosophila* complicate direct application of findings to human systems. Nevertheless, the consistent downregulation observed here supports the interpretation that gluten exposure, particularly in combination with microbial modulation, influences not only immune and repair pathways but also broader developmental signalling networks. Overall, dietary treatments appeared to influence immune signalling genes (*Rel* and *egr*) differently from genes involved in DNA repair (*mre11* and *XRCC1*).

4.3 *mre11* and DNA damage signalling

mre11 expression increased by only 4% with the addition of gluten. As a component of the MRN complex responsible for double-strand break repair, this upregulation suggests activation of DNA damage or inflammatory signalling pathways. This small increase suggests initiation of DNA damage or inflammatory signalling pathways, potentially due to oxidative stress rather than direct genotoxic damage. This is consistent with the idea that gluten may cause mild DNA damage or inflammation, prompting activation of DNA repair pathways.

In contrast, all probiotic treatments reduced *mre11* transcription relative to gluten alone. This reduction exceeded the magnitude of gluten-induced upregulation, suggesting a potential normalising or protective effect. Mechanistically, probiotics may attenuate inflammation, enhance epithelial barrier integrity, or modulate oxidative

stress through metabolite production such as short-chain fatty acids (Rao & Samak, 2013). Under such conditions, reduced transcription of repair genes may reflect decreased cellular stress rather than impaired repair capacity.

Although ANOVA indicated overall group differences, post-hoc testing did not identify significant pairwise contrasts, likely reflecting limited statistical power. These findings should therefore be interpreted as directional trends rather than definitive effects.

The seemingly counterintuitive downregulation of human orthologue *MRE11* after exposure to gluten reported by van der Graaf *et al.* (2021) has been noted elsewhere in relation to DNA-repair scenarios. Tarapara & Shaw's (2025) study on DNA repair pathways in cancer patients described an upregulation of most damage repair genes under disease conditions - with the notable exception of *MRE11*, which was downregulated. Furthermore, their study demonstrated that reduced expression of *MRE11* was “associated with better overall survival” for patients. These findings emphasise that DNA repair gene expression cannot be interpreted linearly; reduced transcription may represent adaptive regulation rather than pathological suppression.

4.4 *XRCC1* and Oxidative Stress-Associated Repair

A non-significant trend toward *XRCC1* upregulation was observed following gluten exposure, with the gluten-only group demonstrating a more pronounced increase than that observed for *mre11*. Although this increase did not reach statistical significance, the directionality and relative magnitude of change are noteworthy. *XRCC1* functions within the base excision repair (BER) pathway, acting as a scaffolding protein that coordinates the activity of enzymes, endonucleases and polymerases involved in the repair of single-strand DNA breaks, particularly those arising from oxidative damage (Caldecott, 2019). The comparatively greater expression of *XRCC1* relative to *mre11* may therefore indicate prioritisation of repair mechanisms associated with oxidative base damage rather than widespread double-strand break formation.

When considered alongside concurrent upregulation of *mre11* and *Rel*, this pattern supports the interpretation that gluten exposure induces an inflammatory or oxidative environment capable of triggering genome maintenance pathways in the absence of extensive DNA damage. In this context, *XRCC1* upregulation appears consistent with adaptive stress reprogramming rather than acute DNA degradation.

Dashnamoorthy *et al.* (2009) argue that the course of damage response in *D. melanogaster* involves pathways with diverse biological functions beyond DNA repair alone, necessitating a systems-level perspective to understand the interplay between repair, survival, and metabolic signalling. Accordingly, interpretation of *XRCC1* expression is most informative when considered alongside other components of the BER pathway and related stress-regulatory networks such as Target of Rapamycin (TOR). While isolated transcriptional measurement cannot fully capture pathway activity, the coordinated trends observed here suggest that *XRCC1* modulation forms part of a broader stress-responsive transcriptional program.

In contrast, essentially no change in *XRCC1* expression was observed in human datasets following gluten exposure. This difference may reflect fundamental variations in immune architecture, tissue type, or pathological mechanisms. In celiac disease, tissue injury results primarily from cytokine-mediated epithelial damage and T-cell activation rather than direct damage to genetic material. Consequently, engagement of BER pathways may not represent a dominant transcriptional feature in human intestinal samples. These cross-species differences emphasise the need for caution in predicting translational similarities while highlighting the value of using model organisms for investigating mechanistic responses.

4.5 *Rel* and context-dependent immune activation

A moderate increase in *Rel* expression (fold change 1.94) was observed following gluten exposure; however, this did not reach statistical significance. While variability between biological replicates limits definitive conclusions, the magnitude of change suggests that gluten may engage innate immune signalling to some extent under the conditions tested. As *Rel* functions as a central transcription factor within the IMD pathway (De Gregorio et al., 2002), even modest upregulation may reflect early or low-grade activation of antimicrobial and inflammatory responses.

Probiotic supplementation produced a variety of effects on *Rel* expression. *C. casei* elicited a pronounced increase (3.43-fold), whereas the active blend and *C. variabile* demonstrated more moderate elevations (1.94 and 1.11 respectively), and *M. gubbeenense* resulted in slight downregulation (0.75). Although these changes were not statistically significant, the variability in both magnitude and direction indicates that immune signalling in this instance is highly responsive to microbial context. Rather than uniformly suppressing inflammation, probiotics appear capable of amplifying or attenuating immune responsiveness depending on strain composition.

When considered alongside modulation of *mre11* and *XRCC1*, the gluten-associated increase in *Rel* expression is consistent with a low-grade inflammatory environment that may contribute to oxidative stress and subsequent engagement of genome maintenance pathways. The comparatively greater upregulation observed with *C. casei* further supports the notion that microbial signals can enhance innate immune activation, potentially influencing downstream stress-response mechanisms. Whether such modulation is beneficial or detrimental depends on cellular context. *Rel* signalling can drive both pro-inflammatory and regulatory pathways, including induction of anti-inflammatory cytokines such as IL-10 (Ganesan et al., 2011). Elevated expression may therefore reflect immune activation rather than pathological overstimulation.

In human celiac disease, NF- κ B signalling is frequently reported to be elevated in response to gluten-mediated inflammation. For example, Maiuri et al. (2003) demonstrated sustained activation of NF- κ B in the intestinal mucosa of untreated patients, contributing to prolonged inflammatory gene expression. However, other studies report more variable findings. Van der Graaf et al. (2021) observed an average 8.5% decrease in *NFKB1* expression following gluten exposure, a result that appears counterintuitive given the gene's role in regulating innate and adaptive immune responses (Lougaris et al., 2016). Notably, Maiuri et al. (2003) also demonstrated that

NF- κ B activation is time-dependent, remaining elevated for several hours following gliadin exposure before declining. These findings suggest that accurate assessment of NF- κ B signalling is highly sensitive to sampling time and disease context.

Given that transcription was measured at a single time point in the present study, temporal fluctuations in *Rel* expression cannot be excluded and may partially account for both the absence of statistical significance and the variability observed across probiotic treatments. Differences in immune architecture, developmental stage, and tissue homogenisation further limit direct comparison between larval *Drosophila* and human intestinal mucosa. Nonetheless, the observed fold changes indicate that *Rel*-mediated signalling forms part of a broader, context-dependent transcriptional response to dietary and microbial stimuli.

4.6 *SlgA*, proline metabolism, and ER stress

Expression of *SlgA* increased by 47% relative to controls following gluten supplementation, suggesting that the introduction of gluten proteins into the diet was associated with increased transcription of genes related to proline metabolism. *SlgA* is not directly connected with immune signalling but participates in the breakdown of proline, a major component of gliadin. The observed upregulation may therefore reflect a metabolic response to increased proline availability or incomplete digestion of gluten peptides.

Under conditions where gliadin fragments accumulate, endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) may occur. ER stress is closely linked to oxidative imbalance, providing a plausible mechanistic bridge between altered proline metabolism, inflammatory signalling, and the activation of DNA repair pathways. Studies in inflammatory bowel disease and gluten-sensitive mouse models have demonstrated that gliadin exposure can induce chronic ER stress and disrupt epithelial barrier function (Rahmani et al., 2021; Ferrari et al., 2021). Additionally, Ferrari et al. (2021) also reported that administration of probiotics caused a reduction in both UPR activation and intestinal inflammation, suggesting that microbial modulation may influence ER-associated stress pathways.

In the present study, probiotic treatments produced variable effects on *SlgA* expression. While the Active diet group showed a slight elevation relative to controls, supplementation with *M. gubbeenense* resulted in statistically significant downregulation, while other strains moved toward baseline levels. The variability in responses suggests that different probiotic strains may influence proline metabolism or ER stress through distinct metabolic or signalling mechanisms, potentially via production of strain-specific metabolites.

Human data provide partial parallels. Van der Graaf et al. (2021) reported a marked increase in expression of the human orthologue *PRODH* in celiac disease patients, consistent with enhanced proline metabolism under inflammatory conditions. Proline dehydrogenase catalyses the conversion of proline to pyrroline-5-carboxylate, a reaction that can contribute to ATP generation under metabolic stress but may also influence reactive oxygen species production (Natarajan & Becker, 2012). Increased

PRODH expression has been associated with oxidative stress responses, suggesting that upregulation of proline metabolism may represent either adaptive metabolic compensation or part of a broader inflammatory cascade.

Although direct comparison between larval *Drosophila* and human intestinal tissue is limited by species and tissue differences, the observed modulation of *SlgA* expression supports the interpretation that gluten exposure affects metabolic and ER-associated stress pathways. When considered alongside changes in *Rel*, *mre11*, and *XRCC1* expression, *SlgA* upregulation therefore suggests coordinated stress adaptation rather than isolated immune activation.

4.7 Ethical considerations

In general, studies using flies as subjects are not restricted by the same ethical framework that would be applied to human patients. That being said, the biological materials employed in this experiment were derived from a previously completed project. Consequently, no live animals were handled during the course of this study - the larvae utilized were obtained from cold storage, thereby precluding the need to breed and sacrifice new specimens.

From the perspective of this experiment as a pilot study or a “proof of concept” approach, the small number of creatures used to investigate initial results can be considered an ideal choice. The number of biological replicates analysed was the minimum from which it was possible to draw any conclusions. Only six *Drosophila* larvae were sacrificed for each category in this study, which allows for some genetic diversity, although in a continuation study the number of biological replicates would need to be increased in order to collect statistical data from which to draw generalised conclusions. Despite *Drosophila* not being subject to the same ethical framework as humans there are still compelling reasons to try and limit the number of specimens sacrificed under research conditions. Cost-effectiveness is always a factor in experimental design, and maintaining an unnecessarily large population of flies would be a drain of time and resources that could be put to better use elsewhere. As living creatures *Drosophila* larvae have the capacity to experience pain and suffering, and minimizing the amount of pain inflicted upon any form of life is always a goal worth striving towards. Using the smallest quantity of individuals necessary in order to achieve satisfactory results is also a core tenant of the 3Rs set of ethical principles, which directs researchers regarding the humane use of animal test subjects.

Alternatives to using model organisms could be either *in vitro* studies or computational models, both of which have been used successfully in the recent past. Freire *et al.* (2019) were able to study the interplay of gut epithelial cells and metabolic secretions from microbiota regarding celiac disease pathogenesis. This team did so by creating CD-organoid monolayers which were exposed to gliadin, whereafter permeability characteristics and pro-inflammatory cytokine production were analysed. A similar approach to investigate molecular and cellular mechanisms related to gliadin exposure which would mimic the intestinal environment could be applied to *Drosophila*. A concept for an experimental setup might be to culture Schneider 2 cells for the creation of a monolayer. Cell medium containing gliadin could be added to the

culture, and after a sufficient incubation period several options would be available for analysis of the cellular reactions. For example, fluorescent microscopy using FITC-dextran could be used to investigate cell permeability, qPCR could be used to measure gene expression, or the medium itself could be analysed for the presence of specific *Drosophila* cytokines.

As an example of the second alternative, a computational study conducted in 2025 by McCreery *et al.* was able to use Constraint-Based Reconstruction and Analysis (COBRA) methods to predict patterns of nutrient absorption and metabolite secretion in celiac disease patients.

While the usefulness of these approaches is illustrated by these studies, they are not without drawbacks. A monolayer based approach to investigating the effects of probiotics would allow the use of human cell lines to visualise local effects but could not be used to study a systemic immune response or the ultimate consequences of nutrition deficiencies for the organism as a whole. Likewise, employing a monolayer approach with *Drosophila* cells would preclude the observation of systemic effects and the holistic impact of gluten on the organism. By using larvae, however, it is possible to assess the broader consequences of gluten intake beyond mere gene expression measurements. Investigating the systemic immune response throughout the organism may offer insights into reactions instigated by gliadin and similar proteins. However, it is crucial to consider that the *Drosophila* model lacks adaptive immunity, which may limit the translatability of findings to humans. Computational models rely on assumptions from previous data or studies, and results ultimately need to be validated against the outcomes of actual animal experimentation.

4.8 Implications for society and healthcare

The primary goal of experimenting with probiotics on *Drosophila* was to gain insights into the molecular mechanisms of action, with the hope that this knowledge would directly translate into useful information for human health and benefit. Because of the existence of conserved orthologous genes between the two species, there is potential for finding similarly structured pathways and processes. Time, organisational complexity, and, perhaps most importantly, cost are also important practical considerations. Effects can be more readily studied in fly models due to their short life cycle and, as mentioned earlier, the complete control over diet and environmental conditions that an experiment with animals affords.

A potential benefit from a study of this type would be the development of a personalised treatment scheme to increase the patient's quality of life. If knowledge were available regarding how particular probiotic supplements influence the expression of specific genes, a gene expression profile derived from a patient's gut tissue could be utilized to map the effects of these probiotics to specific differentially expressed genes and the biological pathways in which they are involved. This mapping could, in principal, facilitate the selection of which probiotics, and at what dosage, might be indicated for restoring the patient's gene expression levels to match those of a healthy control. To effectively implement such a treatment regimen, it would also be essential to obtain accurate information about the patient's medical history and dietary habits, as

well as detailed knowledge of the unique composition of the patient's gut microbiome. Additionally, environmental factors that may impact either the gut microbiota or gene expression should be identified. Furthermore, any known genetic variants carried by the patient that could influence gene expression or the patient's response to any suggested probiotic treatment should be considered.

One example of the use of personalised medicine to combat the effects of celiac disease can be found in the above-mentioned computational study by McCreery *et al.* (2025). The authors utilised the transcriptional profiles of small intestinal epithelial cells taken from CD patients to create genome-scale models of metabolism. The potential of a personalised medical approach has also been championed by Borrego-Ruiz & Borrego (2025) in their research, where they argue that shifting research towards approaches customised to the patient's unique “genetic, immunological, microbial and metabolic profiles” will result in improved therapeutic efficacy while also reducing incidences of adverse outcome. To apply a similar concept to experimentation with *Drosophila melanogaster*, several key steps would need to be undertaken. The workflow could commence with obtaining transcriptional profiles of the test animals under different dietary conditions. Here it would be advantageous to exclusively select gut tissue for analysis, as it is directly involved in metabolic processes and dietary responses. The collected data could then be used to create genome-scale metabolic models, illustrating how various metabolic pathways might be altered by changes in diet. By integrating this information with knowledge about the unique genetic and metabolic profiles of individual test subjects, researchers could gain insights into how these variations affect responses to treatment. Similarities between transcriptional or metabolic profiles in flies and humans could be identified to develop models for potential personalized therapeutic regimens. Understanding the underlying metabolic mechanisms and identifying therapeutic targets in *Drosophila* could provide valuable insights that translate to human health, ultimately contributing to the development of personalised medical approaches.

Aside from an enhancement in the quality of life of individual patients there are several other potential benefits from knowledge gained about the functions of probiotics in overall health. Understanding the role of the microbiota in ameliorating CD symptoms can lead to refinements in nutritional supplements as well as increasing general scientific knowledge about the role of the microbiome in disease presentation. Public health can be made more efficient and affordable through streamlining care with personalised approaches and using probiotics in a preventative manner. As Borrego-Ruiz & Borrego (2025) also mention, “integrating clinical models with microbial biomarkers” has great potential to reduce the financial burdens and feelings of social isolation experienced by these individuals.

4.9 Integrated stress-adaption framework

When considered collectively, the transcriptional patterns observed across *Rel*, *mre11*, *XRCC1*, *SlgA*, and *egr* suggest that gluten exposure induces coordinated physiological rebalancing rather than isolated pathway activation. Although not all individual gene changes reached statistical significance, consistent directional trends

across immune, repair, metabolic, and developmental markers indicate a structured biological response.

A moderate increase in *Rel* expression following gluten exposure is consistent with low-grade engagement of innate immune signalling, while concurrent upregulation of *mre11* and the comparatively stronger upregulation of *XRCC1* support activation of genome maintenance pathways. Given the role of *XRCC1* in base excision repair, particularly in resolving oxidative single-strand DNA damage, this pattern reinforces a model of mild inflammatory or oxidative stress rather than heavy cytotoxic stress. The integration of immune signalling and DNA repair suggests that gluten exposure may create conditions which activate protective maintenance mechanisms without indicating acute cellular damage.

In parallel, suppression of the developmental regulator *egr*, particularly under probiotic supplementation, indicates a shift in physiological priorities. The statistically significant downregulation observed in several probiotic groups supports the interpretation that microbial influences enhance the attenuation of growth-related signalling pathways. This type of alteration is consistent with adaptive stress reprogramming, where resources are temporarily redirected from developmental processes toward immune defence and cellular maintenance.

Probiotic treatments did not uniformly reverse gluten-associated changes; instead, they altered transcriptional patterns in strain-specific ways. Variable effects on *Rel* expression and consistent suppression of *egr* suggest that microbial signalling influences both immune response and developmental regulation. Rather than functioning solely as anti-inflammatory factors, probiotics appear to modify the balance between defence, repair, and growth pathways.

Collectively, these findings support a model in which gluten exposure promotes coordinated stress adaptation characterised by limited immune involvement, activation of repair mechanisms associated with oxidative damage, and suppression of developmental signalling. This systems-level perspective provides a unified framework through which the individual gene-specific observations can be interpreted.

4.10 Future perspectives and methodological considerations

Drosophila flies are, of course, vastly different organisms than humans and while there are similarities between the orthologous genes of the two species, both the immune and digestive systems function quite differently between the two. To further compound the situation, it needs to be emphasised that the RNA extracted for this experiment was taken from *D. melanogaster* larvae and not from adult flies. As the larvae represent a developmental stage in the *Drosophila* life cycle, it is not unreasonable to suspect that many cells or internal systems may have reacted differently to gluten exposure than would their fully developed adult counterparts. RNA used in this experiment was taken from homogenised soft tissue, meaning that results showed gene expression as an average for the organism, but did not offer any insight into the behavior of specific biological systems, including the gut. As mentioned earlier,

van der Graaf *et al.* (2021) used whole blood as the source of the DNA used in their study, which is a good representative tissue but is not ideal for all scenarios.

To use *SlgA* as an example, there was a significant upregulation with gluten exposure. When gluten was combined with the Active diet, the upregulation became less intense but failed to return to baseline conditions; with *C. variabile* there was a non-significant change in overall expression which was close to baseline measurements, whereas *M. gubbeenense* and *C. casei* appeared to have protective effects. Such a wide range of outcomes strongly suggests that for one and the same gene different probiotics must confer different overall effects, or similar effects in distinct ways, either due to production of diverse kinds of metabolites and molecules, or unequal amounts of the same types.

In hindsight there is one important category missing from this experiment which would have helped clarify experimental results - that of a control diet with probiotics but *not* gluten. In general, it is hard to draw concrete inferences about what effect the combination of gluten protein and probiotic may provide when the effect of a probiotic supplement alone is undetermined. As was seen in the case of *Rel*, it is uncertain whether the increase in transcription seen was a result of a reaction to gliadin protein or the components of the probiotics themselves. Additionally, a second methodological concern (not exclusive to the measurement of any single gene) is the reliance on self-reported data regarding food intake. There is no assurance that the information provided by study participants was entirely accurate, which could introduce bias into the findings.

A future version of this experiment should include quantification of cDNA using methods such as qPCR or spectrophotometry to enhance the reliability of the results.

An opportunity for future study might come in the form of a more cross-disciplinary approach to research. As this and other experiments have demonstrated, there is a very clear connection between genetics, nutrition and microbiology involved in the pathology of celiac disease. A collaborative study leveraging the expertise of specialists from each relevant field could be a compelling strategy. For example (and as an extension of personalised medicine), it could be interesting to investigate how patterns of tolerance or adaptation to probiotic supplementation over time and / or dosages of various strains could affect patients with specific haplotypes. It could also be pertinent to investigate if there are unintended, possibly unrecognised as of yet, long-term negative changes in the microbiome which accompany the short-term beneficial effects of probiotic usage. (Leeflang *et al.*, 2025).

4.11 Conclusions

This study investigated whether gluten exposure induces stress- and immune-related transcriptional changes in *Drosophila melanogaster* larvae and whether probiotic supplementation mitigates these effects. Overall, the findings partially support the original hypothesis.

Gluten supplementation consistently altered expression of all five genes examined. The observed pattern—upregulation of *Rel*, *mre11*, *XRCC1*, and *SlgA*,

alongside downregulation of *egr*—is most consistent with the induction of a coordinated adaptive stress response rather than acute toxicity. Immune activation, suggested by increased *Rel* expression, occurred in parallel with modulation of DNA repair genes (*mre11* and *XRCC1*) and increased expression of *SlgA*, which may reflect altered proline metabolism and endoplasmic reticulum stress. The concurrent suppression of *egr* supports the interpretation of a physiological shift away from growth and differentiation toward stress adaptation.

Probiotic supplementation did not uniformly restore transcriptional levels to baseline. Instead, strain-specific effects were observed, with some probiotics attenuating markers of stress while others appeared to enhance immune signalling. These findings suggest that probiotics modulate the balance between immune activation and cellular maintenance pathways rather than acting as simple anti-inflammatory agents.

Although certain parallels were observed between *Drosophila* genes and their human orthologues, biological differences between the two species make direct comparison difficult. The use of homogenated tissues and a limited number of biological replicates further restricts the strength of the findings. Future studies incorporating tissue-specific analysis, larger sample sizes, and additional control groups would strengthen understanding of results.

In summary, gluten exposure in larval *Drosophila melanogaster* was associated with changes in the expression of genes involved in immune signalling, DNA repair, metabolism, and developmental regulation. Although not all observed differences reached statistical significance, the overall directional patterns are consistent with a coordinated transcriptional response characterised by moderate immune engagement, activation of oxidative stress-associated repair pathways, and suppression of growth-related signalling. Probiotic supplementation did not uniformly restore gene expression to baseline conditions but instead modified the response in a strain-dependent manner, suggesting that microbial context influences transcriptional regulation under dietary stress.

Given the use of homogenated tissue, a single sampling time point, and a limited number of biological replicates, these findings should be interpreted with caution. Nonetheless, examining multiple functional gene categories within the same experimental framework provides a broader perspective on how gluten exposure may influence physiological balance. Further investigation incorporating tissue-specific analysis and expanded sample sizes would be necessary to clarify the extent and functional consequences of these transcriptional changes.

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6 Appendices

Appendix 1 RNA purity and concentration

SAMPLE	[ng/μl]	260/280	260/230
<i>C. casei</i> 1	258.177	2.201	1.463
<i>C. casei</i> 2	184.959	2.186	0.512
<i>C. casei</i> 3	344.818	2.254	2.459
<i>C. variabile</i> 4	360.882	2.267	2.130
<i>C. variabile</i> 5	264.152	2.237	1.888
<i>C. variabile</i> 6	344.543	2.271	2.553
<i>M. gubbeenense</i> 7	317.373	2.245	2.464
<i>M. gubbeenense</i> 8	105.278	2.279	1.415
<i>M. gubbeenense</i> 9	129.792	2.147	1.023
Active 10	188.091	2.245	2.045
Active 11	155.205	2.241	1.811
Active 12	200.415	2.246	2.548
Control 13	374.554	2.226	2.068
Control 14	392.748	2.216	1.296
Control 15	276.069	2.235	1.959
Gluten 16	282.817	2.194	1.928
Gluten 17	194.022	2.221	1.650
Gluten 18	289.310	2.259	2.420

Appendix 2 Raw data and fold changes for all experimental genes and human orthologues

Dm02362441_s1	AlphaTub6+					
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sample	Ct1	Ct2	Ct3	AVG Ct	Geometric mean Ct
control 13	29.71	29.69	29.58	29.66	25.35
control 14	30.36	30.28	30.22	30.29	24.82
control 15	29.46	29.75	29.27	29.49	24.40
c.casei 1	29.40	29.34	29.31	29.35	24.98
c.casei 2	30.37	30.09	30.30	30.25	24.98
c.casei 3	30.09	30.51	29.70	30.10	24.99
c.varia 4	30.72	30.32	30.87	30.64	25.13
c.varia 5	29.72	30.60	30.07	30.13	24.90
c.varia 6	30.53	30.67	30.57	30.59	25.52
Dm02151827_g1 Rpl32					
sample	Ct1	Ct2	Ct3	AVG Ct	
control 13	21.56	21.74	21.68	21.66	
control 14	20.36	20.28	20.39	20.34	
control 15	20.17	20.21	20.20	20.19	
c.casei 1	21.17	21.38	21.24	21.26	
c.casei 2	20.47	20.78	20.63	20.63	
c.casei 3	20.86	20.63	20.74	20.74	
c.varia 4	20.68	20.62	20.53	20.61	
c.varia 5	20.57	20.62	20.52	20.57	
c.varia 6	21.30	21.09	21.50	21.30	

Dm01832532_s1	Drs12	$p = 0.0630$								NO ORTHOLOGUE
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	$\Delta\Delta Ct$	$2^{\Delta-\Delta Ct}$	Fold Expression		
control 13	29.45	29.29	29.57	29.44	4.09	-0.71	1.64			
control 14	30.12	30.06	29.74	29.97	5.15	0.35	0.79			
control 15	29.52	29.55	29.66	29.58	5.17	0.37	0.78	Control	1.07	
c.casei 1	29.27	29.15	29.27	29.23	4.25	-0.56	1.47			

c.casei 2	38.05	29.8	30.12	32.66	7.68	2.87	0.14			
c.casei 3	30.09	30.04	30.17	30.1	5.11	0.31	0.81	c.casei	0.8	
c.varia 4	30.28	30.65	30.19	30.37	5.25	0.44	0.74			
c.varia 5	30.07	30.18	30.06	30.1	5.21	0.4	0.76			
c.varia 6	31.35	31.03	30.78	31.05	5.53	0.72	0.61	c.varia	0.7	
m.gubb 7	29.38	29.29	29.16	29.28	3.89	-0.91	1.88			
m.gubb 8	30.14	29.65	29.56	29.78	4.62	-0.18	1.14			
m.gubb 9	28.47	27.84	28.12	28.14	3.1	-1.7	3.25	m.gubb	2.09	
active 10	29.91	30.32	30.29	30.17	4.96	0.16	0.9			
active 11	30.77	30.39	30.83	30.66	5.38	0.57	0.67			
active 12	31.14	31.13	31.12	31.13	5.77	0.97	0.51	Active	0.69	
gluten 16	29.86	30.29	30.35	30.17	4.96	0.16	0.9			
gluten 17	30.32	30.43	32.54	31.1	6.27	1.46	0.36			
gluten 18	30.21	30.71	30.38	30.43	5.48	0.67	0.63	Gluten	0.63	
				AVG ΔCt	4.8					
Dm01793294_g1	GLS	<i>p</i> = 0.118								NO ORTHOLOGUE
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		
control 13	27.02	27.05	26.95	27.01	1.66	0.21	0.86			
control 14	25.99	25.88	25.85	25.91	1.08	-0.36	1.29			
control 15	26.26	25.9	25.85	26	1.6	0.15	0.9	Control	1.02	
c.casei 1	26.61	26.46	26.8	26.62	1.64	0.19	0.87			
c.casei 2	26.71	26.29	26.1	26.37	1.39	-0.06	1.04			
c.casei 3	27.15	27.04	26.54	26.91	1.92	0.47	0.72	c.casei	0.88	
c.varia 4	25.88	26	25.76	25.88	0.75	-0.7	1.62			
c.varia 5	27.04	27.15	26.9	27.03	2.13	0.69	0.62			
c.varia 6	27.16	27.07	26.95	27.06	1.54	0.09	0.94	c.varia	1.06	
m.gubb 7	28.3	27.5	27.32	27.71	2.32	0.87	0.55			
m.gubb 8	26.74	26.5	26.38	26.54	1.38	-0.07	1.05			
m.gubb 9	28.52	28.13	27.9	28.18	3.14	1.7	0.31	m.gubb	0.64	
active 10	26.55	26.67	26.4	26.54	1.33	-0.12	1.08			
active 11	26.35	26.24	26.25	26.28	1	-0.45	1.37			

active 12	25.7	25.43	25.52	25.55	0.19	-1.25	2.39	Active	1.61	
gluten 16	26.36	26.19	26.26	26.27	1.07	-0.38	1.3			
gluten 17	25.6	26.07	25.51	25.73	0.9	-0.55	1.46			
gluten 18	26.14	25.88	26.05	26.02	1.07	-0.38	1.3	Gluten	1.36	
				AVG ΔCt	1.45					
Dm01842882_m1	PI3K21B	<i>p</i> = 0.197								PIK3R2
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		1.680 (<i>p</i> = 0.016)
control 13	28.18	27.45	27.8	27.81	2.46	0.06	0.96			
control 14	27.03	27.02	27.09	27.05	2.22	-0.18	1.14			
control 15	27.04	26.94	26.84	26.94	2.54	0.13	0.92	Control	1	
c.casei 1	27.62	27.69	27.71	27.67	2.69	0.28	0.82			
c.casei 2	27.3	27.13	27.24	27.22	2.24	-0.17	1.12			
c.casei 3	27.54	27.69	27.7	27.64	2.66	0.25	0.84	c.casei	0.93	
c.varia 4	27.33	27.28	27.26	27.29	2.16	-0.25	1.19			
c.varia 5	28.07	27.67	27.54	27.76	2.86	0.46	0.73			
c.varia 6	27.85	27.75	28.16	27.92	2.4	-0.01	1.01	c.varia	0.97	
m.gubb 7	27.56	27.77	27.73	27.69	2.3	-0.11	1.08			
m.gubb 8	27.09	27.16	27.02	27.09	1.93	-0.48	1.4			
m.gubb 9	28.69	28.59	28.26	28.51	3.47	1.07	0.48	m.gubb	0.98	
active 10	27.21	27.25	27.53	27.33	2.12	-0.29	1.22			
active 11	27.08	27.01	26.95	27.01	1.73	-0.68	1.6			
active 12	26.81	27.06	27.14	27	1.65	-0.76	1.69	Active	1.51	
gluten 16	27.35	27.53	27.46	27.45	2.24	-0.16	1.12			
gluten 17	27.25	27.22	27.76	27.41	2.58	0.17	0.89			
gluten 18	26.44	26.74	27.04	26.74	1.79	-0.62	1.54	Gluten	1.18	
				AVG ΔCt	2.41					
Dm01795840_g1	XRCC1	<i>p</i> = 0.0081								XRCC1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.994 (<i>p</i> = 0.992)
control 13	30.16	30	29.81	29.99	4.64	-0.26	1.2			

control 14	29.82	29.58	29.33	29.58	4.75	-0.15	1.11			
control 15	30.06	29.54	29.55	29.72	5.31	0.41	0.75	Control	1.02	
c.casei 1	30.22	30.27	30.42	30.35	5.36	0.46	0.73			
c.casei 2	30.13	30.16	30.33	30.21	5.23	0.32	0.8			
c.casei 3	30.08	29.9	30.23	30.07	5.08	0.18	0.88	c.casei	0.8	
c.varia 4	30.21	30.62	30.25	30.36	5.23	0.33	0.8			
c.varia 5	29.86	30.06	30.14	30.02	5.12	0.22	0.86			
c.varia 6	30.43	30.75	30.62	30.6	5.08	0.17	0.89	c.varia	0.85	
m.gubb 7	30.98	31.25	30.77	31.01	5.62	0.72	0.61			
m.gubb 8	30.56	30.54	30.38	30.49	5.33	0.43	0.74			
m.gubb 9	31.22	31.55	31.39	31.39	6.35	1.44	0.37	m.gubb	0.57	
active 10	29.81	30.04	30.46	30.1	4.89	-0.01	1.01			
active 11	30.64	30.94	30.48	30.69	5.4	0.5	0.71			
active 12	30.92	31.04	30.71	30.89	5.53	0.63	0.65	Active	0.79	
gluten 16	26.51	26.6	26.21	26.41	1.2	-0.53	1.45			
gluten 17	26.39	26.64	26.45	26.49	1.66	-0.07	1.05			
gluten 18	26.44	26.31	26.37	26.37	1.42	-0.32	1.24	gluten	1.25	
				AVG ΔCt	1.73					
Dm01799281_g1	dlg1	<i>p</i> = 0.0041								DLG1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{ΔΔCt}	Fold Expression		0.958 (<i>p</i> = 0.925)
control 13	26.9	27.05	27.1	27.02	1.67	-0.06	1.05			
control 14	26.68	26.4	26.31	26.46	1.64	-0.09	1.07			
control 15	26.32	26.57	26	26.3	1.89	0.16	0.9	Control	1	
c.casei 1	33.12	27.43	27.25	27.34	2.36	0.62	0.65			
c.casei 2	27.14	27	26.63	26.92	1.94	0.21	0.87			
c.casei 3	26.66	27.89	26.4	26.98	2	0.26	0.83	c.casei	0.78	
c.varia 4	26.9	26.59	26.54	26.68	1.55	-0.19	1.14			
c.varia 5	26.69	26.66	26.59	26.65	1.75	0.02	0.99			
c.varia 6	26.74	27.02	27.02	26.93	1.4	-0.33	1.26	c.varia	1.13	
m.gubb 7	33.12	27.43	27.25	27.34	1.95	0.22	0.86			
m.gubb 8	27.14	27	26.63	26.92	1.76	0.03	0.98			

m.gubb 9	26.66	27.89	26.4	26.98	1.94	0.21	0.86	m.gubb	0.9	
active 10	26.9	26.59	26.54	26.68	1.47	-0.27	1.2			
active 11	26.69	26.66	26.59	26.65	1.36	-0.37	1.29			
active 12	26.74	27.02	27.02	26.93	1.57	-0.16	1.12	Active	1.21	
gluten 16	26.51	26.6	26.21	26.41	1.2	-0.53	1.45			
gluten 17	26.39	26.64	26.45	26.49	1.66	-0.07	1.05			
gluten 18	26.44	26.31	26.37	26.37	1.42	-0.32	1.24	gluten	1.25	
				AVG ΔCt	1.73					
Dm03419822_m1	Parp1	$p = 0.123$								PARP1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{ΔΔCt}	Fold Expression		0.832 ($p = 0.560$)
control 13	29.25	29.33	29.31	29.3	3.95	-0.28	1.22			
control 14	29.02	28.68	28.96	28.89	4.06	-0.17	1.13			
control 15	29.08	29.14	29.06	29.09	4.69	0.45	0.73	Control	1.02	
c.casei 1	29.46	29.43	29.47	29.45	4.47	0.24	0.85			
c.casei 2	28.81	29.01	29.07	28.96	3.98	-0.25	1.19			
c.casei 3	29.19	29.27	29.12	29.19	4.21	-0.03	1.02	c.casei	1.02	
c.varia 4	29.57	29.54	29.33	29.48	4.35	0.12	0.92			
c.varia 5	29.25	29.29	29.35	29.3	4.4	0.17	0.89			
c.varia 6	29.7	29.69	29.74	29.71	4.19	-0.05	1.03	c.varia	0.95	
m.gubb 7	29.46	29.43	29.47	29.45	4.07	-0.17	1.12			
m.gubb 8	28.81	29.01	29.07	28.96	3.8	-0.43	1.35			
m.gubb 9	29.19	29.27	29.12	29.19	4.15	-0.08	1.06	m.gubb	1.18	
active 10	29.57	29.54	29.33	29.48	4.27	0.04	0.98			
active 11	29.25	29.29	29.35	29.3	4.01	-0.22	1.17			
active 12	NaN	30.62	29.27	29.95	4.59	0.35	0.78	Active	0.97	
gluten 16	28.75	29	28.57	28.77	3.57	-0.66	1.59			
gluten 17	28.62	28.73	28.74	28.7	3.87	-0.37	1.29			
gluten 18	28.86	29.17	28.7	28.91	3.96	-0.28	1.21	gluten	1.36	
				AVG ΔCt	4.23					
Dm02136251_g1	CASK	$p = 0.0902$								CASK

sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	$\Delta\Delta Ct$	$2^{-\Delta\Delta Ct}$	Fold Expression		1.090 ($p = 0.832$)
control 13	27.43	27.3	27.41	27.38	2.03	-0.15	1.11			
control 14	27.02	26.62	26.66	26.77	1.94	-0.24	1.18			
control 15	27.26	26.56	27.11	26.98	2.57	0.39	0.76	Control	1.02	
c.casei 1	27.79	27.39	27.51	27.56	2.58	0.4	0.76			
c.casei 2	27.76	27.42	NaN	27.59	2.61	0.43	0.74			
c.casei 3	27.32	27.33	27.56	27.4	2.42	0.23	0.85	c.casei	0.78	
c.varia 4	27.04	27.22	27.1	27.12	1.99	-0.19	1.14			
c.varia 5	27.36	27.2	27.24	27.27	2.37	0.19	0.88			
c.varia 6	27.54	27.34	27.5	27.46	1.94	-0.25	1.19	c.varia	1.07	
m.gubb 7	27.79	27.39	27.51	27.56	2.18	-0.01	1			
m.gubb 8	27.76	27.42	NaN	27.59	2.43	0.24	0.84			
m.gubb 9	27.32	27.33	27.56	27.4	2.36	0.18	0.88	m.gubb	0.91	
active 10	27.04	27.22	27.1	27.12	1.91	-0.27	1.21			
active 11	27.36	27.2	27.24	27.27	1.98	-0.2	1.15			
active 12	27.54	27.34	27.5	27.46	2.1	-0.08	1.06	Active	1.14	
gluten 16	27.13	27.05	27.42	27.2	2	-0.19	1.14			
gluten 17	27.02	27.18	26.94	27.05	2.22	0.03	0.98			
gluten 18	27.3	27.34	27.4	27.35	2.39	0.21	0.86	gluten	0.99	
				AVG ΔCt	2.18					
Dm02143923_g1	Mettl3	$p = 0.517$								METTL3
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	$\Delta\Delta Ct$	$2^{-\Delta\Delta Ct}$	Fold Expression		0.705 ($p = 0.130$)
control 13	30.16	30.22	30.31	30.23	4.88	-0.14	1.1			
control 14	29.55	29.55	29.67	29.59	4.77	-0.25	1.19			
control 15	30.05	29.66	29.72	29.81	5.41	0.39	0.76	Control	1.02	
c.casei 1	31.14	30.82	31.22	31.06	6.08	1.06	0.48			
c.casei 2	29.95	30.2	30.29	30.15	5.17	0.15	0.9			
c.casei 3	30.53	29.88	30.16	30.19	5.2	0.18	0.88	c.casei	0.75	
c.varia 4	30.22	30.29	30.36	30.29	5.16	0.14	0.91			
c.varia 5	30.78	30.45	30.28	30.5	5.61	0.59	0.66			

c.varia 6	30.52	30.15	32.58	31.08	5.56	0.54	0.69	c.varia	0.75	
m.gubb 7	31.14	30.82	31.22	31.06	5.67	0.65	0.64			
m.gubb 8	29.95	30.2	30.29	30.15	4.98	-0.04	1.03			
m.gubb 9	30.53	29.88	30.16	30.19	5.15	0.13	0.91	m.gubb	0.86	
active 10	30.22	30.29	30.36	30.29	5.08	0.06	0.96			
active 11	30.78	30.45	30.28	30.5	5.22	0.2	0.87			
active 12	30.52	30.15	32.58	31.08	5.73	0.71	0.61	Active	0.81	
gluten 16	29.67	29.89	29.88	29.81	4.61	-0.41	1.33			
gluten 17	30.04	30	30.02	30.02	5.19	0.17	0.89			
gluten 18	30.42	30.35	30.15	30.31	5.35	0.33	0.79	gluten	1	
				AVG ΔCt	5.02					
Dm01821494_g1	Fen1	$p = <0.001$								FEN1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.549 ($p = 0.202$)
control 13	29.05	28.94	28.83	28.94	3.59	-0.28	1.22			
control 14	28.49	28.51	29.67	28.89	4.07	0.19	0.88			
control 15	28.47	28.55	28.1	28.37	3.97	0.09	0.94	Control	1.01	
c.casei 1	31.32	28.93	29.02	28.98	3.99	0.12	0.92			
c.casei 2	29.3	29.1	28.94	29.11	4.13	0.26	0.84			
c.casei 3	28.87	29.23	28.74	28.95	3.96	0.08	0.94	c.casei	0.9	
c.varia 4	29.16	29.07	29.02	29.08	3.96	0.08	0.95			
c.varia 5	28.73	28.56	28.51	28.6	3.7	-0.17	1.13			
c.varia 6	29.29	29.27	29.14	29.23	3.71	-0.17	1.12	c.varia	1.07	
m.gubb 7	29.4	29.3	29.58	29.44	4.05	0.98	0.51			
m.gubb 8	29.21	28.88	28.9	29	3.83	0.76	0.59			
m.gubb 9	28.91	29.5	28.65	29.02	3.98	0.9	0.53	m.gubb	0.55	
active 10	29.12	28.76	28.71	28.86	3.65	0.58	0.67			
active 11	29.22	29.18	28.92	29.11	3.82	0.74	0.6			
active 12	29.28	29.3	29.5	29.36	4	0.93	0.53	Active	0.6	
gluten 16	28.75	28.74	28.44	28.59	3.39	-0.49	1.4			
gluten 17	28.33	28.47	28.35	28.38	3.55	-0.32	1.25			
gluten 18	28.2	28.23	28.29	28.24	3.29	-0.59	1.51	gluten	1.39	

				AVG ΔCt	3.88					
Dm01822597_m1	CycA	<i>p</i> = 0.315								CCNA2
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.488 (<i>p</i> = 0.042)
control 13	28.83	28.6	28.85	28.76	3.41	-0.45	1.37			
control 14	28.67	28.11	28.02	28.27	3.44	-0.42	1.34			
control 15	29.09	29.08	29.27	29.15	4.74	0.88	0.55	Control	1.08	
c.casei 1	29.64	29.51	29.71	29.62	4.64	0.77	0.59			
c.casei 2	29.1	29.28	29.12	29.17	4.19	0.32	0.8			
c.casei 3	29.22	29.11	29.32	29.22	4.23	0.36	0.78	c.casei	0.72	
c.varia 4	29.48	29.71	29.93	29.71	4.58	0.71	0.61			
c.varia 5	29.7	29.79	29.73	29.74	4.84	0.98	0.51			
c.varia 6	29.69	29.96	30.03	29.89	4.37	0.5	0.71	c.varia	0.61	
m.gubb 7	29.59	29.79	29.69	29.69	4.3	0.44	0.74			
m.gubb 8	29.63	29.23	29.11	29.32	4.16	0.29	0.82			
m.gubb 9	30.77	30.58	30.98	30.78	5.74	1.87	0.27	m.gubb	0.61	
active 10	28.9	29.12	28.95	28.99	3.78	-0.09	1.06			
active 11	29.65	29.71	29.84	29.73	4.45	0.58	0.67			
active 12	29.66	29.9	29.93	29.83	4.47	0.61	0.66	Active	0.8	
gluten 16	28.93	29.22	29.25	29.13	3.93	0.06	0.96			
gluten 17	28.77	28.84	29.09	28.9	4.07	0.2	0.87			
gluten 18	29.77	29.58	29.48	29.61	4.66	0.79	0.58	gluten	0.8	
				AVG ΔCt	3.87					
Dm01809600_g1	Mettl14	<i>p</i> = 0.0427								METTL14
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.949 (<i>p</i> = 0.920)
control 13	31.29	31.11	31.09	31.16	5.82	0	1			
control 14	31.01	30.22	30.34	30.52	5.7	-0.11	1.08			
control 15	30.59	30.14	30.25	30.33	5.92	0.11	0.93	Control	1	
c.casei 1	31.72	31.63	31.29	31.55	6.57	0.75	0.59			
c.casei 2	31.45	30.94	30.68	31.02	6.04	0.23	0.85			

c.casei 3	31.01	30.97	30.6	30.86	5.87	0.06	0.96	c.casei	0.8	
c.varia 4	31.13	31.65	31.04	31.27	6.15	0.33	0.79			
c.varia 5	31.51	30.94	31.14	31.2	6.3	0.49	0.71			
c.varia 6	31.82	31.63	31.45	31.63	6.11	0.3	0.81	c.varia	0.77	
m.gubb 7	32.1	31.83	31.84	31.92	6.54	0.72	0.61			
m.gubb 8	31.8	31.55	31.34	31.56	6.4	0.59	0.67			
m.gubb 9	32.04	31.51	31.14	31.56	6.52	0.71	0.61	m.gubb	0.63	
active 10	31.2	31.01	31.03	31.08	5.87	0.06	0.96			
active 11	31.63	31.73	31.58	31.65	6.36	0.55	0.68			
active 12	31.69	31.6	31.14	31.48	6.12	0.31	0.81	Active	0.82	
gluten 16	28.98	29.1	29.15	29.08	3.87	-0.29	1.22			
gluten 17	29.06	29.04	28.25	28.78	3.95	-0.21	1.16			
gluten 18	29.5	29.79	29.3	29.53	4.58	0.41	0.75	gluten	1.04	
				AVG ΔCt	4.16					
Dm02142679_g1	PI3K92E	p = 0.0964								PIK3CD
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.804 (p = 0.643)
control 13	26.83	27.08	27.05	26.99	1.64	-0.16	1.12			
control 14	27.07	26.49	26.71	26.76	1.93	0.13	0.91			
control 15	26.18	26.48	26.05	26.24	1.83	0.03	0.98	Control	1	
c.casei 1	27.27	27.39	27.3	27.35	2.36	0.56	0.68			
c.casei 2	27.16	26.74	26.53	26.81	1.83	0.03	0.98			
c.casei 3	26.5	26.74	26.33	26.52	1.54	-0.27	1.2	c.casei	0.95	
c.varia 4	27.63	27.12	27.92	27.56	2.43	0.63	0.65			
c.varia 5	26.76	26.94	26.84	26.85	1.95	0.15	0.9			
c.varia 6	27.09	27.44	27.43	27.32	1.8	-0.01	1	c.varia	0.85	
m.gubb 7	27.87	27.71	27.86	27.79	2.4	0.6	0.66			
m.gubb 8	28.07	28.01	28.01	28.03	2.87	1.06	0.48			
m.gubb 9	28.51	28.93	28.47	28.64	3.6	1.8	0.29	m.gubb	0.48	
active 10	26.98	26.65	26.49	26.71	1.5	-0.3	1.24			
active 11	27.44	27.36	27.35	27.38	2.1	0.3	0.81			
active 12	27.03	27	26.73	26.92	1.56	-0.24	1.18	Active	1.08	

gluten 16	26.23	26.13	25.55	25.84	0.64	-1.17	2.24			
gluten 17	26.31	26.52	26.72	26.52	1.69	-0.12	1.08			
gluten 18	26.77	26.84	26.89	26.83	1.88	0.08	0.95	gluten	1.43	
				AVG ΔCt	1.8					
Dm01819480_g1	metro	$p =$ 0.0191								MPP3
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	$\Delta\Delta Ct$	$2^{\Delta\Delta Ct}$	Fold Expression		0.697 ($p = 0.643$)
control 13	30.04	29.96	30.03	30.01	4.66	0.22	0.86			
control 14	29.34	28.88	28.67	28.96	4.14	-0.3	1.23			
control 15	28.82	28.75	29.2	28.92	4.52	0.08	0.95	Control	1.01	
c.casei 1	30.13	30.07	30.03	30.08	5.1	0.65	0.64			
c.casei 2	29.92	29.68	29.49	29.7	4.72	0.27	0.83			
c.casei 3	29.75	29.42	30.09	29.75	4.77	0.32	0.8	c.casei	0.75	
c.varia 4	29.48	29.64	29.76	29.63	4.5	0.06	0.96			
c.varia 5	29.83	29.46	29.66	29.65	4.75	0.31	0.8			
c.varia 6	29.58	29.8	29.98	29.79	4.26	-0.18	1.13	c.varia	0.97	
m.gubb 7	30.46	30.25	30.31	30.34	4.95	0.51	0.7			
m.gubb 8	30.25	30.03	29.73	30	4.84	0.4	0.76			
m.gubb 9	31.19	31.04	31.35	31.19	6.15	1.71	0.3	m.gubb	0.59	
active 10	29.05	29.61	29.41	29.36	4.15	-0.29	1.23			
active 11	29.68	29.66	30.04	29.79	4.51	0.07	0.95			
active 12	29.45	29.77	29.91	29.71	4.35	-0.09	1.06	Active	1.08	
gluten 16	28.53	29.06	29.1	28.9	3.69	-0.75	1.68			
gluten 17	29.04	28.98	29.17	29.06	4.23	-0.21	1.15			
gluten 18	29.25	29.23	29.41	29.3	4.34	-0.1	1.07	gluten	1.3	
				AVG ΔCt	4.44					
Dm01835862_s1	CG32368	$p =$ 0.0756								NO ORTHOLOGUE
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	$\Delta\Delta Ct$	$2^{\Delta\Delta Ct}$	Fold Expression		
control 13	30.46	30.83	30.64	30.64	5.3	-0.26	1.2			
control 14	30.67	30.26	30.28	30.4	5.58	0.03	0.98			

control 15	30.59	30.16	29.82	30.19	5.79	0.23	0.85	Control	1.01	
c.casei 1	30.53	30.52	30.32	30.46	5.48	-0.08	1.06			
c.casei 2	31.2	31.02	30.8	31.01	6.03	0.47	0.72			
c.casei 3	31.32	30.79	30.92	31.01	6.02	0.47	0.72	c.casei	0.83	
c.varia 4	31.82	31.47	31.37	31.55	6.43	0.87	0.55			
c.varia 5	31.42	31.14	31.09	31.22	6.32	0.77	0.59			
c.varia 6	32.32	32	32.23	32.18	6.66	1.1	0.46	c.varia	0.53	
m.gubb 7	30.44	30.55	30.22	30.4	5.02	-0.54	1.45			
m.gubb 8	31.15	31.02	31.1	31.09	5.93	0.37	0.77			
m.gubb 9	29.41	29.1	29.05	29.19	4.15	-1.41	2.65	m.gubb	1.63	
active 10	31.48	31.34	31.35	31.39	6.18	0.63	0.65			
active 11	31.74	31.84	31.73	31.77	6.49	0.93	0.52			
active 12	32.35	32.31	31.78	32.15	6.79	1.24	0.42	Active	0.53	
gluten 16	30.48	30.69	30.44	30.54	5.33	-0.22	1.17			
gluten 17	31.43	31.43	30.91	31.26	6.43	0.87	0.55			
gluten 18	31.28	31.32	31.03	31.21	6.26	0.7	0.61	gluten	0.78	
				AVG ΔCt	5.55					
Dm02139557_g1	lig3	<i>p</i> = 0.0004								LIG3
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{ΔΔCt}	Fold Expression		0.807 (<i>p</i> = 0.378)
control 13	29.23	29.31	29.33	29.29	3.94	0.18	0.88			
control 14	28.35	28.58	28.02	28.32	3.49	-0.26	1.2			
control 15	28.22	28.5	28.01	28.24	3.84	0.08	0.95	Control	1.01	
m.gubb 7	29.31	29.39	29.44	29.38	3.99	0.23	0.85			
m.gubb 8	29.59	29.37	29.39	29.45	4.29	0.53	0.69			
m.gubb 9	30.15	30.74	30.41	30.43	5.39	1.64	0.32	m.gubb	0.62	
active 10	28.89	29.01	28.35	28.75	3.54	-0.22	1.16			
active 11	28.44	28.7	28.44	28.53	3.24	-0.52	1.43			
active 12	28.6	28.71	28.63	28.65	3.29	-0.47	1.38	Active	1.33	
gluten 16	28.29	27.88	28.02	28.06	2.86	-0.9	1.87			
gluten 17	28.01	27.79	27.81	27.87	3.04	-0.72	1.65			
gluten 18	27.89	27.76	27.74	27.8	2.84	-0.92	1.89	Gluten	1.8	

				AVG ΔCt	3.76					
Dm01794373_m1	egr	<i>p</i> = 0.0046								EDA
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.778 (<i>p</i> = 0.755)
control 13	26.87	26.87	26.98	26.91	1.56	-0.4	1.32			
control 14	26.75	27.02	26.87	26.88	2.06	0.1	0.94			
control 15	26.72	26.67	26.62	26.67	2.27	0.3	0.81	Control	1.02	
c.casei 1	28.45	28.17	28.44	28.35	3.37	1.41	0.38			
c.casei 2	27.84	27.71	27.62	27.72	2.74	0.78	0.58			
c.casei 3	28.15	28.12	28.26	28.18	3.19	1.23	0.43	c.casei	0.46	
c.varia 4	28.36	28.39	28.58	28.44	3.32	1.35	0.39			
c.varia 5	27.97	27.93	28.01	27.97	3.07	1.11	0.46			
c.varia 6	28.2	28.14	28.32	28.22	2.7	0.73	0.6	c.varia	0.48	
m.gubb 7	28.7	28.42	28.46	28.53	3.14	1.18	0.44			
m.gubb 8	28.26	28.38	28.2	28.28	3.12	1.16	0.45			
m.gubb 9	29.5	29.68	29.75	29.64	4.6	2.64	0.16	m.gubb	0.35	
active 10	27.51	27.73	27.39	27.54	2.33	0.37	0.77			
active 11	27.97	28.18	28.18	28.11	2.83	0.86	0.55			
active 12	28.04	28.01	28.2	28.08	2.73	0.77	0.59	Active	0.64	
gluten 16	27.54	27.57	27.21	27.44	2.24	0.28	0.83			
gluten 17	27.16	27.19	27.19	27.18	2.35	0.39	0.76			
gluten 18	27.61	27.71	27.84	27.72	2.77	0.8	0.57	gluten	0.72	
				AVG ΔCt	1.96					
Dm01840723_s1	PGRP- SD	<i>p</i> = 0.1602								PGLYRP1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		No data reported
control 13	29.25	29.49	29.28	29.34	3.99	0.05	0.97			
control 14	28.43	28.69	28.75	28.62	3.8	-0.15	1.11			
control 15	28.54	28.39	28.42	28.45	4.05	0.1	0.93	Control	1	
c.casei 1	29.63	29.77	29.7	29.7	4.72	0.77	0.59			
c.casei 2	29.13	29.43	29.61	29.39	4.41	0.46	0.73			

c.casei 3	29.67	29.23	29.52	29.47	4.49	0.54	0.69	c.casei	0.67	
c.varia 4	30.05	30.08	30.05	30.06	4.93	0.98	0.51			
c.varia 5	30.11	29.8	30.15	30.02	5.12	1.18	0.44			
c.varia 6	30.3	30.6	30.97	30.62	5.1	1.15	0.45	c.varia	0.47	
m.gubb 7	29.55	30.33	30.01	29.96	4.58	0.63	0.65			
m.gubb 8	29.54	29.51	29.73	29.59	4.43	0.48	0.72			
m.gubb 9	28.98	28.96	29.07	29	3.96	0.02	0.99	m.gubb	0.78	
active 10	29.48	29.39	29.4	29.42	4.21	0.27	0.83			
active 11	30.12	30.06	30.17	30.12	4.83	0.89	0.54			
active 12	29.88	30.12	30.51	30.17	4.81	0.87	0.55	Active	0.64	
gluten 16	28.26	28.29	28.24	28.26	3.06	-0.89	1.85			
gluten 17	29.46	28.98	29.1	29.18	4.35	0.4	0.76			
gluten 18	29.25	29.44	29.78	29.49	4.54	0.59	0.66	gluten	1.09	
				AVG ΔCt	3.95					
Dm01817703_g1	mre11	p = 0.0387								MRE11
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.749 (p = 0.309)
control 13	29.31	29.6	29.25	29.39	4.04	-0.12	1.09			
control 14	28.98	28.92	28.68	28.86	4.04	-0.12	1.09			
control 15	29.01	28.78	28.65	28.81	4.41	0.25	0.84	Control	1.01	
c.casei 1	30.53	30.03	29.77	30.11	5.13	0.97	0.51			
c.casei 2	30.03	30.04	29.88	29.98	5	0.84	0.56			
c.casei 3	NaN	29.49	29.27	29.38	4.39	0.23	0.85	c.casei	0.64	
c.varia 4	30.11	30.17	30.2	30.16	5.03	0.87	0.55			
c.varia 5	29.67	29.85	29.56	29.69	4.8	0.64	0.64			
c.varia 6	30.01	30.09	29.9	30	4.48	0.31	0.8	c.varia	0.67	
m.gubb 7	30.53	30.03	29.77	30.11	4.72	0.56	0.68			
m.gubb 8	NaN	NaN	29.65	29.65	4.49	0.32	0.8			
m.gubb 9	NaN	29.49	29.27	29.38	4.34	0.18	0.88	m.gubb	0.79	
active 10	30.11	30.17	30.2	30.16	4.95	0.79	0.58			
active 11	29.67	29.85	29.56	29.69	4.41	0.25	0.84			
active 12	30.01	30.09	29.9	30	4.64	0.48	0.72	Active	0.71	

gluten 16	28.98	29.1	29.15	29.08	3.87	-0.29	1.22			
gluten 17	29.06	29.04	28.25	28.78	3.95	-0.21	1.16			
gluten 18	29.5	29.79	29.3	29.53	4.58	0.41	0.75	gluten	1.04	
				AVG ΔCt	4.16					
Dm02134843_g1	Rel	$p =$ 0.1464								NFKB1
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		0.915 ($p = 0.816$)
control 13	25.81	25.69	25.78	25.76	0.41	-0.1	1.08			
control 14	25.57	25.44	25.47	25.49	0.67	0.15	0.9			
control 15	25.3	24.58	24.74	24.87	0.47	-0.05	1.03	Control	1	
c.casei 1	24.69	24.64	24.48	24.6	0.38	-0.9	1.86			
c.casei 2	23.78	23.92	23.8	23.83	1.15	-1.67	3.17			
c.casei 3	23.12	22.98	23.23	23.11	1.88	-2.4	5.26	c.casei	3.43	
c.varia 4	25.6	25.81	25.51	25.64	0.51	-0.01	1			
c.varia 5	24.67	24.86	24.59	24.71	0.19	-0.71	1.63			
c.varia 6	26.44	26.66	26.52	26.54	1.02	0.5	0.71	c.varia	1.11	
m.gubb 7	25.79	25.87	25.67	25.78	0.39	-0.13	1.09			
m.gubb 8	27.1	27.05	27.08	27.08	1.91	1.4	0.38			
m.gubb 9	25.96	26.03	25.73	25.91	0.87	0.35	0.78	m.gubb	0.75	
active 10	24.57	24.65	24.49	24.57	0.64	-1.16	2.23			
active 11	26.34	26.46	26.32	26.37	1.09	0.57	0.67			
active 12	26.33	25.58	25.54	25.82	0.46	-0.06	1.04	Active	1.31	
gluten 16	23.58	23.56	23.58	23.57	1.63	-2.15	4.43			
gluten 17	25.82	25.88	25.18	25.63	0.8	0.28	0.82			
gluten 18	26.25	26.41	26.21	26.29	1.34	0.82	0.57	gluten	1.94	
				AVG ΔCt	0.52					
Dm01799725_m1	SlgA	$p =$ 0.0007								PRODH
sample	Ct1	Ct2	Ct3	AVG Ct	ΔCt	ΔΔCt	2 ^{Δ-ΔCt}	Fold Expression		5.01 ($p = 7.92e-08$)

control 13	27.51	27.33	27.29	27.38	2.03	0.29	0.82		
control 14	26.33	26.31	26.39	26.34	1.52	-0.22	1.17		
control 15	26.12	26	26.12	26.08	1.68	-0.07	1.05	Control	1.01
c.casei 1	27.47	37.5	27.67	27.57	2.59	0.85	0.56		
c.casei 2	26.79	26.65	26.89	26.78	1.8	0.05	0.96		
c.casei 3	26.67	26.64	26.94	26.75	1.76	0.02	0.99	c.casei	0.97
c.varia 4	26.67	26.89	26.9	26.82	1.69	-0.05	1.04		
c.varia 5	26.8	26.95	27.21	26.99	2.09	0.35	0.79		
c.varia 6	27.24	27.12	27.48	27.28	1.76	0.01	0.99	c.varia	0.94
m.gubb 7	27.91	27.86	28.14	27.97	2.58	0.84	0.56		
m.gubb 8	27.33	27.14	27.13	27.2	2.04	0.29	0.82		
m.gubb 9	27.98	28	28.03	28	2.96	1.22	0.43	m.gubb	0.6
active 10	26.3	26.92	26.35	26.52	1.31	-0.43	1.35		
active 11	26.87	26.65	27.09	26.87	1.59	-0.16	1.11		
active 12	26.73	26.86	26.74	26.78	1.42	-0.32	1.25	Active	1.24
gluten 16	26.21	26.36	26.2	26.26	1.05	-0.69	1.61		
gluten 17	25.8	26.25	26.28	26.11	1.28	-0.46	1.38		
gluten 18	26	26.34	26.23	26.19	1.24	-0.51	1.42	Gluten	1.47
				AVG ΔCt	1.74				