



# EFFECT OF B-GLUCANASE SUPPLEMENTATION ON FEED CHARACTERISTICS AND NUTRIENT DIGESTIBILITY IN BROILER CHICKS FED DIETS WITH DIFFERENT LEVELS OF BARLEY

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<b>Received:</b> 08 <sup>th</sup> December 2023 <b>Accepted:</b> 07 <sup>th</sup> January 2024 <b>Published:</b> 08 <sup>th</sup> February 2024	<p>This experiment was conducted to study and understand the effect of threonine on performance, weight gain, feed intake, and liver enzyme activity, using 540 Ross 308 broiler chicks (of both sexes) in a completely randomized design. Nine treatments were used (three levels of threonine supplementation: zero, 10, and 20% of the strain's requirement) and (three levels of barley inclusion: zero, 5, and 10% replacing corn). The experiment was replicated four times. The experimental treatments included: 1 to 3 of the basal corn-soybean meal diet with threonine supplements of zero, 10, and 20%, and 4 to 6) the diet containing 5% barley replacing corn, respectively, with zero, 10, and 20% threonine supplements.</p> <p>The results indicate that the interaction between the level of barley and threonine supplements on feed intake, weight gain, feed conversion ratio, European production index, and mortality rate in the early, final, and overall periods had no significant effect (<math>P&gt;0.05</math>). Adding 5% barley with 10 or 20% threonine to the diet increased glucose, insulin, and IGF-1 levels in the blood. Adding 10% threonine supplement to diets with low barley levels (5%) in this experiment had a positive effect on improving performance and immunity. At high levels of barley (10%), the presence of non-starch polysaccharides (beta-glucans) may affect immune factors and gut morphology. Overall, based on the results of the experiments, the researchers considered the use of 5% barley as a substitute for corn to be beneficial without negative effects on the performance of broiler chicks.</p>

**Keywords:** Threonine, Barley, Broiler Chicks, Immune System, Performance

## INTRODUCTION

Multiple factors, including breeding, nutrition, hygiene, and proper management, have led to improvements in weight gain, feed conversion ratio, and grain yield in broiler chicks. However, this has been associated with a negative correlation between antibody concentration in the blood and immune response through the production of antibodies against red blood cells in broilers. In other words, the bird bears the metabolic and nutritional costs of creating an appropriate immune response, which is inversely related to growth rate (Azzam et al., 2015).

Therefore, optimal growth potential was achieved using balanced diets to meet maintenance and growth requirements, and it was possible to achieve maximum safety response. The immune response of birds can be affected by dietary factors because nutrition plays an important role in the ecological balance and gut health of broilers (Chen et al., 2003). The use of barley has been limited in broiler diets due to its anti-nutritional factors, which include soluble and insoluble non-starch polysaccharides (Evdokia et al., 2010).

The physical and chemical properties of barley vary depending on the cultivar, which causes changes in the shape of the digestive tract, changes in the availability of energy and other nutrients of this edible material, and thus affects performance and immunity in birds and chicks (Lelis et al. 2010 and Sepehri et al. 2014).

Diets rich in non-starch polysaccharides, especially soluble non-starch polysaccharides, can increase viscosity in the small intestine, destroy villus cells, reduce villus height, increase crypt cell production, increase digestive enzyme activity, and change the volatile fatty acid profile and ultimately lead to changes in the gut microbiota (Kegley et al., 2014).

## MATERIAL AND METHODS

### Place and Location of the Experiment and Examination

The current study was conducted in a poultry house located in Ilam city in the spring of 1400 (4/2021) for 42 days. The length and width of the breeding house were 12 × 18 meters and the height of the ceiling to the ground was 3 meters. 16 panels (experimental units) with an area of 4/1 × 4/1 meters each were prepared to conduct this research. These panels were made of pipes and nets and each panel had a hanging waterer and a grain feeder in the form of a plate.

### Preparing the Breeding House

In the beginning, all the equipment was removed from the house, and then the floor, doors, windows, and walls were washed carefully and with a high-pressure water pump to remove any remaining manure and other waste from the house. After drying, it was washed again with a detergent and the house was disinfected with ammonium quaternary products. All parts, sections, and tools in the house were washed, cleaned, and disinfected, including the feeders, waterers, and special nets for the test and examination units (panels). Water and cleaning materials were used and the house was then sprayed with disinfectants after which they were installed. Then all the windows and air inlets of the hall were covered and the fumigation process was carried out with formaldehyde gas. After 48 hours, all doors and windows were opened and the vents were opened to better dissipate and remove the gas.

To maintain the health of the house, a germicidal disinfectant solution was poured into the entrance pool of the house and changed daily.

### Breeding Management

Characteristics of Chicks and Transfer to Experimental Units

500 one-day-old broiler chicks of the Ross 308 strain were purchased from Zarbal Merk Chicken Company in Tonkabon City with an average weight of 54 grams and a parental flock age of 58 weeks. The health characteristics of the chicks according to the chicken health card were as follows:

1- MG Negative 2- MS Negative and 3- sp Negative

Also, the coronavirus or reovirus and infectious bronchitis (encephalomyelitis) vaccines were used in the parent flock.

This experiment was conducted completely randomly and on the first day, we weighed the chicks and divided them randomly between the different treatments. In each panel or section, we put 20 to 30 chicks. The chicks also had full access to water and food and the necessary care was taken according to the methods recommended in the commercial catalog.

### Grain Feed and Drinker

The chicks were randomly placed inside the pens or panels from the moment they entered the house and from the first hour, grain and water were provided to them. After 24 hours of the chicks entering, the water was placed in the house so that its temperature could become similar to the environment or the house. We used conical feeders or drinkers and tray feeders until the age of 12 days. Until day 12, we used nipple drinkers to provide water and we also used feeders with a diameter of 50 cm to provide the chicks with grain. Four-grain meals are added to the feeders daily.

### Lighting and Ventilation of the House

On the first day of breeding, 24 hours of light were applied. After that, 23 hours of light and one hour of darkness. In this research, we used 12 energy-saving lamps in two hexagonal rows, two meters above the ground level. Ventilation is used to balance the temperature, release gases rising from waste and dust, as well as to provide a sufficient amount of oxygen and control the humidity of the floor. Therefore, we used 4 exhaust fans. The windows were placed in the southern part of the house.

### Temperature and Humidity of the House

One of the important goals of chick-rearing cycles is to provide a comfortable environment for them. Two gas heaters were used to heat the house during the first week of the rearing period. The heaters were turned on 24 hours before the chicks were transferred. Also, 4 ordinary thermometers and two minimum thermometers were used to measure and control the temperature of the house. In addition to this, the internal temperature was maintained at 33 degrees Celsius on the first day and at 32 degrees Celsius until the end of the first week. It decreased by 3 degrees Celsius per week with increasing age, so that in the last week of breeding, the internal temperature was 18 degrees Celsius. The humidity in the house at the beginning of the breeding and reproduction period is about 60-50% and in the following weeks, the humidity in the house reached a higher percentage. At the end of the period, it reached about 65-60 degrees Celsius.

### Vaccination Program

To prevent common poultry diseases such as bronchitis<sup>1</sup>, Newcastle<sup>2</sup>, and Gumboro<sup>3</sup>, a vaccination program was implemented according to the recommendations of the Regional Veterinary Directorate (Table 3-1). To prevent and reduce stress caused by vaccination, a multivitamin solution was injected into the water 24 hours before and after vaccination. Drinking water was kept out of the reach of chickens for two hours. All of this comes according to the orders of the Animal and Poultry Organization in the region.

Table shows vaccination programs

Type of Injection	Age of Injection (Day)	Method of Use
Bronchitis	1	Drinking
Influenza	7	Subcutaneous Injection
Newcastle (1B)	7	Eye Drop
Bronchitis	11	Eye Drop
Gumboro	14	Drinking

### Experimental Diets

The experimental diets were prepared according to the recommendations of Ross flocks and at different growth and rearing periods based on corn and soybeans and in two periods: the first (1-21 days) and the final (22-42 days). National Research and Studies Institute (NRC, 1994) tables were used to evaluate the nutrients in the feed rations. The feed intake, composition, and chemical composition of the rations were presented.

### Mortality and Damage

All experimental units were inspected daily and once injuries were observed, the carcasses of the damaged birds were collected, weighed, and recorded. The experimental units were taken into account and when calculating the daily weight gain and the amount of feed in the sections and experimental units, they were considered and processed.

## THE RESULTS AND DISCUSSIONS

### 4-1- Effect of Experimental Diets on the Performance and Efficiency of Broiler Chicks

#### 4-1-1- Feed (Grain) Intake

The data and information related to the effect of the different experimental treatments on feed and grain intake by broiler chicks during the growth period are presented in Table (1).

**Table 1- Effect of Barley Level and Threonine Supplement on Feed Intake by Broiler Chicks (g)**

Experimental Treatment		Period		
Barley Level (%)	Threonine Supplement	Initial (1-21 days)	Final (22-42 days)	Total Period (1-42 days)
zero	zero	1017	3450	4467
zero	10	996	3370	4365
zero	20	1128	3597	4725
5	zero	1064	3428	4493
5	10	1080	3429	4508
5	20	1213	3389	4602
10	zero	1243	3526	4769
10	10	1257	3331	4589
10	20	1196	3389	4624
SEM		41/7	136/5	145/1
Sources of Variation		P-Value		
Barley Level		<0/05	>0/05	>0/05
Threonine Supplement		>0/05	>0/05	>0/05
Threonine × Barley Lev. Sup.		>0/05	>0/05	>0/05

a,b,cThe numbers in each column have a significant purity with their corresponding numbers (OP <0/05 )

.Standard Error of Means =SEM

The results showed that the type of cereal in the basal diet, threonine supplement level, and their interaction at different rearing periods including 1-21 days, 22-42 days, and 1-42 days (whole rearing period) had no significant effect on the amount of feed consumed by broilers. ( $P>0.05$ ).

During the 1-21 day period, broilers fed a diet containing 10% barley consumed more feed than other diets ( $P>0.05$ ), so broilers fed diets containing 0, 5, and 10% barley consumed 1047, 1192, and 1232 grams of feed, respectively ( $P<0.05$ ).

#### 4-1-2- Increase in weight

Table 2 - The effect of ration barley level and threonine supplement on body weight and weight gain of broiler chicks (g)

Experimental Treatment		Traits (cycle)			
Barley Level (%)	Threonine Supplement	Body weight ( 1-21 days	Increased body weight (days 21-1)	increase weight the body (days 42-22)	All period (days 42-1)

zero	zero	882/5	839/5	1454/8	2337/2
zero	10	859/5	816/5	1400/0	2259/2
zero	20	894/5	851/5	1513/7	2407/7
5	zero	882/0	839/0	1548/0	2430/0
5	10	909/0	866/0	1432/7	2341/7
5	20	888/5	845/5	1335/0	2223/2
10	zero	867/7	824/7	1531/0	2398/2
10	10	883/5	840/5	1423/7	2307/2
10	20	891/3	848/3	1488/8	2380/00
SEM		18/1	18/2	73/0	71/9
Sources of Variation		P-Value			
Barley Level		>0/05	>0/05	>0/05	>0/05
Threonine Supplement		>0/05	>0/05	>0/05	>0/05
Threonine Sup. *Barley Lev.		>0/05	>0/05	>0/05	>0/05

.(0P </05 )a,b,cThe numbers in each column have a significant purity with their corresponding numbers

.Standard Error of Means =SEM

Table 2 shows the effect of experimental diets on body weight at the initial, growth, final, and full periods. The results showed that the type of cereal in the basal diet, threonine supplement level, and their interaction at different rearing periods, including 1-21 days, 22-42 days, and 1-42 days (whole rearing period) had no significant effect on body weight or weight gain of broilers ( $P>0.05$ ).

#### 4-1-3 Effect of Experimental Diets on Liver Enzyme Activity

Table 3 shows the effect of experimental diets on liver enzyme activity (alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase) in broilers. The results showed that the activity of alkaline phosphatase and alanine aminotransferase in broilers was significantly affected by the main effect of dietary barley level and the interactive effect of dietary barley level and threonine supplements ( $P<0.05$ ), but the threonine supplement level alone did not significantly affect the activity of alkaline phosphatase in broilers ( $P>0.05$ ).

Regarding alkaline phosphatase, the effect of dietary barley level on alkaline phosphatase activity was such that the inclusion of 5 and 10% barley in the broiler diet reduced the activity of this enzyme and had a significant difference with the control group (116 and 112 vs. 137 units). However, in terms of alanine aminotransferase, only in the 5% barley diet, did the amount of enzyme activity decrease significantly (4.22 units in the 5% barley group vs. 5.3 units in the control group and 10% barley). There was no significant difference between the control group and 10% barley.

The effect of dietary threonine supplements on the activity of the aspartate aminotransferase enzyme was significant, and there was a significant difference between broilers receiving the control diet (4.4 units) and broilers receiving 10% of the threonine supplement (4.6 units) while receiving 20%. The supplement: Threonine of aspartate aminotransferase enzyme (5.6 units) had a significant difference ( $P<0.05$ ). The interaction effect of barley level and threonine supplement on alkaline phosphatase activity was also significant and did not follow the same trend as the main effects. Therefore, among the experimental treatments, the lowest alkaline phosphatase activity belonged to the 5% barley group with 10%. Threonine supplement was added and the highest amount to broilers, which was done by adding 10% threonine to the control group ( $P<0.05$ ). However, in the case of alanine aminotransferase activity, the lowest enzyme activity belonged to the 5% barley group without threonine supplements and the highest level belonged to broilers that received 10% barley feed with 20% threonine supplements. It seems that in this experiment, the inclusion of 10% barley in the diet, as well as adding threonine more than 10%, increased the activity of this enzyme.

The effect of aspartate aminotransferase enzyme activity in broilers was significant for the main effect of dietary barley level and the interactive effect of dietary barley level and threonine supplements ( $P<0.05$ ). Regarding the effect of dietary barley level on aspartate aminotransferase enzyme activity, there was no significant difference between broilers receiving the control diet and 5% barley (63 units), and there was a significant difference between the group receiving 10% barley feed and the others. The lowest amount belongs to the 10% barley group (54 out of 63 units ( $P<0.05$ )). The interaction effect of barley level and threonine supplement on aspartate aminotransferase enzyme activity was also significant. Therefore, among the experimental treatments, the highest aspartate aminotransferase enzyme activity belonged to the control group and 10% threonine supplement, and the lowest value belonged to the control group for broilers that received 10% barley with 10% threonine supplement ( $P<0.05$ ).

Table 2 - The effect of ration barley level and threonine supplement on body weight and weight gain of broiler chicks (g)

Experimental Treatment		Traits (cycle)		
Barley Level (%)	Threonine Supplement	(ALP (U/L)	AST (U/L)	ALT (U/L)
zero	zero	143/0ab	66/0abc	5/1b
zero	10	150/8 <sup>a</sup>	77/2 <sup>a</sup>	5/6 <sup>ab</sup>
zero	20	117/0 <sup>bcd</sup>	46/0 <sup>d</sup>	5/3 <sup>ab</sup>
5	zero	104/0 <sup>d</sup>	54/0 <sup>bcd</sup>	3/6c
5	10	99/8 <sup>d</sup>	58/2 <sup>bcd</sup>	4/1bc
5	20	133/2 <sup>abc</sup>	76/2 <sup>a</sup>	5/1abc
10	zero	128/5 <sup>abcd</sup>	70/5 <sup>ab</sup>	4/6bc
10	10	104/5 <sup>cd</sup>	42/5 <sup>d</sup>	4/2bc
10	20	115/0 <sup>bcd</sup>	50/8 <sup>cd</sup>	6/3 <sup>a</sup>
SEM		1/15	8/12	1/15
Sources of Variation		P-Value		
Barley Level		>0/05	>0/05	>0/05
Threonine Supplement		>0/05	>0/05	>0/05
Threonine Sup. × Barley Lev.		>0/05	>0/05	>0/05

a,b,c The numbers in each column have a significant purity with their corresponding numbers (OP < 0.05)

Standard Error of Means = SEM, ALT = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase.

Alibeik et al. (2017) showed that adding a 30% threonine supplement did not affect the amount of aspartate aminotransferase and alanine aminotransferase. The researchers showed that plasma alanine aminotransferase activity increased with a threonine-imbalanced diet and the highest activity was observed in the late diet with excess threonine levels (2.85%) (Gao et al., 2014; Zimonja, and Svihus, 2009).

Contrary to our results, some researchers have reported that high levels of threonine did not hurt alanine aminotransferase levels (Valizadeh et al., 2014). Regarding the effect of dietary barley level on aspartate aminotransferase enzyme activity, there was no significant difference between broilers receiving the control diet and 5% barley (63 units), and there was no significant difference between the group receiving 10% barley feed and the others. The lowest amount belonged to the 10% barley group (54 out of 63 units) (P < 0.05).

The interaction effect of barley level and threonine supplement on aspartate aminotransferase enzyme activity was also significant. Therefore, among the experimental treatments, the highest aspartate aminotransferase enzyme activity belonged to the control group and 10% threonine supplement, and the lowest value belonged to the group that received 10% barley with 10% threonine supplement.

Aspartate aminotransferase is produced in the liver and catalyzes the transfer of amino groups between alpha-amino acids and alpha-keto acids. The activity of this enzyme increases in case of damage to the lungs, heart, kidneys, brain, and muscles. Alanine aminotransferase is also produced in the liver and catalyzes the transfer of amino groups between alanine and alpha-ketoglutarate to meet physiological needs. The activity of this enzyme increases during liver damage.

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