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Utilizing Enzymes in Feed with Date Seed Waste Meal and Their Impact on The Reproductive Performance of Indigenous Laying Hens

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استخدام الإنزيمات في العلف مع مخلفات نوى التمر المجروش وتأثيرها على الأداء التناسلي للدجاج المحلي البياض

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Abstract:
This study pointed to investigate the influence of including changed levels of date waste meal (DSWM) in the diets of laying hens (local strain) aged 24-39 weeks. The DSWM levels tested were 0.0 (control), 100 kg/ton, and 200 kg/ton. Sub-treatments for the 100 and 200 kg DSWM levels included adverse control group, phytase, Optizyme, and phytase plus Optizyme. The parameters studied were reproductive performance, egg quality, and reproductive efficiency. Results showed that the laying rate, egg weight, and egg mass were significantly enhanced by including 100 kg DSWM in isocaloric, isonitrogenous diets for laying hens related to the control group and the 200 kg DSWM level. The 200 kg DSWM had no adverse effects associated to the control. The laying rate and egg mass were greatly boosted by the addition of optzyme, however the addition of phytase over multienzyme did not result in any additional rise in these parameters. Hens given 100 kg DSWM saw increases in laying rate and egg mass thanks to enzymes; on the 200 kg DSWM diet, Optizyme showed the greatest impact. Up to 200 kg of the DSWM level in the meals of the laying hens did not have a negative effect on the feed conversion ratio (FCR); the only independent variable that significantly increased FCR was Optizyme, when compared to both the control group and the group that received phytase supplements. For laying hens given up to 200 kg DSWM, Optizyme increased FCR by about 4.5% and produced the best FCR of all the experimental groups. When compared to the control and the 200 kg DSWM diet, the dietary addition of 200 kg DSWM significantly reduced the yolk index and Haugh Unit Score, but had no adverse influence on the eggshell quality criteria for shell quality. With up to 200 kg of DSWM in the diet, there was a considerable improvement in hatchability and fertility and a significant decrease in embryonic mortality and piping chicks. The efficiency of reproduction was not negatively impacted by enzymes. The most effective reproductive treatment among the experimental treatments was feeding

a diet containing 200 kg DSWM, either with or without phytase supplementation. In conclusion, laying hens between the ages of 24-39 weeks could be fed a 200 kg DSWM diet supplemented with Optizyme without experiencing negative effects on productivity, egg quality, or reproductive function.

Keyword: Laying Hens; Date Seed Waste Meal; Egg Quality; Reproductive Performance.

الملخص

أشارت هذه الدراسة إلى معرفة تأثير ادخال مستويات متغيرة من مخلفات نوى التمر المطحون في اعلاف الدجاج البياض (السلالة المحلية) التي تتراوح أعمارها بين 24-39 أسبوعًا. مستويات مخلفات نوى التمر المطحون التي تم اختبارها كانت كالاتي (0.0 (الكنترول)، 100 كجم/طن، و200 كجم/طن). شملت المعاملات الفرعية لمستويات 100 و200 كجم من مخلفات نوى التمر المطحون مجموعة التحكم السلبية، الفيتاز، أوبتيزيم، والفيتاز مع أوبتيزيم. المعايير التي تم دراستها كانت الأداء التناسلي، جودة البيض، والكفاءة التناسلية. أظهرت النتائج أن معدل انتاج البيض، وزن البيض، وكتلة البيض قد تحسنت بشكل كبير من خلال إضافة 100 كجم من مخلفات نوى التمر المطحون على حساب الاحتياجات الغذائية المتساوية السعرات الحرارية والمتساوية النيتروجين للدجاجات البياضة مقارنة بمجموعة التحكم ومستوى 200 كجم من مخلفات نوى التمر المطحون. استخدام مخلفات نوى التمر المطحون التي تزن 200 كجم لم يكن لها أي آثار سلبية مرتبطة بالنسبة لمجموعة الكنترول. معدل وضع البيض وكتلة البيض قد زادا بشكل كبير بفضل إضافة الأوبتزايم، ومع ذلك، فإن إضافة الفيتاز على الإنزيمات المتعددة لم تؤدِ إلى أي زيادة إضافية في هذه المعايير. الدجاجات التي تم إعطاؤها 100 كجم من مخلفات نوى التمر المطحون اعطت زيادات في معدل وضع البيض وكتلة البيض بفضل الإنزيمات؛ الاعلاف التي تحتوي على 200 كجم من مخلفات نوى التمر المطحون، بينما اضافة الاوبتيزيم كان له أكبر تأثير. 200 كجم من مخلفات نوى التمر المطحون في اعلاف الدجاج البياض لم يكن له تأثير سلبي على نسبة معامل التحويل الغذائي؛ المتغير المستقل الوحيد الذي زاد بشكل كبير في مجموعة الاوبتيزيم عند مقارنته بكل من المجموعة الكنترول والمجموعة التي اخذت انزيم الفيتاز. بالنسبة للدجاج البياض الذي تم إعطاؤه حتى 200 كجم من مخلفات نوى التمر المطحون في وجود الاوبتيزيم ادى الى تحسين معامل التحويل الغذائي بحوالي 4.5% وأنتج أفضل نسبة تحويل غذائي من بين جميع المجموعات التجريبية. عند المقارنة بمجموعة الكنترول والعلف الذي يحتوي على 200 كجم من مخلفات نوى التمر المطحون، فإن الإضافة الغذائية لـــ 200 كجم من مخلفات نوى التمر المطحون قللت بشكل كبير من مؤشر الصفار ودرجة وحدة هيو، لكنها لم تؤثر سلبًا على معايير جودة قشرة البيض. إضافة 200 كجم من مخلفات نوى التمر المطحون في اعلاف الدجاج اذى الى حدوث تحسن كبير في نسبة الفقس والخصوبة وانخفاض ملحوظ في وفيات الأجنة وزيادة الكتاكيت الفاسقة. لم تتأثر كفاءة التكاثر سلبًا فبي وجود الإنزيمات. أفضل نتائج الصفات التناسلية الأكثر فعالية بين المعاملات التجريبية هو استخدام اعلاف تحتوي على 200 كجم من مخلفات نوى التمر المطحون، سواء مع أو بدون إضافة انزيم الفيتاز. يمكن تغذية الدجاجات البياضة التي تتراوح أعمارها بين 24-39 أسبوعًا على اعلاف تحتوي على 200 كجم من مخلفات نوى التمر المطحون مع إضافة انزيم الاوبتيزيم دون حدوث أى تأثيرات سلبية على الإنتاجية أو جودة البيض أو الصفات التناسلية.

الكلمات المفتاحية: دجاج البياض، وجبة مخلفات نوى التمر، جودة البيض، الأداء التناسلي.

Introduction

During the COVID-19 crisis, the lockdowns, closed borders, and traffic restrictions highlighted the importance of utilizing agricultural and agro-industrial by-products to diminish environmental pollution, [1,2]. Date by-products, common in arid regions, can be used as alternative feedstuffs for livestock and poultry diets [3]. Utilizing presented feed components can provide a sustainable explanation. In dry and semiarid areas, date (*Phoenix dactylifera*) is a major crop, with the Middle East accounting for 70% of global date production. Date production has augmented from 1.8 million tons in 1961 to 5.4 million tons in 2001. Date by-products like whole cull dates, date stones, date pulp, and pressed cakes are accessible for productions [4]. Date seeds are hard, oblong bodies found in the fruit, comprising 10-20% of its weight. Date seed meal (DSM) is a carbohydrate and fat-rich feed ingredient commonly used in livestock feed, [5]. However, its application in monogastric animal diets is restricted by the high crude fibre content of distillers' dried grains containing soluble. According to studies, broiler diets containing up to 5% DSM may contain it without having a detrimental effect on performance. Enzyme supplementation can improve nutrient digestibility in poultry diets containing DSM. DSM also has functional properties like mannans, phenolic compounds, and antioxidant activity. Including DSM in poultry diets can improve performance and feed utilization. Although the impacts of these inexpensive feed additives on chicken production performance differ throughout research, they do present viable alternatives in poultry diets.

Due to its high fibre content, date seed meal is not often used in poultry diets. Nonetheless, processing techniques have been developed to enhance chicken performance and feed utilisation efficiency [6].

Experiments were carried out in a study by [6]. to look into the impact of phytase and/or multienzymes on the way laying hens use date seed meal (DSM). In meals with additional phytase (500 FTU/kg) and/or multienzymes (0.1%), maize was substituted with DSM. Laying performance and eggshell quality decreased with complete corn substitution, but hatchability and fertility were unaffected. Phytase and multienzyme supplementation did not fully restore laying performance. Performance of hens is unaffected by the inclusion of DSM in meals up to 30% with multienzyme supplementation. Research conducted in 2004 by [7] revealed that the productivity and quality of eggs produced by laying hens were not adversely affected by substituting 16% of their diet with date waste meal or 10% with date pit meal. However, using more than 20% date seeds decreased egg quality [8] found that using date pits improved maturity in pullets and reproduction in layers. According to [9], adding date pits to laying hen meals up to 10% of the time would not have a negative impact on health or productivity; however, adding more dates would cause the gut content viscosity to increase and decrease feed intake. Older birds benefit more from date fiber, and date waste should be added in higher amounts to finisher diets. Ducks and laying hens benefit more from date waste diets than broiler chickens. Overall, incorporating date waste up to 5-10% in poultry diets can have positive effects on productivity. The research expected to explore the possessions of incorporating varying levels of DSM (0, 100 kg, and 200 kg) with or without formulating the diet based on phytase and/or optizyme supplementation on the performance of laying hens aged 24 to 39 weeks.

Material and methods

The study was conducted at the Poultry Research Station of the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, from September 2023 to June 2024.

Housing and management:

A total of 270 local strain laying hens at 24 weeks old and 54 males (for artificial insemination) were used in this trial. The hens were individually weighed, leg banded, and casually assigned to 9 experimental diet clutches, each further divided into three replicates of 10 hens each. Between 24 and 39 weeks of age, during the laying season, the diets of the animals were supplemented with 0, 100, and 200 kg per ton of date seed waste meal (DSWM). As a reference, a control group receiving a diet devoid of DSWM (0% level) was included in the experimental design. It was intended for the diets to be isonitrogenous and isocaloric. Feed analysis was conducted for moisture, ash, crude protein, ether extract, and crude fiber following [10] methods, with nitrogen-free extract intended by variance (Table 1).

Table 1. An analyzed the nutrient composition (in kg) and metabolizable energy (ME) content of the diets fed to laying hens from 24 to 39 weeks of age.

Date seed waste meal level in the laying hen diets (24-39 week of age)			
Ingredients (kg/Ton)	0.0	100	200
Yellow corn	649	575.8	446.5
Soybean meal (44%)	218.5	229	237
Wheat bran	50	0.0	0.0
Date stone	0.0	100	200
Soybean meal oil	0.0	6	25
Dicalcium phosphate	15	15.5	16
Limestone	61	64.5	66
Sodium chloride	3	3	3
Vit+Min Mix. [1]	3	3	3
L-Lysine	0.0	1.8	1.9
Dl-methionine	0.5	1.4	1.6
Total	1000	1000	1000
Intended analysis[2], (%)			
ME kcal/kg	2728	2734	2725
CP	15.1	15.1	15.10
Methionine	0.35	0.39	0.40
TSAA	0.66	0.66	0.66
Lysine	0.91	0.91	0.92
Calcium	2.97	2.96	2.96
Available phosphorus	0.35	0.35	0.35

Determined analysis[3] (%)			
Dry matter	90.4	89.89	89.73
CP	15.2	14.91	14.76
EE	2.89	3.75	6.21
CF	3.15	3.58	4.44

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12000 IU; vitamin E, 20 IU; menadione, 1.3 mg; Vit. D_3, 2500 ICU; riboflavin, 5.5 mg; Ca pantothenate, 12 mg; nicotinic acid, 50 mg; choline chloride, 600 mg; vitamin B_{12}, 10 µg; vitamin B_6, 3 mg; thiamine, 3 mg; folic acid, 1.0 mg; d-biotin, 50 µg. Trace mineral (milligrams per kilogram of diet): Mn, 80; Zn, 60; Fe, 35; Cu, 8; Se, 0.60. [2]Calculated values [11]. [3]Determined values [10].

Each DSWM-containing diet group was further divided into four subgroups: one fed an unsupplemented diet, one with microbial phytase supplementation (500 U of Ronozyme phytase®), one with 0.1% Optizyme-P5® supplementation, and one with a combination of phytase and Optizyme. The diets were expressed to meet recommended stages of CP, ME, calcium, available phosphorus (AP), lysine, and total sulfur amino acids (TSSA) according to [11] guidelines. Water and food were given out on a regular basis. Egg laying rate, egg weight, feed consumption, and viability were recorded for each group. The feed conversion ratio (FCR) and egg mass were determined throughout the experiment. Egg quality characteristics and reproductive efficiency were evaluated at 28, 32, and 36 weeks of age.
Measurements during the laying period:

Hen-day egg production (HD) % = (Egg number / Period (days)) x 100

Every day, eggs were gathered and tallied in order to precisely track production levels. The number of eggs multiplied by the average egg weight yielded the egg mass. By deducting the amount of feed left over from the amount delivered, the weight of feed consumed (measured in grammes) per replicate per time was determined. The feed eaten was divided by the egg mass to determine the feed conversion ratio (FCR), which is the amount of feed (in kilogrammes) needed to produce one kilogramme of eggs. Using four eggs per replicate at 28, 32, and 36 weeks of age, the quality of the eggs was evaluated three times using the techniques of [12] and [13].

Egg Shape Index:
According to [14]; [15], the Egg Shape Index was computed by dividing the egg's transverse diameter by its length and then multiplying the result by 100. These measurements were taken, with a caliper, to the closest millimetre.

Egg Shape Index = (width / length) x 100.

Eggshell Weight:

Shell Percentage = (Shell Weight / Egg Weight) x 100.

Shell Thickness:
The average thickness of the shell with membranes was determined in millimeters using a micrometer at the midsection and also at the two poles of the egg.
Albumen Percentage:

The albumen (%) = albumen weight (g)/ egg weight (g) × 100.

Haugh Unit Score:
A tripod micrometre was used to measure the albumen height to the closest 0.01 mm. To avoid the chalazae, the height was measured twice on different sides of the yolk. The Haugh unit score was determined by averaging these measurements.

Yolk Index:
Following the approach of [16], the yolk index—this is the ratio of the yolk's height to width—was determined after the albumen and yolk were separated. To get the yolk percentage, the yolk weight was calculated, divided by the weight of the egg, and then multiplied by 100.

Fertility, Hatchability, and Embryonic Mortality:
Throughout the study, 28, 32, and 36 weeks of age were used to assess hatchability and fertility. Over a seven-day period, eggs were gathered and kept in an egg chamber with a dry bulb temperature of 15.5ºC and a relative humidity of 70%. In automatic incubators, incubation was conducted at 37.6ºC with a relative humidity of 55%, and hatching occurred at 36.8ºC with a relative humidity of 65%. Infertile eggs were removed and the eggs were candled on day eighteen. To differentiate between eggs that were infertile and those that contained dead embryos, eggs that had not hatched by day 21 were

opened. The entire egg set's fertility and hatchability were determined using the technique outlined by [17]. Analytical Statistics: The General Linear Model technique in the Statistical Analysis System [18] was utilised to analyse the data by one-way ANOVA. The model used was Yik = µ + Ti + eik, in which Y is the dependent variable, T is the effect of the experimental treatments, and e is the random error of the experiments. Duncan's new multiple range test was used to assess differences between means [19].

Results and discussion

Laying performance

Laying rate

The table 2 shows the interaction effect between enzyme supplementation and varying levels of date seed waste meal (DSWM) on egg production rates. A significant interaction effect was observed during the 24-27, 28-31, and 32-35 weeks of age, as well as throughout the entire experimental period.

Table 2 Enzymes and dietary amounts of DSWM affected the percentage laying rate (head/day) of laying hens between the ages of 24 and 39 weeks.

Treatments	Laying rate (%) during different age Period /week				
	24-27	28-31	32-35	36-39	24-39
(+) Control	56.5^{a}	60.2^{b}	64.7^{b}	67.9	62.3^{b}
Date seed waste meal 100 kg					
(-) Control	51.7^{b}	54.6^{c}	60.1^{c}	62.3	57.2^{c}
Phytase	57.3^{a}	60.7^{b}	64.6^{b}	65.7	62.1^{b}
Optizyme	48.5^{c}	66.4^{a}	68.5^{a}	70.8	63.6^{ab}
Phytase+Optizyme	49.0^{bc}	65.8^{a}	72.7^{a}	73.2	65.2^{a}
Date seed waste meal 200 kg					
(-) Control	56.1^{a}	59.8^{bc}	63.0^{bc}	63.2	60.6^{bc}
Phytase	52.7^{b}	59.6^{bc}	64.2^{b}	68.2	61.2^{b}
Optizyme	60.8^{a}	64.7^{a}	68.6^{a}	68.9	65.8^{a}
Phytase+Optizyme	52.9^{b}	61.2^{b}	67.8^{ab}	71.7	63.5^{ab}
SEM	0.85	1.32	1.61	1.52	0.71
P value	**0.0001**	0.002	0.0001	0.0971	0.0006

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).

Without enzyme supplementation, incorporating 100 kg DSWM led to an 8.2% decrease in laying rate compared to the positive control. In contrast, adding 200 kg DSWM resulted in a smaller decline of 2.7% compared to the 8.2% decrease with 100 kg DSWM. This difference may be due to the higher fat content in 200 kg DSWM, which likely helped balance energy levels and improve overall performance and energy utilization, [20]; [21] and [22].

The study originates that adding phytase expressively augmented the laying rate of hens fed a 100 kg DSWM diet, resulting in an 8.6% overall improvement. However, the impact of phytase on hens fed a 200 kg DSWM diet was less pronounced. Multienzyme supplementation improved the laying rate of hens on a 100 kg DSWM diet by 11.1% after 27 weeks of age compared to the regulator group. Similarly, hens on a 200 kg DSWM diet showed an 8.6% increase in laying rate with multienzyme supplementation. The reduced effect of multienzymes on the 200 kg DSWM diet may be due to higher oil levels balancing the diet's energy value. Additionally, the type of fat present in the diet can affect gut viscosity and the digestibility of fatty acids, potentially limiting enzyme effectiveness on viscous grains, [23]; [24]; [25].

A combination of phytase and multienzymes showed superior performance compared to phytase and multienzymes alone, but this was only evident after hens reached 31 weeks of age on a 10% DSWM diet and after 35 weeks of age on a 200 kg DSWM diet. This combination led to a modest 2.5% increase in laying rate throughout the experimental period for hens on the 100 kg DSWM diet, the 20% DSWM diet did not negatively impact egg production. These results indicate that multienzymes effectively enhance laying rates in hens fed both 100 kg and 200 kg DSWM diets. Notably, hens on a 200 kg DSWM diet supplemented with a multienzyme mixture (Optizyme) achieved the highest laying rate for the duration of the experiment. Furthermore, hens on a 100 kg DSWM diet supplemented with a combination of phytase and multienzymes achieved a laying rate comparable to those on the 20% DSWM diet with multienzymes. These results are similar to the findings of [26] who found that multienzymes combinations were effective in improving (P < 0.05) AMEn. Comparable outcomes were documented by [27]; [28]; [29] and [30]. They revealed that multienzymes mixture containing different carbohydrase and protease improved egg production of laying hens. In summary, hens fed a diet containing 200 kg DSWM supplemented with multienzymes, as well as those on a diet with 100 kg DSWM supplemented with a combination of phytase and multienzymes, showed the highest laying rates. This indicates that enzyme supplementation has a significant influence on improving egg production in hens during the early phase of their production cycle, particularly between 24 and 39 weeks of age.

Egg weight

The table shows the combined impact of enzyme supplementation and date seed waste meal (DSWM) on egg weight Table 3. A significant interaction was noted between the levels of enzyme supplementation and DSWM during the periods of 28-31 weeks and 32-35 weeks, as well as across the entire experimental duration of 24-39 weeks of age. Notably, at the unsupplemented level of 100 kg DSWM, the egg weight increased by 6.9% and 9.6% for the age groups of 24-27 weeks and 32-35 weeks, respectively, resulting in an overall increase of 6.6% compared to the positive control. On the other hand, compared to the positive control, the unsupplemented 200 kg DSWM level consistently maintained egg weights over the study period; nonetheless, it was much lower than the unsupplemented 100 kg DSWM level. In comparison to their negative controls, hens fed a 100 kg DSWM diet showed a considerable drop in egg weight with enzyme supplementation, while hens fed a 200 kg DSWM diet showed a modest decrease. However, during the 28–31-week period, enzyme supplementation resulted in a slight increase in egg weight for hens consuming a diet with 200 kg DSWM. These findings suggest that hens tend to prioritize nutrient allocation to support laying rates over egg weight in response to enzyme supplementation. It is well known that there is a negative correlation between laying rate and egg weight [31]; [32].

Table 3 Effects of dietary levels of DSWM and addition of enzymes on egg weight (g) of laying hens during 24-39 week of age

Treatments	**Egg weight (g) during different age periods (Week)**				
	24-27	**28-31**	**32-35**	**36-39**	**24-39**
(+) Control	41.1	43.3^{b}	45.0^{c}	47.0	44.1^{c}
Date seed waste meal 100 kg					
(-) Control	42.6	46.5^{a}	49.3^{a}	49.9	47.0^{a}
Phytase	41.8	44.5^{ab}	47.3^{b}	48.6	45.5^{b}
Optizyme	40.7	45.2^{a}	48.1^{ab}	49.9	46.0^{ab}
Phytase+Optizyme	41.2	44.6^{ab}	47.0^{b}	49.6	45.6^{b}
Date seed waste meal 200 kg					
(-) Control	41.3	42.9^{c}	44.8^{c}	47.2	44.0^{c}
Phytase	40.9	43.2^{b}	44.9^{c}	46.8	44.0^{c}
Optizyme	40.8	43.1^{b}	44.4^{c}	46.2	43.6^{d}
Phytase+Optizyme	40.7	43.2^{b}	44.5^{c}	46.6	43.8^{d}
SEM	0.34	0.43	0.43	0.54	0.19
P value	0.076	0.03	0.03	0.082	0.02

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).

Egg mass:

The table below illustrates the interaction between enzyme supplementation and date seed waste meal (DSWM) on egg mass. Between 24-27 and 28-31 weeks of age, there was a significant contact among the levels of enzyme supplementation and DSWM on egg mass; from 24 to 39 weeks of age, there was a trend towards significance (P<0.07). In particular, over the 24–27-week period, the addition of 10% DSWM to unsupplemented meals led to a 5.2% decrease in egg mass in comparison to the positive control. This trend continued throughout most of the experimental duration. On the other hand, the addition of 200 kg DSWM had a more pronounced negative impact on egg mass after 31 weeks of age, leading to a 3.6% decrease over the entire experimental period compared to a 2.2% decrease with 100 kg DSWM. This difference could be due to the increased egg weight detected in hens fed the 100 kg DSWM diet, while egg production remained consistent between the two DSWM levels.

Moreover, the inclusion of phytase significantly improved the laying number of hens on diets containing 100 kg DSWM throughout the experimental period, resulting in an overall increase of 5.2%. In contrast, the result of phytase on the laying rate of hens fed 200 kg DSWM was less pronounced. Notably, supplementation with multienzymes led to a substantial increase in the laying rate of hens on the 10%

DSWM diet after 27 weeks of age, showing a 9.3% improvement matched to the negative control above the intact trial period, demonstrating greater efficacy than phytase.

Table 4 The impact of different dietary levels of DSWM and the inclusion of enzymes on the egg mass (in grams) of laying hens between 24 and 39 weeks of age.

Treatments	**Egg mass (g) during different age periods (Week)**				
	24-27	**28-31**	**32-35**	**36-39**	**24-39**
(+) Control	23.2^{b}	26.0^{b}	29.2	31.8	27.6
Date seed waste meal 100 kg					
(-) Control	22.0^{c}	25.4^{c}	29.5	31.1	27.0
Phytase	24.0^{a}	27.0^{b}	30.5	31.9	28.4
Optizyme	19.8^{c}	30.1^{a}	33.0	35.3	29.5
Phytase+Optizyme	20.2^{c}	29.2^{a}	34.2	36.4	30.0
Date seed waste meal 200 kg					
(-) Control	23.1^{b}	25.7^{c}	28.2	29.7	26.7
Phytase	21.6^{c}	25.8^{c}	28.9	31.9	27.0
Optizyme	24.8^{a}	27.9^{ab}	30.5	31.9	28.8
Phytase+Optizyme	21.5^{c}	26.4^{b}	30.2	33.4	27.9
SEM	0.40	0.64	0.82	0.81	0.36
P value	**0.0001**	**0.005**	**NS**	**NS**	**0.07**

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).

Additionally, multienzyme supplementation improved the egg mass of hens on the 200 kg DSWM diet, resulting in a 7.9% increase in egg mass associated to the respective regulator during the course of the trial duration. This suggests that the enzyme mixture had a more significant effect on diets containing 100 kg DSWM than on those with 200 kg DSWM. It was also indicated that gut viscosity could influence the type of fatty acids present and reduce their digestibility, thereby limiting the effectiveness of enzymes on viscous grains, [33]; [34]; [35]. The communication effect among enzymes and date seed waste meal (DSWM) on egg mass is summarized in Table 4. Notably, the combination of phytase and multienzymes showed superior performance during specific phases of the laying period compared to using either enzyme individually. This was particularly evident after the hens reached 31 weeks of age on a diet containing 100 kg DSWM and after 35 weeks on a diet with 200 kg DSWM. Throughout the study, the combination of phytase and multienzymes led to a 5.6% increase in egg mass for hens on a 100 kg DSWM diet and a 3.3% increase for those on a 200 kg DSWM diet. In contrast, combining multienzymes and phytase resulted in a 1.7% increase in egg mass for hens fed 100 kg DSWM compared to using multienzymes alone, indicating that multienzymes were effective in improving egg mass on both 100 kg and 200 kg DSWM diets. The maximum egg mass was detected in hens fed a 100 kg DSWM diet supplemented with both multienzymes and phytase, showing an 8.7% increase over the positive control, although this group did not significantly differ from those receiving multienzymes alone.

Feed intake

Results for the interaction effect between enzymes and date seed waste meal (DSWM) on feed intake are presented in Table 5. Over the course of the trial, which lasted from 24 to 39 weeks of age, there was a substantial interaction between the enzymes and the dietary level of DSWM. The results showed that the inclusion of 200 kg DSWM in unsupplemented controls controlled to a reduction in feed intake, which became more pronounced after 28 weeks of age. Specifically, the inclusion of 200 kg DSWM reduced feed intake by 2.9% and 3.7% compared to the negative and positive controls, respectively. The addition of enzymes caused variations in feed intake between the 100 kg and 200 kg DSWM levels, suggesting that the age of the hens influences the impact of enzymes on feed consumption. Overall, feed intake for the entire experimental period increased by approximately 1.5% with the adding of phytase or multienzymes associated to the unsupplemented control. However, a combination of phytase and multienzymes did not result in further increases in feed intake at the 100 kg DSWM level. In contrast, phytase and multienzymes significantly increased feed intake by approximately 3.5% in hens fed 200 kg DSWM individually. The combination of phytase and multienzymes did not lead to additional improvements in feed intake. In addition to the enhanced nutrient utilization, the increased feed intake in enzyme-supplemented groups may explain the improved laying performance observed in these groups. Similarly, [36]; [37],[38] and [39] found that enzymes significantly increased feed intake of laying hens.

Table 5 The impact of different dietary levels of DSWM and the inclusion of enzymes on the feed intake (in grams per hen per day) of laying hens between 24 and 39 weeks of age.

Treatments	Feed intake (g/h/d) during different age periods				
	24-27	**28-31**	**32-35**	**36-39**	**24-39**
(+) Control	103.6^{c}	106.9^{b}	109.0^{b}	112.0^{a}	107.9^{ab}
Date seed waste meal 100 kg					
(-) Control	104.8^{c}	106.6^{b}	106.5^{c}	110.2^{b}	107.0^{b}
Phytase	104.1^{b}	107.9^{a}	110.9^{a}	111.3^{ab}	108.6^{a}
Optizyme	104.2^{b}	108.2^{a}	110.2^{a}	111.5^{ab}	108.5^{a}
Phytase+Optizyme	105.2^{a}	106.8^{b}	108.6^{b}	109.4^{b}	107.5^{b}
Date seed waste meal 200 kg					
(-) Control	103.0^{c}	105.3^{bc}	104.4^{d}	105.0^{c}	103.9^{c}
Phytase	103.3^{c}	106.4^{b}	108.9^{b}	111.4^{ab}	107.5^{b}
Optizyme	104.2^{b}	105.5^{bc}	108.1^{b}	110.8^{b}	107.2^{b}
Phytase+Optizyme	104.1^{b}	103.3^{c}	110.1^{a}	111.7^{a}	107.9^{ab}
SEM	0.34	0.24	0.28	0.13	0.14
P value	**0.002**	**0.0004**	**0.0001**	**0.0001**	**0.0001**

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).

Feed conversion ratio:

The data in Table 6 show the interaction effect between enzymes and DSWM on FCR. A significant interface was seeing among enzyme supplementation and DSWM levels on FCR during weeks 24-27, 32-35, and 24-39 of age, with a trend towards significance during weeks 28-31. In the 24–27-week period, inclusion of 100 kg DSWM without enzyme supplementation caused in a 6.9% impairment in FCR matched to the positive regulator group. This trend continued throughout most of the experimental period, leading to 2.0% impairment in FCR for the entire study. However, inclusion of 200 kg DSWM did not negatively impact FCR during the experimental period. This lack of negative effect with 200 kg DSWM could be due to increased oil supplementation to maintain energy levels, [40]; [41]; [42] which could overcome the negative effect of DSWM crude fibre and/or NSP on energy availability for egg production.The addition of phytase improved the feed conversion ratio (FCR) of hens fed diets containing 100 kg of dried spent wheat malt (DSWM) during weeks 24-27 and 28-31, resulting in a 4.2% improvement over the entire experimental period compared to the negative control.

Table 6 Enzymes and dietary levels of DSWM affected the laying hens' feed conversion ratio (g feed/g egg) over a 24-39-week period.

Treatments	Feed conversion ratio (g/g) during different age periods (Week)				
	24-27	**28-31**	**32-35**	**36-39**	**24-39**
(+) Control	4.49^{c}	4.13	3.80^{ab}	3.56	3.99^{ab}
Date seed waste meal 100 kg					
(-) Control	4.80^{b}	4.26	3.64^{b}	3.58	4.07^{ab}
Phytase	4.38^{c}	4.04	3.67^{b}	3.53	3.90^{b}
Optizyme	5.33^{a}	3.67	3.39^{c}	3.18	3.89^{b}
Phytase+Optizyme	5.27^{a}	3.71	3.26^{c}	3.10	3.84^{b}
Date seed waste meal 200 kg					
(-) Control	4.50^{c}	4.07	3.77^{ab}	3.65	4.00^{ab}
Phytase	4.82^{b}	4.23	3.96^{a}	3.56	4.14^{a}
Optizyme	4.24^{c}	3.85	3.63^{b}	3.54	3.82^{b}
Phytase+Optizyme	4.87^{b}	4.07	3.73^{ab}	3.45	4.03^{ab}
SEM	0.089	0.100	0.11	0.09	0.05
P value	**0.0001**	**0.06**	**0.003**	**0.099**	**0.003**

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).

Nevertheless, phytase did not improve the FCR of pullets fed 20% DSWM, and in fact, had a negative impact, possibly due to increased feed consumption. Multienzyme supplementation improved the FCR of hens fed 100 kg DSWM after 27 weeks of age, leading to a 4.4% enhancement over the entire experimental period associated to the negative controller, similar to the influence of phytase in diets containing 100 kg DSWM. Additionally, multienzyme addition to the 200 kg DSWM diet significantly improved FCR compared to phytase, resulting in a 4.5% and 7.7% improvement over groups fed diets containing 20% DSWM without or with phytase addition, respectively. This suggests that multienzymes consistently improve the FCR of hens fed 100 and 200 kg DSWM. Moreover, there was no cumulative effect between phytase and multienzymes, as evidenced by the fact that the combination of the two did not produce better outcomes than multienzymes alone. It appears that multienzymes alone are sufficient for improving the FCR of hens fed up to 200 kg DSWM.

The increased FCR of the groups supplemented with enzymes is consistent with the findings of [43] and [44], [45] and [46]. They indicated that the enhancement in nutrient utilization could not be attributed only to the liberation of nutrients complexes to cell wall, but likewise owed to elimination of the adverse result of NSP on nutrient utilization. In conclusion, multienzymes enhanced feed conversion ratio (FCR) by approximately 4.5% in laying hens fed up to 200 kg of dried swine waste meal (DSWM). Additionally, both multienzymes and phytase improved FCR by 5.7% in a diet containing 100 kg of DSWM compared to the negative control. Phytase and multienzymes also improved FCR in the 100 kg DSWM diet, but to a lesser extent.

Egg Quality:

The effects of the interaction between the level of date seed waste meal (DSWM) and enzyme supplementation on the quality of eggs produced by laying hens are displayed in Table 7. On the other egg quality criteria, there was no significant interaction; however, there was a significant effect on the egg shape index.

Table 7 Impact of dietary DSM levels and enzyme addition on hens' egg quality standards throughout 24-39 week of age.

Treatments	Egg quality criteria					
	ShI	**YI**	**HU**	**SW**	**S**	**ST**
(+) Control	70.5^{c}	38.6	87.7	5.03	9.98	0.458
Date seed waste meal 100 kg						
(-) Control	74.2^{a}	38.4	86.2	5.56	10.52	0.478
Phytase	75.3^{a}	40.3	86.4	5.53	10.51	0.472
Optizyme	70.9^{c}	38.4	84.7	5.45	10.51	0.453
Phytase+Optizyme	72.5^{b}	38.1	85.0	5.33	10.31	0.479
Date seed waste meal 200 kg						
(-) Control	72.1^{b}	33.7	81.9	4.92	9.74	0.490
Phytase	71.9^{b}	34.8	82.8	4.99	9.80	0.481
Optizyme	73.2^{ab}	33.1	81.6	5.15	10.24	0.482
Phytase+Optizyme	74.6^{a}	35.9	84.3	4.87	9.59	0.481
SEM	1.12	1.10	1.19	0.12	0.17	0.003
P value	**0.02**	**0.045**	**0.091**	**0.08**	**0,103**	**0.056**

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05).
ShI=Shape index; YI= Yolk index; HU= Haugh unit; SW= Shell weight (g); S=Shell (%); ST= Shell Thick. (mm).

Hens fed 100 or 200 kg DSWM without enzyme supplementation had higher egg shape index, with 100 kg DSWM showing a greater effect. However, multienzymes and a combination of phytase and multienzymes decreased egg shape index in hens fed 100 kg DSWM, while increasing it in hens fed 200 kg DSWM. The best Haugh unit score was observed in hens fed the control diet, while the best shell weight and percentage shell were from hens fed 100 kg unsupplemented diet. This suggests that including 100 kg DSWM improved shell quality, indicating better calcium utilization for eggshell formation. Also, [47] and [48] found that shell quality was improved due to inclusion of dates and attributed this to sucrose contents of dates, which was found to improve calcium utilization [49].

Reproductive efficiency:

The data in Table 8 show the impact of the interaction between date seed waste meal (DSWM) level and enzyme supplementation on the reproductive efficiency of laying hens. There was no notable interaction between the two variables DSWM and enzyme supplementation on reproductive efficiency, except for a significant interaction observed in fertility and hatchability. Inclusion of unsupplemented 100 kg or 200 kg DSWM increased fertility significantly, with 200kg DSWM having a greater influence than 100kg DSWM compared to the unsupplemented control.

Table 8 The impact of different dietary levels of DSWM and the inclusion of enzymes on the reproductive characteristics of laying hens between 24 and 39 weeks of age.

Treatments	Reproductive traits				
	F	H	EED	LED	P
(+) Control	90.8^{c}	80.9^{d}	1.53	0.76	7.65
Date seed waste meal 100 kg					
(-) Control	93.9^{b}	90.5^{c}	0.51	0.76	2.29
Phytase	92.1^{bc}	87.7^{c}	0.25	2.04	2.04
Optizyme	93.9^{b}	91.1^{b}	0.25	0.76	1.78
Phytase+Optizyme	94.1^{b}	92.6^{b}	0.25	0.76	1.02
Date seed waste meal 200 kg					
(-) Control	98.0^{a}	94.8^{a}	0.51	1.02	1.53
Phytase	97.4^{a}	94.8^{a}	0.51	1.27	0.76
Optizyme	95.2^{b}	91.5^{b}	0.51	1.02	2.04
Phytase+Optizyme	93.1^{b}	87.7^{c}	0.51	1.27	3.57
SEM	1.06	1.67	0.31	0.54	0.90
P value	**0.03**	0.01	0.94	0.082	0.069

$^{a-c}$ Means within a column not sharing similar superscripts are significantly different (P<0.05), NS (P>0.05). F=Fertility; H=Hatchability; EED= Early embryonic dead; LED=Late embryonic dead P= Piped

Enzymes did not significantly affect fertility of hens fed 100 kg DSWM, while multienzymes without or with phytase decreased fertility of hens fed 200 kg DSWM. Results indicated that hatchability of total egg set was highest in hens fed 200 kg DSWM without or with phytase compared to the control. In conclusion, feeding a diet containing 200 kg DSWM without or with phytase administration resulted in the best reproductive efficiency, highlighting the positive impact of DSWM on fertility and hatchability. Also, in a study conducted by [50], it was discovered that the fertility and hatchability of eggs improved when dates waste was included in the quails' drinking water, with or without yeast administration. Additionally, there was a decrease in non-pipping embryos and embryonic mortality.

Conclusion and application:

Supplementing Optizyme to a 200 kg DSWM diet during the laying period (24-39 weeks of age) did not negatively impact bird performance. This enzyme mixture enhances the utilization of NSP in DSWM, offering an economical biotechnological solution to enhance the utilization of low-quality agro-industry by-products. This allows for maximum utilization in the nutrition of laying hens, reducing the need for imported feedstuffs and saving on foreign currency expenditure.

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