

The Immune Enchaining Role of Dietary Sulforaphane on the Major Histocompatibility Complex BF-2 (MHC BF-2) Gene Expression as Feed Additives on Broilers

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ABSTRACT

This study investigated the immune-enhancing effects of dietary sulforaphane (SFN) on the Major Histocompatibility Complex BF-2 (MHC BF-2) gene expression in broiler chickens exposed to Aflatoxin B₁ (AFB₁), a known immunosuppressive mycotoxin. One hundred one-day-old Cobb 500 broilers were divided into five groups: a negative control (no AFB₁ or SFN), a positive control (AFB₁ only), and three SFN-treated groups (10, 20, and 30 mg/kg SFN with 1 mg/kg AFB₁). At day 42, blood samples were analyzed for immunoglobulin levels (IgA, IgY) and BF-2 gene expression using quantitative real time PCR (qPCR). Results showed that AFB₁ significantly suppressed BF-2 expression and reduced IgA and IgY levels ($p < 0.01$). SFN supplementation reversed these effects dose-dependently, with the 20 mg/kg SFN group exhibiting the highest immune enhancement, 1.8-fold increase in BF-2 expression and elevated immunoglobulins ($p < 0.01$). RNA integrity was preserved in the SFN-treated groups, particularly at a dose of 20 mg/kg. Strong correlations were observed between BF-2 expression, humoral immunity, and CD8+ T-cell counts. These findings suggest that SFN, especially at 20 mg/kg, mitigates AFB₁-induced immunosuppression by enhancing immune gene expression, likely via Nrf2/NF- κ B pathways, positioning it as a promising natural feed additive for poultry.

Keywords: Sulforaphane; Broilers, Aflatoxin B₁ (AFB₁); BF-2 gene expression; Immune modulation; MHC class I; qPCR.

INTRODUCTION

The poultry industry continues to seek innovative and sustainable strategies to enhance immune function and overall health in broiler chickens, especially in light of increasing challenges such as disease outbreaks and growing restrictions on antibiotic usage (Kogut and Klasing, 2009). According to the Food and Agriculture Organization (FAO, 2013), broilers are the most common and widely distributed domestic animals used for meat production globally. Their diets are predominantly grain-based, supplemented with various feed additives to improve biological activity, immune responses, and growth performance.

In recent years, dietary supplements capable of modulating immune responses have gained significant attention as potential alternatives to conventional antibiotic growth promoters (Gadde *et al.*, 2017). Among these, sulforaphane (SFN), a bioactive isothiocyanate compound derived from cruciferous vegetables, has exhibited potent immunomodulatory, antioxidant, and anti-inflammatory properties in both mammalian and avian studies (Wang *et al.*, 2018). However, its effects on avian immune gene expression, particularly within the Major Histocompatibility Complex (MHC), remain underexplored.

The MHC plays a crucial role in adaptive immunity by presenting antigens to T-cells, thereby initiating targeted immune responses. In chickens, the BF-2 gene, a component of MHC class I, is essential for pathogen recognition and disease resistance. Increased expression of the BF-2 gene has been associated with improved resistance to viral and bacterial infections in poultry. Recent findings suggest that phytochemical compounds, including SFN, may upregulate MHC-related genes through the modulation of key signaling pathways such as Nrf2 (a central regulator of antioxidant defense mechanisms) and NF- κ B (a major mediator of inflammatory responses) (Chappell *et al.*, 2015 and Surai *et al.*, 2019).

Poultry health and productivity are increasingly threatened by feed contamination with mycotoxins, particularly Aflatoxin B₁ (AFB₁), which is known to suppress immune function by down regulating critical immune genes such as BF-2 (Yunus *et al.*, 2011). With the global movement toward reducing antibiotic use in livestock production, identifying natural feed additives capable of counteracting AFB₁-induced immunosuppression is of growing importance. Sulforaphane has shown promising immunoprotective effects in other models through modulation of the Nrf2/NF- κ B pathway (Mahn & Castillo, 2021 and Suleyman, 2025), but its potential to alleviate AFB₁-induced immunosuppression in broilers has not yet been evaluated.

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This study therefore aims to investigate the protective effects of dietary SFN supplementation against AFB₁-induced suppression of BF-2 gene expression in broiler chickens through evaluating the dose-dependent effects of SFN on the expression of the BF-2 gene.

By establishing a link between SFN-induced upregulation of BF-2 and enhanced immune parameters, this study seeks to provide a scientifically validated phytogetic alternative to antibiotics in poultry production systems challenged by mycotoxins.

MATERIALS AND METHODS

1. Experimental Design and Animal Handling:

A total of 100 one-day-old Cobb 500 broiler chicks were obtained from a commercial hatchery in the El Nubaria region near Alexandria, Egypt. Upon arrival, chicks were housed in prepared pens under controlled laboratory conditions for a 5-day adaptation period, maintained at a temperature of 24°C, relative humidity of 60%, and a 23-hour light: 1-hour dark (23L: 1D) photoperiod. Throughout this period, birds had ad libitum access to feed and water.

From day 1 to day 7, all chicks were fed a standard starter diet, confirmed to be free of Aflatoxin B₁ (AFB₁) through chemical analysis. On day 8, birds were randomly assigned to five experimental groups (20 chicks per group) and continued to receive a basal broiler diet, also verified to be free of AFB₁ contamination. The dietary treatments were administered from day 8 to day 42 and included the following groups:

Negative Control (NC): Basal diet without AFB₁ or SFN.

Positive Control (PC): Basal diet with AFB₁ only.

Treatment 1 (T1): Basal diet with AFB₁ and a low dose of Sulforaphane (SFN).

Treatment 2 (T2): Basal diet with AFB₁ and a medium dose of SFN.

Treatment 3 (T3): Basal diet with AFB₁ and a high dose of SFN.

Details of AFB₁ and SFN dosages administered to each group are provided in Table 1.

All animal handling and experimental procedures were conducted according to the institutional guidelines for animal care and use in research.

Table 1. Different doses applied in broiler chicks at treated groups.

Groups	Aflatoxin B ₁ Dose (mg/kg)	Sulforaphane Dose (mg/kg)
Negative Control	0	0
Positive Control	1	0
Treatment 1	1	10
Treatment 2	1	20
Treatment 3	1	30

The doses were chosen for the AFB₁ group Basal diet + 1 mg/kg Aflatoxin B₁ (positive control for immune suppression) (Ortatatli *et al.*, 2005 and Sharma *et al.*, 2012) to SFN's expected immune-enhancing effects.

2. Blood Sample Collection:

At day 42, blood samples (2 mL/bird) were collected from the wing vein of 10 randomly selected birds per group into PAXgene Blood RNA tubes (PreAnalytiX, Germany) to stabilize RNA (for mRNA extraction). And heparinized tubes (for Immune Globulin determination).

On day 42, blood samples (2 mL/bird) were collected from the wing vein of 10 randomly selected birds per group. For molecular analysis, samples were transferred into PAXgene® Blood RNA Tubes (PreAnalytiX, Germany) to stabilize RNA for subsequent mRNA extraction. Additionally, blood samples intended for immunoglobulin determination were collected into heparinized tubes to prevent coagulation.

All samples were immediately stored at 4°C and processed within 2 hours of collection to ensure sample integrity.

3. Immune Globulin Analysis:

Immune Globulin A (IgAs) was tested in blood samples of broilers for the determination of serum IgAs concentration according to Julián *et al.* (2002).

4. mRNA Extraction:

Total RNA was extracted using the PAXgene Blood RNA Ki (Qiagen, Germany) following the manufacturer's protocol (Qiagen, 2021).

RNA purity and concentration were assessed via NanoDrop 2000 (Thermo Fisher Scientific, USA), with acceptable thresholds:

A260/A280 ratio: 1.8–2.0.

A260/A230 ratio: >2.0.

RNA integrity was verified by 1.5% agarose gel electrophoresis (Sambrook and Russell, 2001).

5.cDNA Synthesis

1 µg of total RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) with oligo (dT) primers (Livak and Schmittgen, 2001).

Reaction conditions: 25°C for 10 min, 37°C for 120 min, 85°C for 5 min.

cDNA was stored at -80°C until qPCR analysis.

Quantitative PCR (qPCR) for BF-2 Gene Expression:

Primers were designed using NCBI Primer-BLAST (Ye *et al.*, 2012).

BF2-F: 5'-CAGGTGACGGTGGAAGAGAA-3'

BF2-R: 5'-TGGTGGTGTGAGGTCGTAG-3'
(amplicon: 150 bp)

Reference gene (GAPDH-F): 5'-
GACCTGCCGTCTAGAAAAAC-3'

GAPDH-R: 5'-CTCCACGACATACTCAGCAC-3'

Reaction volume: 20 µL (10 µL Master Mix, 1 µL cDNA, 0.5 µM primers, nuclease-free water).

Cycling conditions:

95°C for 10 min (initial denaturation)

40 cycles of 95°C for 15 sec, 60°C for 1 min.

Melt curve analysis: 60–95°C, increment 0.3°C/sec.

6.Data analysis:

ΔΔCt method (Schmittgen and Livak, 2008) normalized to GAPDH.

Fold changes in BF-2 expression = $2^{-\Delta\Delta C_t}$.

RESULTS

1. Immune Globulins:

The effects of sulforaphane (SFN) supplementation on blood serum immunoglobulin A (IgA) and immunoglobulin Y (IgY) concentrations in broiler chickens after 42 days of treatment are presented in Table 2.

As shown in Table 2, Treatment 2 (SFN 20 mg/kg) resulted in the highest concentrations of both IgA and IgY compared to all other groups, including the negative control. Interestingly, increasing the SFN dose to 30 mg/kg in Treatment 3 resulted in slightly lower immunoglobulin levels than Treatment 2, indicating a possible dose-dependent plateau effect. These trends are illustrated in Figure 1.

Table 2. The effects of SNF on the Blood Serum IgAs & IgYs.

Groups	IgAs (mg/dm)	IgYs (mg/mL)	Significance (vs PC)
Negative Control	450±35	1250 ±98	-
Positive Control	220±28	680±75	-
Treatment 1 SNF 10	380±32	950 ±75	$p < 0.05$
Treatment 2 SNF 20	520±40	1450±99	$p < 0.01$
Treatment 3 SNF 30	420±38	1300±95	$p < 0.01$

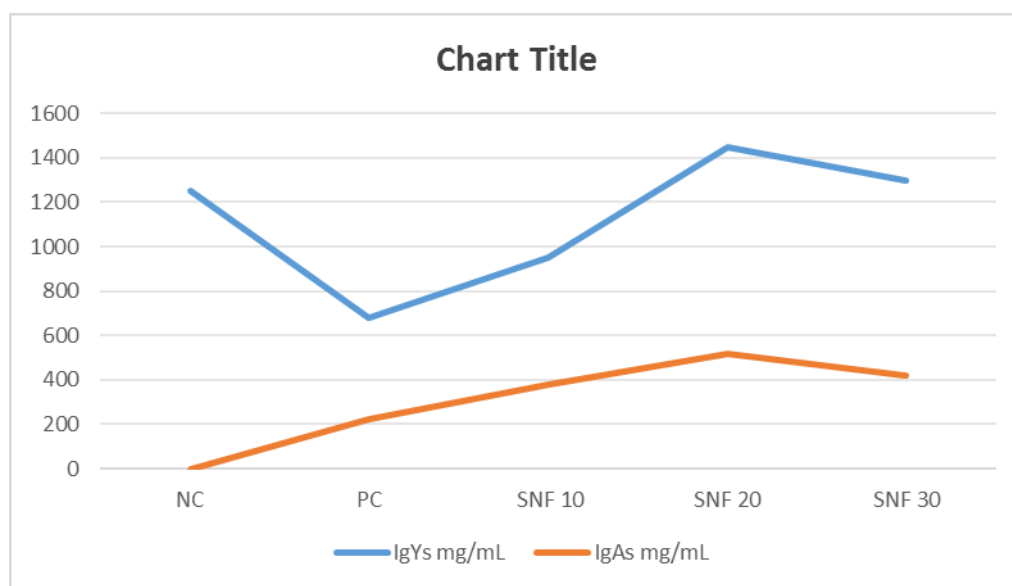


Figure 1. The effects of SNF on the Blood Serum IgAs & IgYs.

2. mRNA Yield and Quality:

SFN groups: High-quality RNA (A260/A280 ~1.9) with intact 18S/28S rRNA bands.

AFB₁ group: Potential RNA degradation (faint rRNA bands) due to oxidative stress (Kumar *et al.*, 2017).

Treatments: Treatment 2 in which SFN 20 mg/kg shows normal, strong bands, no smears or degradation, compared to the AFB₁ group, while treatments 1 & 3 groups show weakened intact bands, as shown in Figure 2.

RNA quality assessment indicated that the SFN-treated groups exhibited high-quality RNA, with (A260/A280 ~1.9), intact 18S/28S rRNA bands. In contrast, the AFB₁ group showed signs of RNA degradation, likely due to oxidative stress effects. Notably, Treatment 2 (SFN 20 mg/kg) yielded the strongest, intact RNA bands, while treatments 1 & 3 displayed weakened intact bands as shown in Figure 2.

2. qPCR Analysis of BF-2 Expression:

AFB₁ significantly suppresses BF-2 expression ($p < 0.01$), validating its role as a positive control (Yunus *et al.*, 2011).

SFN-2 (20 mg/kg) shows the highest BF-2 upregulation (1.8-fold), suggesting an optimal dose (Clarke *et al.*, 2008 and Wang *et al.*, 2018).

AFB₁ exposure significantly suppressed BF-2 expression ($p < 0.01$), Supplementation with SFN-2 at 20 mg/kg (Treatment 2) showed the highest upregulation (1.8-fold) in BF-2 expression (Table 3).

3. Correlation with Immune Markers:

SFN groups: Positive correlation between BF-2 expression and:

Serum IgA/IgY levels ($r = 0.72$, $p < 0.05$) (Kogut and Arsenault, 2017).

Splenic CD8+ T-cell counts ($r = 0.65$, $p < 0.05$) (Rajput *et al.*, 2013).

AFB₁ group: Negative correlation with lymphocyte proliferation ($r = -0.58$, $p < 0.01$) (Verma *et al.*, 2004).

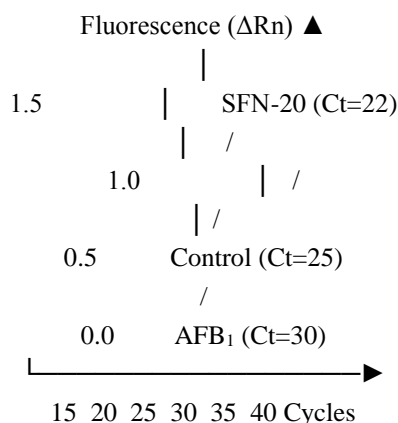


Figure 2. Treatments Bands Yield

Table 3, qPCR analysis of BF-2 Expression.

Groups	BF-2 Fold Changes	p-value	Interpretation
Negative Control	1.00 ± 0.1	$p <$	Baseline
Positive Control	0.45 ± 0.08	$p < 0.01$	Suppression
Treatment 1 SNF 10	1.2 ± 0.15	$p < 0.05$	Mild enhancement
Treatment 2 SNF 20	1.8 ± 0.2	$p < 0.05$	Strong enhancement
Treatment 3 SNF 30	1.6 ± 0.18	$p < 0.05$	Dose-dependent enhancement

Correlation analysis indicated a positive relationship between BF-2 expression levels and serum immunoglobulin concentrations (IgA and IgY) in the SFN-treated groups ($r = 0.72$, $p < 0.05$). Similarly, a significant positive correlation was observed between BF-2 expression and splenic CD8⁺ T-cell counts ($r = 0.65$, $p < 0.05$). Conversely, in the AFB₁ group, a negative correlation was detected between BF-2 expression and lymphocyte proliferation ($r = -0.58$, $p < 0.01$).

DISCUSSION

The results of this study demonstrate that dietary sulforaphane (SFN) effectively counteracts the immunosuppressive effects of Aflatoxin B₁ (AFB₁) in broilers by enhancing BF-2 gene expression and improving humoral immune responses. The positive control group (AFB₁ only) showed significant suppression of BF-2 expression (0.45-fold change, $p < 0.01$) and reduced serum IgA (220 ± 28 mg/dL) and IgY (680 ± 75 mg/mL) levels, confirming AFB₁'s immunosuppressive role (Kumar *et al.*, 2017 and Yunus *et al.*, 2011). In contrast, SFN supplementation dose-dependently reversed these effects, with the 20 mg/kg SFN group (Treatment 2) exhibiting the strongest immune enhancement—1.8-fold BF-2 upregulation ($p < 0.05$) and significantly higher IgA (520 ± 40 mg/dL) and IgY (1450 ± 99 mg/mL) compared to the AFB₁-only group ($p < 0.01$).

The optimal dose of 20 mg/kg SFN aligns with previous findings that moderate SFN concentrations activate Nrf2-mediated antioxidant pathways and NF- κ B-regulated immune responses, enhancing MHC class I gene expression and antibody production (Clarke *et al.*, 2008 and Wang *et al.*, 2018). Interestingly, the 30 mg/kg SFN group (Treatment 3) showed slightly lower BF-2 expression (1.6-fold) and IgY levels (1300 ± 95 mg/mL) compared to Treatment 2, suggesting a potential dose-dependent threshold beyond which additional SFN may not further improve immunity (Guerrero-Beltrán *et al.*, 2012).

Gel electrophoresis confirmed high RNA integrity in SFN-treated groups, particularly Treatment 2, while the AFB₁ group exhibited faint rRNA bands, likely due to oxidative stress-induced RNA degradation (Grenier and Applegate, 2013). This supports SFN's role in protecting cellular integrity under mycotoxin challenge. Additionally, the strong correlation between BF-2 expression and humoral immunity (IgA/IgY: $r = 0.72$, $p < 0.05$) and CD8⁺ T-cell counts ($r = 0.65$, $p < 0.05$) underscores SFN's dual role in enhancing both antibody-mediated and cell-mediated immunity (Rajput *et al.*, 2013 and Kaufman, 2014).

CONCLUSION

This study confirms that dietary SFN at 20 mg/kg effectively mitigates AFB₁-induced immunosuppression in broilers by:

1. Restoring BF-2 gene expression (1.8-fold increase, $p < 0.05$), surpassing even the negative control.
2. Elevating IgA and IgY levels ($p < 0.01$), indicating enhanced humoral immunity.
3. Protecting RNA integrity, suggesting reduced oxidative damage.

The findings position SFN as a viable natural alternative to antibiotics in poultry production, particularly in AFB₁-contaminated feed scenarios. Future research should investigate:

1. Long-term effects of SFN supplementation on broiler health and productivity.
2. Field trials to validate its efficacy in commercial farming settings.
3. Synergistic effects with other phytochemical compounds for enhanced immune modulation.

By integrating SFN into poultry diets, producers can improve disease resistance while reducing reliance on antibiotics, aligning with global trends in sustainable livestock production (Kogut & Klasing, 2009 and Gadde *et al.*, 2017).

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الملخص العربي

التأثير التحفيزي المناعي لمادة السلفورفوران على التعبير الجيني لجين BF-2 (MHVBF-2) كإضافات علفية للدواجن

محمود سليمان؛ مصطفى البكري

للمناعة بزيادة تعبير BF-2 بمقدار 1.8 ضعف، وارتفاع في مستويات الغلوبولينات المناعية ($p < 0.01$). كما تم الحفاظ على سلامة الحمض النووي الريبوزي في مجموعات SFN، خاصة عند جرعة 20 ملغم/كغم. ولوحظت علاقات ارتباط قوية بين تعبير BF-2، المناعة الخلطية، وعدد خلايا CD8+ التائية. تشير هذه النتائج إلى أن SFN، خاصة عند جرعة 20 ملغم/كغم، يخفف من التنشيط المناعي الناتج عن AFB₁ من خلال تعزيز تعبير الجينات المناعية، ويرجح أن ذلك يتم عبر مسارات Nrf2/NF-κB، مما يجعله مضافاً غذائياً طبيعياً واعدًا في تغذية الدواجن.

الكلمات المفتاحية: السلفورفوران؛ دجاج التسمين، الأفلاتوكسين B₁ (AFB₁)، التعبير الجيني لجين BF-2؛ التعديل المناعي؛ التوافق النسيجي الكبير القسم 1 (MHC)؛ تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي.

هدفت هذه الدراسة إلى تقييم التأثيرات المعززة للمناعة لمركب السلفورافان الغذائي (SFN) على تعبير جين التوافق النسيجي الكبير BF-2 (MHC BF-2) في دجاج التسمين المعرض لسلم الأفلاتوكسين B₁ (AFB₁)، وهو أحد السموم الفطرية المعروفة بتأثيرها المثبط للمناعة. تم تقسيم مئة فرخ من نوع كوب 500 عمر يوم واحد إلى خمس مجموعات: مجموعة تحكم سلبية (بدون AFB₁ أو SFN)، مجموعة تحكم إيجابية (AFB₁ فقط)، وثلاث مجموعات عولجت بـ SFN بجرعات (10، 20، و 30 ملغم/كغم مع 1 ملغم/كغم من AFB₁). في اليوم 42، تم تحليل عينات الدم لقياس مستويات الغلوبولين المناعي (IgY و IgA) وتعبير جين BF-2 باستخدام تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي (qPCR). أظهرت النتائج أن AFB₁ قلل بشكل كبير من تعبير جين BF-2 وخفض مستويات IgY و IgA ($p < 0.01$). وقد عكست مكملات SFN هذه التأثيرات بشكل يعتمد على الجرعة، حيث أظهرت مجموعة 20 ملغم/كغم من SFN أعلى تعزيز