IN VITO HYDROLYSIS OF YEAST BIOMASS AND FFECT OF VARYING LEVELS OF HYDORLYZED YEAST ON THE PRODUCTION PERFORMANCE OF BROILERS

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ABSTRACT: The present research study was conducted to assess the effects of supplementation of hydrolyzed yeast (HY) in drinking water (DW) on the production performance, welfare indices, and development of immune organs in broilers. For this a total of 480 broiler chicks were allocated (randomly) into four treatment groups viz: group A was control provided 0ml/L HY in DW, group B was provided 0.2ml/L HY in DW , group C was provided 0.4ml/L HY in DW, and group D was provided 0.6ml/L HY in DW. The HY was supplemented in drinking water for 8 hours as per scheme, afterward the fresh water without HY was offered to groups. Each group was consisted of three replicates (40 birds each). The inclusion of HY in water had effect ($p < 0.05$) on FI (FI), body weight gain (BWG) feed conversion ratio (FCR), foot pad dermatitis score (FPDS) and development of immune organs (bursa; $p < 0.01$ and spleen; $p < 0.05$). Broilers receiving 0.4ml/L HY in DW for 8 hours showed the best growth efficiency and lowest FCR values. Bursa and spleen development was increased in with increasing HY levels (ml/L) in DW. The results of the trial demonstrated that supplementing HY in DW, especially @ 0.4–0.6ml/L levels improves the production performance, livability%, FPDS and health of immune organs without any adverse effects on birds welfare. Therefore it is recommended that HY at levels of up to 0.6ml/L of DW can be used as a natural functional additive to enhance performance, welfare and immunity in poultry.

Keywords: Hydrolyzed Yeast, production performance, footpad score, bursa fabricius, spleen, mortality.

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INTRODUCTION

The poultry industry is continually searching natural and cost-effective alternatives to synthetic growth promoters to insure animal welfare, public health and sustainable global poultry production. Among the promising bioactive feed additives, HY has gained attention for its multifunctional role in enhancing growth, gut health, and immunity in poultry (Gómez et al., 2011; Al-Sahlany et al., 2020; Ashayerizadeh et al., 2025; Abbas et al., 2025). HY, obtained from Saccharomyces cerevisiae, comprised of nucleotides, β-glucans, MOS, peptides, and amino acids which works as antioxidants, microbial modulators and immune boosters (Abbas, 2025; Wang *et al*., 2022; Comi r *et al.*, 2025; Perricone *et al*., 2022).

Water supplementation of additive in poultry production especially during moderate weather and summer is an efficient method of additive delivery which ensure uniform intake with minimum nutrients loss. A number of researches have reported that HY derivatives can improve production performance, antioxidant capacity and intestinal health of poultry (Bar-Dagan et al., 2023; Min *et al*., 2024; Al-Abdullatif, 2024; Comi *et al*., 2025; Perricone *et al*., 2022). However, research data regarding the direct effect of HY in DW on broiler growth and immune organs development is scanty, especially under the local geonetical conditions of Pakistan.

The spleen and bursa of Fabricius of chicken are important immune organs which are responsible for the maturation B- cells and T-cells respectively. Therefore the relative weights of these immune organs are often considered as systemic and health response to nutritional responses (Qureshi and Havenstein, 1994; Wang *et al*., 2022a). An improvement in development of these bursa, thymus and spleen reflects better modularization and health status.

Therefore, the present study was designed to investigate the effects of different levels of HY supplementation in DW on growth performance, feed conversion efficiency, foot pad condition, and immune organ development (bursa and spleen) of broiler chickens.

Materials and methods

1-Protein quantification of *Saccharomyces cerevisiae* hydrolysates through Bradford and digestibility confirm in vitro through SDS PAGE: The present

study focused on the preparation and characterization of an enzymatically hydrolyzed *S. cerevisiae* protein hydrolysate and evaluating its degree of hydrolysis, digestibility, and peptide size distribution by SDS-PAGE analysis. The work further sought to measure crude protein concentration before electrophoretic investigation using the Bradford assay for standard protein loading and appropriate comparative interpretation of findings.

Alkaline-assisted enzymatic hydrolysis was first performed by bursting *Saccharomyces cerevisiae* cells with an alkaline potassium-phosphate buffer to destabilize the cell wall and solubilize intracellular contents; the disrupted biomass was subsequently incubated for internal proteases digestion to perform controlled proteolysis and further hydrolysis of released proteins into soluble peptides and free amino acids (*Vaithanomsat, et al., 2022).* This two-step procedure including mild alkaline lysis and protease digestion is supplemented release and solubilization of proteins in cells without increasing the need for harsh mechanical disruption, and allowing modification of hydrolysis conditions (pH, temperature and time) to control extent of hydrolysis. Other methods for preparation of yeast hydrolysates from *S. cerevisiae*, alkaline-lysis and internal protease exposure has the advantages of enzymatic hydrolysis (better control, milder conditions, improved functional and nutritional quality) and chemical pre-treatment (increased cell permeability). In contrast, classical autolysis uses endogenous yeast enzymes and low heat to induce break-down but is time-consuming and less controllable; plasmolysis (salt or solvent-induced) and mechanical methods (ultrasonication, bead milling, high-pressure homogenization) can cause rapid disruption but perhaps require subsequent clarification and are susceptible to damaging heat-labile ingredients (Rigou *et al., 2022).*

Saccharomyces cerevisiae yeast biomass was treated with controlled enzymatic hydrolysis under optimized conditions for maximum conversion of cellular proteins into smaller, soluble peptides. The enzyme treatment was carried out under conditions that favor the activity of proteolytic enzymes, and in the appropriate pH and temperature to facilitate good cleavage of peptide bonds. After hydrolysis, centrifugation of the mixture was carried out to yield away undigested residues, and the supernatant containing soluble protein hydrolysate was collected for analysis. Bradford assay was employed to analyze for soluble protein content of the hydrolysate (*Rekowski et al., 2022).* The assay is based on the colorimetric binding of Coomassie Brilliant Blue dye to protein molecules in a color change proportional to protein concentration. A calibration curve with bovine serum albumin (BSA) was done under normal conditions, and spectrophotometric measurement of the hydrolysate samples' absorbance at 595 nm was carried out. Crude protein concentration so calculated was used for quantifying the amount of protein required for gel electrophoresis.

For analysis by SDS-PAGE, 30 µg per well of total protein from each sample of hydrolysates were loaded with a volume of 25 µL. As molecular weight standard, a PageRuler Prestained Protein Ladder ranging from 10–180 kDa was used as standard.

Electrophoresis was carried out under steady voltage until complete migration of dye front was achieved. Following electrophoresis, the gel was Coomassie Brilliant Blue R-250 stained to visualize protein bands. The molecular weight profile of hydrolysate peptides was determined by matching positions of sample bands with the relevant molecular weight markers of the protein ladder. The intensity and occurrence or lack of the bands at higher molecular weight were considered as a measure of hydrolysis level, while the intensity and position of lower bands were a measure of protein degradation and peptide generation.

Bradford assay confirmed the high degree of soluble protein concentration of the *S. cerevisiae* protein hydrolysate, which confirmed efficient enzymatic degradation and release of intracellular peptides. Based on these quantifications, 30 µg protein was accurately loaded into every well to have comparable characteristics across samples. The SDS-PAGE pattern of the yeast protein hydrolysate exhibited distinct bands predominantly located below the 10 kDa marker of the protein ladder, indicating extensive enzymatic hydrolysis of yeast proteins into short peptides (Marson *et al., 2022).* The complete loss of the bands of higher molecular weights confirmed that the proteolytic digestion effectively degraded the complex yeast proteins into less complex and more digestible peptide fractions. The Coomassie-stained gel showed a clear, intense band below 10 kDa, without any evidence of aggregation or under digestion, confirming the purity and solubility of the hydrolysate.

Prevalence of peptides under 10 kDa suggests the creation of very digestible and bioavailable fractions of proteins. Low-molecular-weight peptides are found to be readily absorbed by membranes of the intestine and typically associated with improved nutritional efficiency. Such observations demonstrate that *S. cerevisiae* hydrolysate can deliver a consistent supply of peptides of high biological value. The results concur with previous research that enzymatic hydrolysis of yeast proteins produces peptide fractions of heightened functional capacity, like antioxidant capacity, metal chelation capability, and improved digestibility. The hydrolysis efficiency demonstrated here reflects the optimized enzymatic conditions and justifies the potentiality of yeast biomass as a feedstock for protein hydrolysate manufacturing for feed and nutraceutical applications.

2- EFFECT OF VARYING LEVELS OF HYDROLYIS YEAST ON PRODUCTION PERFORMANCE OF BROILERS

Birds, housing, and experimental diets: A total of 480 day old male broiler chicks (Ross 308) were purchased from commercial hatchery after they were vaccinated for infectious bronchitis (IB) and reared in the semi controlled shed at the Riphah College of Veterinary Sciences, Lahore, Pakistan. Before the arrival of chicks the house was cleaned and disinfected and strict bio-security measure were activated. The chicks were randomly divided into four experimental groups in a way that each group was consisted of three replicates (40 birds each). Birds of group A were provided 0ml/L HY in DW, group B was provided 0.2ml/L HY in DW , group C was provided 0.4ml/L HY in DW, and group D was provided 0.6ml/L HY in DW. The HY was supplemented in drinking water for 8 hours as per scheme, afterward the fresh water without HY was offered to each group. The brooding temperature of the house were maintained using electric heaters whereas feeding and drinking arrangements were provided using automatic pan feeders lines, and nipple drinkers lines. The feed and water was provided *ad libitum* throughout the experimental period (42 days). A complete record of dead birds was maintained to determine the mortality% during study period. Commercial broiler starter feed was provided from 0–10 days, then the broiler grower feed was used from 11–24 days, and the broiler finisher feed was provided from 25–35 days period. Vaccination of the flock was adopted as followings: On day 5th the birds were vaccinated against ND/H9 killed vaccine via sub-cut injection and on day 10th birds were vaccinated against infectious bursal disease (IBD) through DW. On the 18th day birds were provided ND Lasota through DW and finally the ND clone vaccine was done on day 26th via DW. The shed temperature was maintained at 33°C on day 1 and then reduced gradually 0.3 per day until it reached 24°C on day 16^{th}. Relative humidity (RH) of 55-60% was maintained during the experimental period. The lighting schedule described by Abbas *et al*., 2014 was adopted. For this A light of 20 lux for 23 hours was provided during first week and 23 hours light of 5 lux intensity was provided thereafter until market age. A complete record of daily feed consumption and weekly body weights were measured for each replicate which was then converted into weekly and overall record FCR of each pen was calculated by dividing the total feed consumed over the total body weight gain (Kg/Kg)of birds in each group. The dead birds in each replicated were considered as feed intake divided by the body weight gain. A complete record of mortality for each pen was maintained. On 42th day of experimental period, two birds per replicate were taken randomly, leg tagged for identification and fasted for about 12 hours. These birds were then weighed individually, slaughtered, eviscerated to determine the spleen and bursa Weights

Also on day 42 birds of each pen were examined for foot pad dermatitis scoring system as described by Michel *et al*. (2012). Each individual bird was assigned as 0 (No lesion) or 1(mild lesion) or 2 (severe lesion). To calculate the FPD Score total numbers of score 0 or scores 1 and scores 2 were counted for each pen. Then multiplied scores 1 with 0.5 and scores 2 with 2 and then made a sum of the result which was then multiplied by 100 and divided by the total numbers of birds examined. The formula of FPDS is mentioned below

FPDS= [(0x feet with score 0) + (0.5 x feet with score 1) + (2 x feet with score 2)]/total number of birds ×100

RESULTS AND DISCUSSION

A-Protein quantification of *Saccharomyces cerevisiae* hydrolysates through Bradford and digestibility confirm in vitro through SDS PAGE: *Saccharomyces cerevisiae* hydrolysates are now a prominent source of available nutrients, especially for the production of low-cost and environmentally safe protein supplements for food, animal feed, and biotechnology applications. *Saccharomyces cerevisiae*, also known as baker's yeast, is one of the most promising because it has a high protein content, safety, and nutritional profile (*Que et al., 2024)*. Yeast biomass is a source of trace minerals, vitamins, and essential amino acids, and on enzymatic hydrolysis, its proteins get efficiently degraded into free amino acids and short-chain peptides (*Jock et al., 2022)*. Hydrolyzed peptides are very digestible, exhibit enhanced absorption rates, and functional properties such as immunomodulatory and antioxidant effects (Hou *et al., 2022)* In addition, yeast protein hydrolysates can also serve as a perfect substitute for animal proteins in various food and nutritional products to promote sustainable food production systems.

Functionally, the yeast protein hydrolysate offers various advantages to aquaculture and animal nutrition. Its short peptide chain is easily digested and assimilated at a faster rate, and its well-balanced amino acid profile leads to growth, immune health, and metabolic performance (Gilbert *et al., 2022)*. Its high solubility and low allergenicity render it a suitable alternative to animal-based protein sources in feed products. Moreover, being derived from renewable microbial biomass, the hydrolysate is a contribution towards green protein production. Collectively, quantitation with Bradford assay and SDS-PAGE characterization were convincing proof of effective enzymatic breakdown of S. cerevisiae proteins and the formation of intensely digestible peptide fractions (*Dos Santas et al., 2022)*. The resulting hydrolysate also has great promise as an environmentally friendly, low-cost,

and nutritionally augmented protein ingredient for various industrial and biological applications, including poultry and dairy feed, aquaculture feeds, and functional food additives.

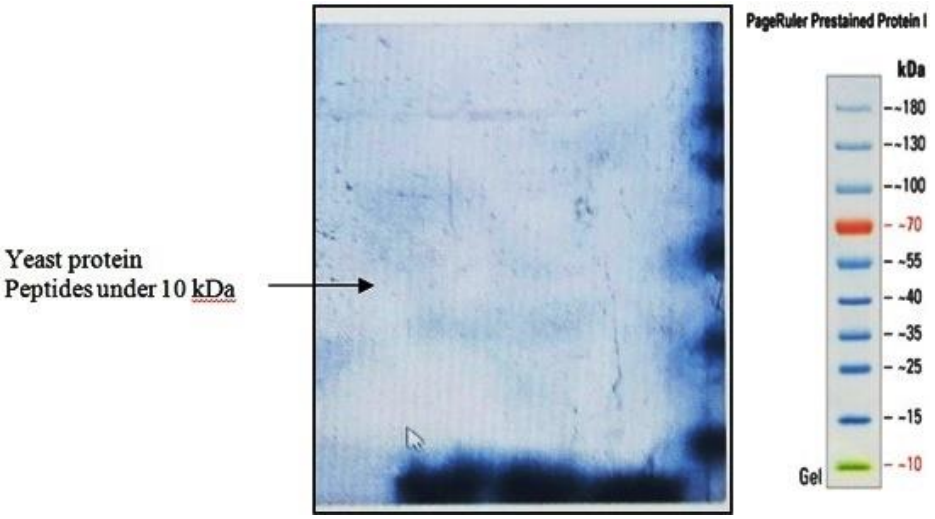

Figure: SDS-PAGE analysis of protein hydrolysates. Lane M represents the protein molecular weight marker (ladder). Lane 1 shows the sample loaded with 20 µL of hydrolysate, Lane 2 corresponds to 25 µL of hydrolysate, and Lane 3 represents 30 µL of hydrolysate.

B-EFFECT OF VARYING LEVELS OF HYDROLYIS YEAST ON PRODUCTION PERFORMANCE OF BROILERS

1-Feed intake: The FI data of birds under different levels of HY supplementation in DW (8 hours) are given in Table 1. Statistical analysis of FI data revealed significant effect ($p < 0.05$) of HY in broilers. The values (mean ± SE) for FI were 3700.67 ± 43.96 g/bird in the control group (represented by A), 3634.33 ± 18.85 g/bird in birds receiving 0.2ml/L HY (represented by B), 3517.67 ± 80.38 g/bird in the 0.4ml/L yeast group (represented by C), and 3576.67 ± 52.08 g/bird in the 0.6ml/L yeast group (represented by D).

Table 1: Effect of HY in DW on FI of broiler.

Group	Treatment	Mean ± SE
A	Control	3700.67 ± 43.96 A
B	0.2ml/L HY in DW	3634.33 ± 18.85 AB
C	0.4ml/L HY in DW	3517.68 ± 80.38 B
D	0.6ml/L HY in DW	**3576.67**±52.08AB

The analysis indicated that the control group consumed more feed ($p < 0.05$) than birds receiving 0.4ml/L HY, whilst differences amongst other groups were non-significant. Results showed slightly reducing trend in FI with increased levels of HY in DW. The inclusion of HY in DW in the current study had a significant effect on FI ($p < 0.05$), with the control group (A) having higher intake than the group receiving 0.4ml/L HY (C). This decline in FI at an intermediate yeast level is consistent with prior research showing that yeast additives can modulate appetite or nutrient utilization efficiency.

According to the research results of Sampath *et al*. (2021) published in *PMC* supplementation of HY in the diets of broilers did not affect FI ($p>0.05$) over the trial period suggesting that under certain conditions, yeast inclusion can maintain feed consumption (FC) by improving physiological outcomes. Al-Abdullatif *et al*. (2024) similarly reported positive effect of HY supplementation on intestines redox balanc during heat stress yet FC changes were modest (Al-Abdullatif, 2024).In this study the birds of control group had the highest FI, whereas the decreases FI observed in broilers provided with HY supplemented DW might be more efficient nutrients utilization.

Another evidence supporting the effect of yeast culture causing an improved in growth and FI in broilers comes from the study of Sun *et al*. (2019). Also, Gómez *et al*. (2011) in a study checked increased ($p < 0.10$) FI effect of enzymatically HY.

The decreased FI of broiler in groups supplemented HY in DW in the present study might be due to improved digestibility of nutrients, GIT health, or efficient metabolism with better microflora balance and nutrient absorption which caused the birds need less intake requirement to meet physiological demands. Ashayerizadeh et al. (2025) determined an improved production performance and immunity in broilers supplemented with HY,.

2-Body weight gain: The mean BWG of broilers subjected to various levels of HY supplemented in DW is presented in Table 2. The data analysis disclosed that

inclusion of HY affected the BW of birds ($p < 0.05$). The highest BWG was noted in birds received 0.4ml/L HY (2460.67 g/bird), and next higher BW was recorded in birds received DW supplemented by 0.6ml/L HY (2453.33 ± 39.01 g/bird). The birds of controlled group exhibited the lowest BW (2389 g/bird), whereas birds supplemented 0.2ml/L HY in DW gained intermediate results i.e. 2411.33 ± 8.41 g/bird. ANOVA confirmed significant effect of treatment effects i.e. $p = 0.036$ and Tukey's test further revealed that broilers in groups C and D had higher ($p < 0.05$) BWG than birds of control group. These results demonstrated that supplementing HY in DW at moderate levels I.e. 0.4–0.6ml/L may enhance BW in broilers.

Table 1: Effect of HY in DW on BW of broiler

Group	Treatment	Mean ± SE
A	Control	2389.00 ± 47.28B
B	0.2ml/L HY in DW	2411.33 ± 8.41B
C	0.4ml/L HY in DW	2460.67 ± 40.17AB
D	0.6ml/L HY in DW	2453.33±39.01A

The improved BWG of broilers provided DW supplemented with HY in this study agrees with previous findings that yeast based products can enhance production performance by supporting physiological mechanisms. HY contains functional nutrients like β-glucans, mannan-oligosaccharides, and nucleotides which promote gut health, nutrients uptake, and immune response (Gómez *et al*., 2011). Therefore these components work as prebiotics and support the growth of friendly micro flora in the gut whereas inhibit the count of pathogenic bacteria, hence these help to improve the efficiency of nutrients utilization .

The higher BW of broilers (at 0.4–0.6ml/L HY in DW) noted in this study proposes that these levels of HY inclusion help to optimize the balance between nutrients metabolism and gut health that might be due to better mucosal integrity or villus height of intestine helping better absorption capacity and/or transportation of nutrients (Wang *et al*., 2022; Comi *et al*., 2025). Moreover, Ashayerizadeh et al. (2025) reported that yeast-derived peptides and nucleotides rapidly absorb by hepatocytes, and enterocytes to support synthesis of protein and energy metabolism.

Furthermore, HY help to the regulate immune and oxidative stress response. Components of yeast (β-glucans) modulate the natural immune response and stimulate lymphocytes and macrophages activity, thus help the birds to maintain homeostasis during physiological and/or environmental stress (Wang *et al*., 2022; Al-Abdullatif *et al*., 2024).

The present findings also show that supplementation of HY in DW at moderate (0.4ml/L) level produced the best improvements, and that using further higher concentration (0.6ml/L) of HY did not produce any additional body weight gain. Such type of effect has also been observed in other trials where further excessive inclusion levels did not provide extra benefit either due to decreased palatability or nutrients efficiency (Wang *et al*., 2022; Gómez *et al*., 2011).

3-Feed conversion ratio: The FCR of broilers supplemented with HY in DW is shown in Table 3. The lowest (best) FCR was observed in birds supplemented with 0.4ml/L HY (1.429), followed by group D (1.458) which was provided water supplemented with 0.6ml/L HY . The birds of control group exhibited the poorest FCR (1.550), whilest the birds of group B who used water added with 0.2ml/L HY showed intermediate (1.507) FCR.

Table 3: Effect of HY in DW on FCR of broiler

Group	Treatment	Mean ± SE
A	Control	1.550 ± 0.43A
B	0.2ml/L HY in DW	1.507 ± 0.01A
C	0.4ml/L HY in DW	1.429 ± 0.013B
D	0.6ml/L HY in DW	1.458±0.0002AB

The improvement in FCR of birds used HY supplemented water observed in the current study is aligned with the results of work of previous scientists who demonstrated the positive effects of yeast and its derived components on efficiency of nutrient utilization and metabolism of animals (Gómez *et al*., 2011). Ashayerizadeh et al. (2025) also documented similar results of supplementation of yeast hydrolysate (YH) on FCR, immunity and feed efficiency in broilers. Likewise, use HY in broiler has been well documented to improve nutrients utilization and villus height which might be responsible of better FCR (Comi *et al*., 2025). Moreover, Al-Abdullatif *et al*. (2024) reported beneficial effect HY on growth rate and FCR in broiler kept under heat stress conditions. The findings of the current study confirm that supplementing HY especially at 0.4–0.6ml/L inclusion in DW yielded best FCR suggesting that HY can promote better immunity, gut health, immune modulation, and nutrient absorption.

4-Spleen weight: HY supplementation in DW affected ($p < 0.05$) spleen weight of broilers (Table 4). The lowest (1.18 g) spleen weight was noted in birds of group A (control), whilst the highest (1.38 g) spleen weigh was recorded in birds of group C who received 0.4ml/L HY in DW. The data regarding the spleen weight again support the idea that moderate (0.4ml/L) level HY supplementation in DW support to optimizes immune organs development.

The spleen (blood and lymphoid filtration organ) is an important immune organ that play vital role in cell-mediated immune response therefore, increased spleen

weight found in birds received DW with HY suggests better immune response and lymphatic tissues proliferation. HY is enriched with immunity supporting bioactive compounds like β-glucans and MOS, which might stimulated production of macrophages and lymphocytes (Ashayerizadeh *et al*., 2021) and enhanced innate as well as adaptive immune response through secretion of cytokines and antioxidants (Wang *et al*., 2021; 2022; Gómez *et al*., 2011). Similarly, Comi *et al*. (2025) observed an improved gut health and immunity function in broiler due to YH. The present findings agrees with the research results of Al-Abdullatif (2024), who determined that YH supplement enhanced oxidative stability and immunity during heat stress period.

Table 1: Effect of HY in DW on spleen weight of broiler

Group	Treatment	Mean ± SE
A	Control	1.18 ± 0.09B
B	0.2ml/L HY in DW	1.30 ± 0.03AB
C	0.4ml/L HY in DW	1.38 ± 0.02A
D	0.6ml/L HY in DW	1.30 ± 0.03AB

5-**Bursa weight:** Effect of HY supplementation had shown positive ($p < 0.01$) effect on weight of bursa in broilers (Table 5).The lowest development (1.80 g) of bursa was recorded in the broilers of control group , whilst the highest (2.37 g) growth of the organ was recorded in birds provided DW supplemented with HY @ 0.6ml/L.

Table 5: Effect of HY in DW on bursa of broiler

Group	Treatment	Mean ± SE
A	Control	1.80 ± 0.03C
B	0.2ml/L HY in DW	1.95 ± 0.06BC
C	0.4ml/L HY in DW	2.28 ± 0.07AB
D	0.6ml/L HY in DW	2.37 ± 0.08A

Bursa of Fabricius is an important lymphoid organ responsible for the production of B-lymphocyte and production of antibody, so its development is indication of immune development and competence. A increased bursa development following HY levels aligns with other findings as Ashayerizadeh et al. (2025) reported development of lymphoid tissue and immune cells proliferation due to YH and derivatives. Gómez *et al*. (2011) and Comi and coworker (2025) also observed an increase in lymphoid organ weight when broilers were provide enzymatically HY. Similarly, Al-Abdullatif (2024) noted an improvement in development of immune organ and oxidative status in broilers due to HY during heat-stress .

6-Mortality: The mortality percentage during 42 days period was non-significant ($P>0.05$) and ranged between 3.33 % to 5.83 % across the groups. Birds received HY showed lower mortality rates compared to the control group. The broilers used DW 0.6 % HY exhibited lowest mortality i.e. 3.33 % whereas control group showed 5.83 % (highest) mortality. The findings revealed that inclusion of HY in DW may improve the survivability of broiler flock. This might be due to unique composition of HY (MOS, nucleotides, functional peptides and β-glucans) which prepared the birds to better fight against the disease challenges and improve the gut health which led to decrease mortality.

Table 6: Effect of HY in DW on mortality rate of broiler

Group	Treatment	Mean ± SE
A	Control	5.83 ± 0.72A
B	0.2ml/L HY in DW	4.17 ± 0.72A
C	0.4ml/L HY in DW	4.17 ± 0.72A
D	0.6ml/L HY in DW	3.303± 0.72A

Al-Abdullatif (2024) reported similar findings HY caused improvement in oxidative status of birds which led lower down the mortality. Similarly Wang *et al*. (2021) observed an improved gut integrity and better immunity in broiler due to HY supplementation. In this trial decreased in mortality with increasing HY levels is attributed due to better micro flora balance as also documented by Wang *et al*. (2022). Although the changes were non-significant ($P>0.05$) the gradual downward decreasing trend of mortality in broiler indicates a supportive biological response (Gunun *et al*., 2022)

7-Foot pad dermatitis score: The foot pad score (%) showed a positive response to HY supplementation in DW (Table 7). The control group had the poor (17.5%) FPDS, whereas birds of group B supplemented with 0.2% HY in DW showed the best FPDS (10.83%).

Table 1: Effect of HY in DW on FPDS of broiler

Group	Treatment	Mean ± SE
A	Control	17.5 ± 2.89A
B	0.2ml/L HY in DW	9.17 ± 2.91AB
C	0.4ml/L HY in DW	13.33 ± 3.33AB
D	0.6ml/L HY in DW	10.87 ± 1.67B

The overall trend indicates a reduction in foot pad lesion percentage with increasing levels of hydrolyzed yeast, suggesting a possible improvement in welfare and litter condition, even though the differences were statistically marginal. The improvement in FPDS due to supplementation of HY might be due to improved gut health, better nutrients absorption, and maintenance

of good litter quality. Yeast based bioactive compounds e.g. β-glucans and MOS have potential to enhance intestinal integrity, thereby help to reduce moisture contents of excreta and ammonia emission in the house which positively improve the FPDS and prevent dermatitis in foot pad (Gómez *et al*., 2011; Wang *et al*., 2022). The FPDS trend in this study agrees with the findings of the research conducted by Ashayerizadeh et al. (2025) and Comi *et al*. (2025) as they reported improved villus height, health and immunity in broilers fed YH which in turn enhanced litter quality and FPDS. Al-Abdullatif *et al*. (2024) similarly found that supplementation of HY can improve physiological response and oxidative stability and welfare of birds under stressful conditions.

8-Economics of Production

Groups	Total Cost	Revenue	Profit	% increase due to HY levels used
A	326.11	373	46.68	-
B	317.40	373	55.60	+37.7
C	304.68	373	68.32	+54.4
D	307.87	373	65.13	+43.4

The economic assessment of broiler production of various groups revealed gradual increase in profitability with increasing levels of HY in DW up to 0.4%. The production cost/Kg of live body weight was decreased from Rs 326.11 (control group) to Rs 304.68 when HY used at 0.4% in DW, this might be due to improved feed efficiency, high survive-ability, and better weight gain. Consequently, the per kilogram profit margin of live broiler was increased from Rs 46.89 (control) to Rs 68.32 in the group provide 0.4% HY in DW, pointing out 54% improvement in the economic return. Though the highest level of HY (0.6%) showed a slight decrease in the net profit as compared to 0.4% level of HY, it still performed well than control and group B (0.2% HY), confirming that moderate inclusion of HY is economically optimal. These findings agree with previous studies e.g., Al-Abdullatif, 2024; Gunun *et al*., 2022 who reported improved FCR and economics of production in poultry and livestock.

Conclusion: HY supplementation in DW caused positive effect on the production performance, FPDS and development of immune organs in broilers especially at 0.4ml/L inclusion of HY in DW for 8 hours. Spleen weight and bursa weight were increased with the increase HY levels, indicated better immune and physiological development. These results of the study confirm that HY acted as a natural immunomodulator and growth promoter/enhancer, improving the health status and performance of broiler chickens without adverse effects. Therefore HY supplementation in DW at 0.4 ml/L is recommended to get optimal economical production and welfare of the poultry birds. However, future research should focus to explore the molecular mechanisms and micro flora modulation related to immunity enhancement due to the HY.

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