

## **Effect of Purslane Powder on the Performance and Immunity System of Broiler Chickens**

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### **ABSTRACT**

Purslane (*Portulaca oleracea*) was selected as the target plant for the present study due to its high nutritional value and antioxidant and antimicrobial properties. The aim was to investigate the effect of adding purslane powder on flock performance and immune response against Newcastle and infectious bursal disease. Purslane seeds were purchased from a medicinal company in India and, after preparation, used as a 1% powder. The experiment was conducted using 180 one-day-old Ross 308 broiler chickens, with the aim of investigating the effect of purslane powder on the performance and immunity system of the birds. The experiment was carried out in a completely randomized design, with three treatments, four replicates and 15 birds each. The experimental treatments comprised a negative control (basic feed without purslane powder or vaccination), a positive control (basic feed without purslane powder but vaccinated against Newcastle and infectious bursal disease virus (IBDV)), and a purslane group (basic feed with 1% purslane powder and vaccination). The results of the experiment demonstrated that performance indicators such as total weight at the end of the period, body weight gain, and European Production Efficiency Factor decreased with vaccination and increased significantly with the use of purslane powder in vaccinated chickens ( $p < 0.05$ ). Antibody titer against Newcastle disease virus in vaccinated chicks received purslane powder was more than vaccinated group without purslane, but the difference was not significant ( $p > 0.05$ ). The study also revealed that the antibody titer against the IBD vaccine increased in both vaccinated groups; however, the titer in the purslane group was significantly higher than in the group that received only vaccination ( $p < 0.05$ ). The findings of this study indicate that the supplementation of broiler feed with purslane can enhance the performance of broiler flocks, strengthen the immune response against ND and IBD vaccination, and positively impact the population of intestinal microflora.

**Keywords:** Immunity System, Infectious Bursal Disease Virus, Purslane, Newcastle Disease, Vaccine.

## 1. Introduction

The global poultry industry has made significant contributions to food security by providing affordable protein sources. Concurrent with the advancements in this sector, there has been an increased demand for healthy and antibiotic-free products, resulting in the enactment of legislation prohibiting the use of antibiotics in several countries. The prohibition of antibiotic use in conjunction with the prevalence of bacterial and viral diseases has inflicted substantial harm on poultry farms, a problem that researchers are endeavoring to address. These efforts encompass the implementation of proper breeding programs, the production of effective vaccines, and the utilization of appropriate compounds such as medicinal plants, probiotics, prebiotics, and acidifiers. Notable among the diseases that pose a significant threat to poultry farms are Newcastle disease and infectious bursal diseases, which affect bird health and efficiency, thereby causing substantial economic losses to the poultry industry annually (1, 2). Infectious bursal disease (IBD) is an acute viral disease that causes significant damage and high mortality (1,3). The acute form of the disease occurs in chickens between 3-5 weeks old and is associated with symptoms such as diarrhea, immobility, loss of appetite, tremors, loss of balance and death. The immunodeficiency induced by IBD in chickens renders the flock vulnerable to a multitude of viral, bacterial, and parasitic infections, consequently resulting in indirect economic losses. Purslane (*Portulaca oleracea*) is a plant that is ubiquitous and is employed as both an edible vegetable and a medicinal plant in Iran and several other countries. This plant is the most widely used medicinal plant documented by the World Health Organization (4). Purslane is a significant source of omega-3 fatty acids (Simopoulos and Salem, 1986), and a comprehensive review of the plant reveals that it is a rich source of various important phytochemicals, including flavonoids, alkaloids, terpenoids, proteins, carbohydrates, vitamins such as A, C, E, and B, carotenoids, and minerals such as phosphorus, calcium, magnesium, and zinc. It is of particular significance due to its high concentration of omega-3 fatty acids, specifically  $\alpha$ -linolenic acid, gamma-linolenic acid, and linoleic acid, which are not typically synthesized in terrestrial plants. Purslane is renowned for its ethnomedicinal and pharmacological applications, which are attributed to its anti-inflammatory, antidiabetic, skeletal muscle relaxant, antitumor, hepatoprotective, anticancer, antioxidant, anti-insomnia, analgesic, gastroprotective, neuroprotective, wound healing and antiseptic properties. The numerous benefits of purslane have led to its recognition as a promising health-promoting food source, attracting significant scientific interest worldwide. A study revealed that the incorporation of 0.2% purslane extract into broiler feed enhanced flock performance, augmented the population of *Lactobacillus*, and diminished the population of *Escherichia coli* within the gastrointestinal tract (5). Purslane has been demonstrated to exert a favorable influence on cecal microflora (6). The present study

investigates the effects of dietary supplementation of purslane powder (PP) on performance, blood indices, and antioxidant status in broilers with triiodothyronine (T3)-induced ascites. The study concludes that supplementation of purslane in the feed improves oxidative status and reduces ascites incidence without impairing growth performance in broiler chickens (7). It is reported that the intake of 2% and 3% purslane could significantly increase body weight gain and lead to a reduction in FCR. Furthermore, purslane feeding has been shown to increase the relative abundance of beneficial bacteria in the chicken intestines (8). Therefore, due to its high nutritional value and antioxidant and antimicrobial properties, purslane was selected as the target plant for the present study in order to investigate the effect of adding purslane powder on flock performance and immune response against Newcastle and infectious bursal disease.

## 2. Materials and Methods

### 2.1. Preparation of purslane seed

Purslane seeds were procured from Shree Herbals, a company based in India that specialises in Ayurvedic medicine. Following the requisite preparation, the seeds were utilised as a powder at a concentration of 1% in G3.

### 2.2. Experiment Design

The experiment was conducted with 180 broiler chickens of the Rass 308 strain, which were of uniform weight. The chickens were randomly allocated to three groups, with four replications and 15 chickens per replication for a duration of 35 days. The control group was fed only a basal diet. Group 2 was fed a basal diet and vaccinated. Group 3 was fed a basal diet containing 1% purslane powder and vaccinated (Table 1 for further details). The European Production Efficiency Factor was calculated according to the following formula (10). In order to assess the immune response of broiler chickens, blood samples were collected from the wing vein of five birds per replication on days 17 and 35 of rearing. The blood samples were then transferred into microtubes and stored in a refrigerator until ELISA testing could be performed. On the 35th day of the experiment, five chickens from each group were randomly selected and euthanised. The relative weight of the spleen and bursa was then measured, and the whole cecum of the broiler chickens from the different experimental groups was collected under high health conditions and sent to the laboratory. Serial dilutions were prepared and cultured in nutrient agar medium and kept at 37°C for 24 hours. The plates were then counted to give a numerical value between 25 and 250.

### 2.3. Statistical Analysis

The statistical analysis of the data was conducted utilising the SAS (Statistical Analysis System – Version 9.1) software. In order to ascertain significant differences between means, two-way ANOVA and least significant difference (LSD) post hoc tests were performed. Chi-squared was used to evaluate significant differences

between proportions.  $P < 0.05$  is considered statistically significant (SAS/STAT 2010).

**3. Results**

The impact of incorporating purslane in the diet of vaccinated broiler chickens against Newcastle and Gambro diseases on flock performance and immune response is illustrated in Table 2. The vaccination process resulted in a decline in bird weight, body weight gain, feed consumption, and European efficiency factor. Conversely, the feed conversion ratio exhibited an increase that reached statistical significance ( $p < 0.05$ ). The incorporation of purslane powder in the diet of vaccinated chickens resulted in an increase in bird weight, body weight, and European efficiency factor, while concurrently leading to a decrease in feed conversion ratio ( $p < 0.05$ ). However, feed consumption remained unaltered. The highest feed consumption was observed in the control group.

**4. Discussion**

The experiment was designed to evaluate the use of medicinal plants in order to strengthen the immune system and reduce the effects of vaccination stress. Medicinal plant compounds may affect the immune system in two ways: directly (by stimulating the lymphatic tissues) and indirectly (by changing the microbial population of the digestive system). The results showed that the relative weight of the bursa increased with vaccination, but no significant difference was observed with the addition of purslane powder. The relative weight of the spleen was found to be unresponsive to vaccination; however, it exhibited a significant increase in response to the incorporation of purslane powder. The body weight gain and the European performance indicators of the broiler chickens in groups 2 and 3 were observed to decrease, while in group 3, an increase was recorded. This increase was found to be

significant, with a 71% and 86% rise, respectively. Researchers hypothesise that the use of purslane in the feed of broiler chickens has a positive effect on appetite and weight gain, which is thought to be a result of the way in which it affects the central and peripheral nervous systems through interference with opioid receptors. It has been posited by some researchers that a 5% increase in purslane in broiler feed results in a significant rise in both feed consumption and weight gain, with the potential to enhance antioxidant status in these chickens (12, 13, 14). The present research lends support to this hypothesis, demonstrating a positive effect of a 1% purslane powder addition on feed consumption, body weight gain, and FCR. The observed increase in feed consumption was attributed to the enhanced palatability of the feed resulting from the incorporation of purslane. This, in turn, led to an improvement in weight gain (15). In a particular study, *Portulaca oleracea* (purslane) extract (POE) supplementation was found to enhance the performance and nutritive value of quails in terms of certain substances (CF, DM, OM, NFE, TDN, and ME) (16,17). It is also notable that POE did not cause any harmful effects on birds' liver and kidney functions. Furthermore, this extract promotes immunity and antioxidant status, while minimizing the harmful microbial load in the quails' intestines. It is therefore recommended that POE be used as a feed additive. The researchers believe that the increase in body weight is due to the improvement of digestibility and absorption of nutrients resulting from the presence of purslane extract (16,17). An increase in the antibody titer is indicative of stimulation of the host's immune system, with vaccination representing the most effective method of preventing damage caused by infectious diseases in humans and animals. Furthermore, it has been demonstrated that vaccination creates a strong immune response, thereby providing long-term protection against infection.

**Table 1.** Vaccination program.

| Treatments | Vaccine ND  | Vaccine IBD                  | Basic feed | Purslane 1% |
|------------|---|------------------------------|------------|-------------|
| G1         | -   | -                            | +          | -           |
| G2         | 1st day spray and repeated at 10 and 28 days <i>via</i> drinking water (DW) | 14 + 24 days <i>via</i> (DW) | +          | -           |
| G3         | 1st spray and repeated at 10 and 28 days <i>via</i> DW                      | 14+ 24 <i>via</i> (DW)       | +          | +           |

1. Newcastle vaccine in one day as a spray and in 10 and 28 days in drinking water, **ND + IB** (Boehringer Ingelheim.- Germany).
2. Infectious bursal disease vaccine at 14 and 24 days orally, (Boehringer Ingelheim- Germany).

**Table 2.** The effects of purslane on the performance of broiler chickens at 35 days old.

| Groups  | Total Weight(g) | Weight gain(g) | Feed consumption(g) | FCR    | EPEF   |
|---------|-----------------|----------------|---------------------|--------|--------|
| G1      | 1092.5 b        | 1052.3 b       | 2855 a              | 2.71b  | 115.1b |
| G2      | 955.1c          | 915.1c         | 2655.3 b            | 2.90a  | 94.1c  |
| G3      | 1608.3a         | 1568.3a        | 2613.7b             | 1.67c  | 275.7a |
| SEM     | 84.80           | 84.81          | 34.66               | 0.16   | 24.47  |
| P-value | 0.0001          | 0.0001         | 0.0003              | 0.0001 | 0.0001 |

\* Different letters in each column indicate significant differences ( $P < 0.05$ ).

In the present study, an increased antibody titer against Newcastle diseases was observed in chickens receiving purslane powder, in comparison to the vaccinated group that did not receive purslane powder in their diet. This finding suggests a positive effect on antibody production. In a related study, the researchers observed an increase in the concentrations of IgM and IgA in quail fed with purslane compared to the control group, which they attributed to the premature maturation of humoral immune responses (16). This finding is consistent with the results of the present study on the Newcastle vaccine. In addition, some researchers have reported a positive effect of bioherbal supplements in broiler feed on the level of Newcastle-specific antibody. In another study, the simultaneous use of Eucalyptus and mint plant essential oils at a dose of 250 ml per 1000 liters of drinking water was beneficial on the titer of Newcastle and influenza in broiler chickens (2). The researchers showed different levels of purslane extract (11). In A study determined that the addition of vitamin E and L-carnitine, either alone or in combination with purslane, to a low-protein diet can enhance the immune system and health of broiler chickens under cold stress by creating synergistic effects (14). Purslane can also function as an immune modulator and antioxidant agent (4). The results of the present study demonstrate that the incorporation of purslane at a rate of 1% into the diet of broiler chickens results in an augmentation of the weight of the bursa of Fabricius, spleen, and intestinal microbial population. The findings further reveal that the population of cecum bacteria

in vaccinated broiler chickens decreases following the administration of purslane powder in conjunction with vaccination against Newcastle and infectious bursal disease. In a study by Ghorbani et al. (2014), it was concluded that broiler immunity was not affected by the inclusion of purslane extract in the feed, but that there was a positive effect on cecal microflora composition (11). It was reported that the population of *E. coli* decreased significantly in the group that received purslane powder compared to the control group, and that the population of *Lactobacillus* bacteria increased significantly (12). The use of purslane extract has been shown to increase the population of lactobacilli and bifidobacteria in the cecum contents of broiler chickens (5). Researchers hypothesize that the increase in lactobacilli population in response to purslane powder is attributable to the phenolic compounds present in purslane, as these compounds are capable of being metabolised by lactobacilli to provide energy for cell metabolism. The present study corroborates the findings of previous research that has demonstrated an increase in beneficial bacteria, such as *Lactobacillus*, in the intestine, and a concomitant decrease in harmful bacteria, such as *Enterobacteriaceae* (*Escherichia-Shigella*), when purslane is incorporated into the feed of broiler chickens. The study concluded with the following findings: the supplementation of broiler feed with purslane had a positive effect on flock performance, enhanced the immune response against viral diseases, and had a beneficial effect on the population of microflora in the intestines (table 5).

**Table3.** The effect of purslane powder in broiler chickens against Newcastle diseases.

| Group   | 17 days( Newcastle Dis. Titer) |                | 35 days             |                   |
|---------|--------------------------------|----------------|---------------------|-------------------|
|         | G1                             | 1 <sup>b</sup> |                     | 1.02 <sup>b</sup> |
| G2      | 3011.8 <sup>a</sup>            |                | 3032.1 <sup>a</sup> |                   |
| G3      | 3016.7 <sup>a</sup>            |                | 3112.8 <sup>a</sup> |                   |
| SEM     | 284.3                          |                | 270.1               |                   |
| P-value | 0.0001                         |                | 0.0001              |                   |
| LSD     | 261                            |                |                     |                   |

\*Mean values of logarithmic count for different products with different superscript letters in the same rows are significantly different at (P<0.05).

**Table4.** The effect of purslane powder in chickens against infectious bursal diseases vaccination by ELISA method.

| Group   | Age                 |                     |
|---------|---------------------|---------------------|
|         | 17 days             | 35 days             |
| G1      | 0.11 <sup>c</sup>   | 0.21 <sup>c</sup>   |
| G2      | 2050.8 <sup>b</sup> | 3284.1 <sup>b</sup> |
| G3      | 3041.4 <sup>a</sup> | 3402.5 <sup>a</sup> |
| SEM     | 236.2               | 293.5               |
| P-value | 0.0001              | 0.0001              |

\* Different superscript letters in the same rows are significantly different at (P<0.05).

**Table 5.** The effect of purslane powder on the relative weight of spleen, bursa of faricus (%) and the microbial population of the cecum (log cfu/g) of broiler chicks at 35 days of age.

| Type equation here Group |                   |                   |                   |
|--------------------------|-------------------|-------------------|-------------------|
|                          | Burs of Fabricus  | Spleen            | Intestine         |
| G1                       | 1.68 <sup>b</sup> | .03 <sup>b</sup>  | 2.68 <sup>b</sup> |
| G2                       | 2.39 <sup>a</sup> | 0.05 <sup>b</sup> | 2.39 <sup>c</sup> |
| G3                       | 3.03 <sup>a</sup> | .12 <sup>a</sup>  | 3.03 <sup>a</sup> |
| SEM                      | 0.17              | 0.01              | 0.17              |
| P-value                  | 0.0001            | 0.0001            | 0.0001            |

\* Different superscript letters in the same rows are significantly different at (P<0.05).  
SEM: standard error of the mean.

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### Authors' Contribution

Study concept and design: M. M, K. SR.  
Acquisition of data: K. SR, M. M, B. Z.  
Analysis and interpretation of data: M. M, G. MR.  
Drafting of the manuscript: M. M, K. SR, B. Z.  
Critical revision of the manuscript for important intellectual content: M. M, VAZ. KY.  
Statistical analysis: VAZ. KY, G. MR, K. SR.  
Administrative, technical, and material support: K. SR, M. M, VAZ. KY, B. Z.

### Ethics

The present study adheres to the ethical guidelines of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran (EE/1401.2.25.36771/Scu.ac.ir).

### Conflict of Interest

The authors declare that there is no conflict of interest.

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### Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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