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Full Length Research

Effect of feeding diet types containing *Pleurotus ostreatus* (oyster mushroom) on gut and reproductive traits of pullets

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ABSTRACT: In response to the growing concerns of the health of people worldwide, there has been increased pressure to find safe, natural alternatives to antibiotic growth promoters in poultry production. The current experiment compared the impacts of the dietary inclusion and feed form of oyster mushrooms on the gut and development of the reproductive organs of the pullets. 320 fourteen-week-old pullets were randomly divided into four dietary treatments (8 replicates) in a 2 × 2 factorial layout that included 2 feed forms (mash and pellet) and 2 inclusion levels of oyster mushroom (0 and 1 g/kg). Each treatment had ten replicates of eight birds each. Reproductive organ parameters were assessed at weeks 16, 20, and 24 by slaughtering two birds per replicate. Data collected included oviduct length, follicle number, follicle weight, ovary number, and ovary weight. Results showed no observable reproductive organ development at week 16. At week 20, oyster mushroom inclusion of 1 g/kg (1.00 g) significantly ($p < 0.05$) reduced follicle weight compared to control (1.75g), while other parameters were not significantly affected. At week 24, feed form significantly ($p < 0.05$) influenced ovary number and ovary weight, with pellet-fed birds showing higher values (7.50) and (37.50g) compared to mash-fed birds. However, oyster mushroom inclusion had no significant effect ($p > 0.05$) on most reproductive parameters. It was concluded that pelletized feed enhances reproductive organ development in pullets, while dietary inclusion of *Pleurotus ostreatus* at 1 g/kg has a limited influence.

Keywords: Feed type, follicle, layers, pellets, poultry.

INTRODUCTION

The poultry industry remains one of the fastest-growing sectors of animal agriculture globally, contributing significantly to food security, employment, and the supply of high-quality animal protein. In Nigeria, poultry production plays a vital role in bridging the animal protein gap due to its affordability and rapid turnover rate (Food and Agriculture Organisation, 2019; Daramola and Omokpariola, 2025). Antibiotics have reportedly been used as growth promoters and infection preventatives in poultry (Azizi *et al.*, 2024), but their excessive use has contributed to the development of antibiotic-resistant bacteria, prompting global restrictions on antibiotics in animal feeds. The increasing restriction on the use of antibiotic growth promoters (AGPs) due to antimicrobial resistance concerns has necessitated the search for safe and sustainable alternatives (Agbai *et al.*, 2023).

Natural feed additives such as probiotics, phytogenics, and medicinal mushrooms have gained considerable attention as viable alternatives. These natural products are increasingly recognised not only for improving growth performance and immunity but also for their beneficial influence on reproductive development and function in poultry and other livestock species (Abdelli et al., 2021). Among these, oyster mushroom (OM) (*Pleurotus ostreatus*) is of particular interest due to its rich content of bioactive compounds, including β-glucans (polysaccharides), Phenolic compounds, Ergothioneine, Flavonoids (antioxidants), and antimicrobial agents, which have been reported to enhance immune response and gut health in poultry (Salahuddin *et al*., 2025). Previous studies have demonstrated that mushroom supplementation can improve growth performance and disease resistance (Suberu *et al*., 2024); however, its effect on reproductive development in pullets remains inadequately (Alabi *et al*., 2025). Previous studies have demonstrated that mushroom supplementation can improve growth performance and disease resistance (Suberu et al., 2024); however, its effect on reproductive development in pullets remains inadequately explored (Alabi *et al*., 2025).

Reproductive efficiency is a critical determinant of productivity in layer birds, as optimal development of the ovary and oviduct is essential for egg production and quality. The onset of sexual maturity and reproductive performance is influenced by several factors, including nutrition, genetics, and management practices (Assersohn *et al*., 2021; Tan *et al*., 2021). Nutritional interventions using functional feed additives have been explored to enhance reproductive development, but responses vary depending on dosage and dietary composition.

Feed form is another important factor influencing poultry performance. Pelletized diets have been reported to improve feed intake, nutrient digestibility, and overall performance compared to mash diets due to reduced feed wastage and improved palatability (Abdollahi *et al*., 2013; Abu *et al*., 2023). Despite these reported benefits, variations in responses have been observed due to differences in dosage, bird species, environmental conditions, and composition of phytogenic products. Some studies have reported inconsistent effects, indicating that the efficacy of natural antibiotics depends on inclusion level, bioavailability, and interaction with other dietary components (Aminullah et al., 2025). Therefore, this study was designed to evaluate the effects of feed form and dietary inclusion of oyster mushroom (*Pleurotus ostreatus*) on the reproductive organ development of pullets.

MATERIALS AND METHODS

Location of the study

The experiment was conducted at the Poultry Unit, Directorate of University Farms (DUFARMS) at the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm approximately lies at 7° 13' 47''N latitude and 3° 26' 21'' E longitude, experiencing an average annual rainfall of about 1,456 mm.

Processing of oyster mushroom and pelletized feed

Pleurotus ostreatus (fresh oyster mushrooms) were sourced within the Abeokuta metropolis at the fruit and vegetable market centre. It was oven dried at 60°C, milled and forced through a 5mm sieve, and afterwards added to the diet of the birds by hand mixing. The feed ingredients were milled to form mash feed; part of the mash was stored for the experiment, while the other part was further ground to pass through a 1 mm sieve screen. Water was utilised to achieve a suitable paste consistency for manual execution of the extrusion process, and the paste was homogenised through kneading for 10 mins. Mobile extrusion equipment produced pellets of 2 mm dimension through its operation. The extruder strands were oven dried at 300°C for 24 hours and was stored in a plastic drum at room temperature.

Experimental birds and management

Three hundred and twenty (320) Harco Black pullets at the point of cage were procured from Sam farm, Kobape, Ogun State. The birds were randomly allocated to four experimental treatments of 80 birds each in a 2*2 factorial arrangement (2 forms of feed, mash and pellet by 2 levels of Oyster mushroom inclusion, 0 g/kg and 1 g/kg), each repeated eight times with ten birds in each replicate. The birds were reared in an intensive deep litter system where they had *ad libitum* feeding and water. The study was conducted following a strict medication and vaccination regimen. The duration of the experiment was 10 weeks. The experimental diets (shown in Table 1) were given to each bird at the rate of 125 g per day.

Data collection

Gut morphology

At the 24th week, two (2) birds were randomly selected from each replicate (total of 96 birds), placed on a top-loading meter balance, starved for 12 hours and subsequently slaughtered. Subsequently, the body cavities were opened, and the wet weight of the liver was measured, along with the lengths of the duodenum, jejunum, ileum and colon.

Reproductive organ development evaluation

At the 16th, 20th and 24th weeks, two (2) birds were randomly selected from each replicate (total of 96 birds), starved for 12 hours and subsequently slaughtered. The ovary and oviduct were removed from each bird and weighed, and the number of developing follicles was counted and weighed using a sensitive scale.

Statistical analysis

To determine significance ($p<0.05$) among treatments

Table 1. Percentage composition of experimental diet.

Ingredients (%)	Growing Phase				Laying Phase			
	T1	T2	T3	T4	T1	T2	T3	T4
	Grower's mash	Grower's mash	Grower's pellet	Grower's pellet	Layer's mash	Layer's mash	Layer's pellet	Layer's pellet
Maize	46.20	46.20	46.20	46.20	40.20	40.20	40.20	40.20
Groundnut cake	10.00	10.00	10.00	10.00	15.00	15.00	15.00	15.00
Wheat offal	26.00	26.00	26.00	26.00	22.00	22.00	22.00	22.00
Soya bean meal	12.00	12.00	12.00	12.00	14.00	14.00	14.00	14.00
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	2.00	2.00	2.00	2.00	3.00	3.00	3.00	3.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium Phosphate	3.00	3.00	3.00	3.00	5.00	5.00	5.00	5.00
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100	100
Oyster mushroom	**0g/100kg**	**100g/100kg**	**0g/100kg**	**100g/100kg**	**0g/100kg**	**100g/100kg**	**0g/100kg**	**100g/100kg**
Determined Analysis.								
Metabolizable Energy (MJ/kg)	11.05	11.05	11.05	11.05	10.65	10.65	10.65	10.65
Crude protein (%)	18.10	18.10	18.10	18.10	20.00	20.00	20.00	20.00
Crude fibre (%)	4.20	4.20	4.20	4.20	4.10	4.10	4.10	4.10
Crude fat (%)	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Calcium	1.80	1.80	1.80	1.80	2.90	2.90	2.90	2.90
Methionine (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysine (%)	0.80	0.80	0.80	0.80	0.90	0.90	0.90	0.90
Phosphorus	0.50	0.50	0.50	0.50	0.80	0.80	0.80	0.80

means, the experiment was laid out in a 2 × 2 factorial arrangements depicting two feed types along with two levels of oyster mushroom inclusion. Tukey test from minitab® version 17.1.0 (Minitab, 2013) was used to separate the means.

RESULTS

Effect of feed form and *Pleurotus ostreatus* on gut morphometry of pullets at 24 weeks

There were no significant ($p>0.05$) differences in the gut morphometry of organs except the caecum length and proventriculus percentage that were influenced by the feed forms (Table 2). Caecum length obtained from birds fed mash feed was significantly ($p<0.05$) higher than that of birds fed pelletized feed, while birds fed pelletized feed had a higher proventriculus percentage.

Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive traits of pullets at week 16

There was no reproductive organ development of pullets in the main effect of feed form and oyster mushroom inclusion at the 16th week, as shown in Table 3.

Interaction of feed type and *Pleurotus ostreatus* inclusion on reproductive traits of pullets at week 16

Table 4 shows no reproductive organ development of pullets in the interaction of feed form and oyster mushroom inclusion at the 16th week.

Table 2. Effect of feed form and *Pleurotus ostreatus* inclusion on gut morphometry of pullets at 24 weeks

Parameter	Feed form				Oyster mushroom inclusion			
	Mash	Pellets	SEM	P-Value	0 g/kg	1 g/kg	SEM	P-Value
Live weight (g)	1093.80	1178.10	36.40	0.11	1168.80	1103.10	36.40	0.21
Duodenum (%)	0.009	0.011	0.000	0.05	0.010	0.009	0.0054	0.07
Duodenum (cm/100 g LW)	2.540	2.500	0.088	0.76	2.6004	2.440	0.088	0.20
Jejunum (%)	0.017	0.018	0.001	0.52	0.016	0.018	0.001	0.09
Jejunum (cm/100 g LW)	5.387	4.963	0.286	0.30	4.932	5.418	0.286	0.24
Ileum (%)	0.012	0.012	0.000	0.81	0.012	0.012	0.000	0.78
Ileum (cm/100 g LW)	4.938	5.089	0.269	0.69	4.745	5.282	0.269	0.16
Caecum (%)	0.007	0.007	0.000	0.59	0.007	0.007	0.000	0.87
Caecum (cm/100 g LW)	3.152^{a}	2.685^{b}	0.099	0.00	2.872	2.965	0.099	0.51
Proventriculus (%)	0.003^{b}	0.004^{a}	0.000	0.00	0.004	0.003	0.000	0.15
Pancreas (%)	0.001	0.001	0.000	0.56	0.001^{b}	0.001^{a}	0.000	0.00

SEM: Standard error of mean; ^{ab}: means on the same row with different superscript differ significantly.

Table 3. Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 16

Parameter	Feed form				Oyster mushroom inclusion			
	Mash	Pellets	SEM	P-value	Control	1 g/kg	SEM	P-value
Oviduct (cm/100 g)	-	-	-	-	-	-	-	-
No of Ovary	-	-	-	-	-	-	-	-
Ovary weight (g)	-	-	-	-	-	-	-	-
No. of follicle	-	-	-	-	-	-	-	-
Follicle weight (g)	-	-	-	-	-	-	-	-

Table 4. Interaction of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 16

Parameter	Mash		Pellets			
	Control	1 g/kg	Control	1 g/kg	SEM	P-value
Oviduct (cm/100 g)	-	-	-	-	-	-
No of Ovary	-	-	-	-	-	-
Ovary weight (g)	-	-	-	-	-	-
No. of follicle	-	-	-	-	-	-
Follicle weight (g)	-	-	-	-	-	-

Table 5. Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 20.

Parameter	Feed form				Oyster mushroom inclusion			
	Mash	Pellets	SEM	P-value	Control	1 g/kg	SEM	P-value
Oviduct (cm/100 g)	0.739	1.383	0.437	0.163	1.297	0.725	0.397	0.328
No of Ovary	5.05	7.09	0.160	0.216	6.26	6.09	0.165	0.512
Ovary weight (g)	0.65	0.95	0.43	0.363	1.75^{a}	1.00^{b}	0.49	0.048
No. of follicle	-	-	-	-	-	-	-	-
Follicle weight (g)	-	-	-	-	-	-	-	-

SEM: Standard error of mean; ^{ab}: means on the same row with different superscript differ significantly

Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive traits of pullets at week 20

The effect of the feed forms did not significantly ($p>0.05$) affect the reproductive organs of the pullets at the 20th week (Table 5). The inclusion of 1 g/kg of oyster mushroom in the diet of the birds however, significantly ($p>0.05$) decreased the follicle weight relative to the

Table 6. Interaction of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 20.

Parameter	Mash		Pellets		SEM	P-value
	Control	1 g/kg	Control	1 g/kg		
Oviduct (cm/100 g)	1.12	0.85	2.521	1.765	0.437	0.620
No of ovary	12.00	15.00	19.20	12.00	3.02	0.539
Ovary weight (g)	1.00	1.20	2.01	1.00	0.47	0.696
No. of follicle	-	-	-	-	-	-
Follicle weight (g)	-	-	-	-	-	-

Table 7. Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 24

Parameter	Feed form				Oyster mushroom inclusion			
	Mash	Pellets	SEM	P-value	Control	1 g/kg	SEM	P-value
Oviduct (cm/100 g)	1.278	2.565	0.807	0.246	2.394	1.449	0.699	0.383
No of follicle	9.50	13.07	2.16	0.316	11.38	11.20	2.46	0.960
Follicle weight (g)	1.50	1.75	0.48	0.693	2.25	1.00	0.43	0.068
No. of Ovary	1.000^{b}	7.500^{a}	0.520	0.003	3.250	5.250	0.581	0.112
Ovary weight (g)	0.50^{b}	37.50^{a}	3.12	0.003	18.50	19.50	3.49	0.879

SEM: Standard error of mean.

control. Other parameters were not significantly (p>0.05) affected by the inclusion of 1 g/kg of oyster mushroom. No collectable ovaries at this stage.

Interaction of feed type and *Pleurotus ostreatus* inclusion on reproductive trait of pullets at week 20

The feed form and oyster mushroom inclusion also did not significantly affect (p > 0.05) the reproductive organs of the pullets at 20 weeks of age (Table 6). Moreover, no ovaries were fully developed at this point.

Effect of feed type and *Pleurotus ostreatus* inclusion on reproductive traits of pullets at week 24

The feed form effect was only significant (p<0.05) on the number of ovaries and ovary weight of the egg-type chickens at 24 weeks of age (Table 7). None of the other reproductive parameters examined exhibited significant differences among feed forms (p > 0.05). The dietary inclusion of oyster mushrooms did not cause any significant effect (p > 0.05) on the development of the reproductive organs of the pullets.

DISCUSSION

The current experiment showed that feed form had a great impact on caecum length and relative weight of the proventriculus at the age of 24 weeks. Birds fed mash feed had longer caeca than those fed pellets. This finding is in line with Zduńczyk *et al.* (2015), who found that pellet diets decrease the caecum length and weight in birds. A decrease in caecal growth with pellet feeding has been explained by lowering the fibre fraction and changing the physical characteristics of the pellet diets, which lowers the necessity of creating vast hindgut fermentation. Efranji *et al*. (2023) also observed that the mash feeds have higher intact fibre particles that take longer to ferment in the gastrointestinal tract, thus triggering the growth of the caecal and fermentation. On the other hand, pellet-fed birds were found to have a very high percentage of proventriculus as compared to mash-fed birds. This observation is consistent with previous studies, which have reported that pelleting changes the physical properties of feed, which may influence the secretory activity and absolute size of the proventriculus (Zduńczyk *et al.,* 2015). The higher proventriculus weight of pellet-fed birds could be an adaptation to gastric functionality, with pellet food being smaller and more compact.

The highest impact of feed form was seen on the development of the reproductive organs at the age of 24 weeks. Birds that were fed on pellet feed had many more ovary counts and ovary weight than birds that were fed on mash feed. This observation conforms with Alabi *et al*. (2025), who indicated that pelletized feed enhanced the growth of reproductive organs in pullets, whereby pellet-fed birds had significantly higher reproductive organ counts (7.50) and ovary weight (37.50 g) compared to those on a mash diet. The mechanism of this increase is presumably associated with the improved nutrient density and digestibility of feeds in pellets that facilitate increased availability of nutrients in reproductive tissue development during the critical period before sexual maturity.

What is interesting, though, is that there were no significant differences in the oviduct length, follicle number or follicle weight of pellet-fed birds in the current study, which is in contrast to the significant difference in the ovarian parameters. The partial response indicates that the effects of the feed forms on ovarian development may be more sensitive than their impact on oviductal growth. Interestingly, Gous and Morris (2001) concluded that pellet-fed pullets were 6 per cent heavier at 20 weeks but displayed a 6-day retardation in age at 50 per cent lay relative to mash-fed birds, showing that a faster growth rate does not always lead to earlier sexual maturity. The researchers concluded that the delayed sexual maturity with the use of short photoperiods is mainly because of the light impact on the development of the gonadal organs, but not because of the limitation of the feed intake.

Inclusion of *Pleurotus ostreatus* in the diet at the level of 1 g/kg was not associated with any significant change in most gut morphometric parameters, with the only exception of a slight but significant increase in pancreatic relative weight. The relative insignificance of effects on intestinal morphology might be explained by the relatively low level of inclusions employed in this study. Törős *et al*. (2024) thoroughly examined the possibilities of *Pleurotus ostreatus* as a poultry feed supplement with its prebiotic and antimicrobial effects that have a beneficial impact on gut microbiota and intestinal morphology. These positive influences are mediated by bioactive compounds such as 2 glucan, chitin, polyphenols, and flavonoids, which increase the growth of advantageous gut bacteria and help to increase the absorption of nutrients (Wang *et al*., 2022). The lack of major alterations in the duodenum, jejunum, ileum and caecum sizes in the current study indicates that an increased inclusion rate or extended duration of supplementation may be required to induce some morphological variations.

Inclusion of *Pleurotus ostreatus* in the diet had minor effects on reproductive development. When the weight of the follicles was measured at the age of 20 weeks, it was found that there was a significant decrease in the follicle weight of the mushroom-supplemented birds compared to the control group. Nevertheless, this was a short-term effect that became no longer significant at 24 weeks. The lack of meaningful effects on reproductive parameters at 24 weeks is consistent with the results of Karageorgou *et al*. (2024), who found no meaningful effects of oyster mushroom waste supplementation on laying hen performance or egg quality but positive effects on yolk fatty acid profile and oxidative stability. Equally, Alabi *et al*. (2025) discovered that the addition of 1 g/kg oyster mushroom did not affect the development of reproductive organs in pullets. The small and temporary decrease in follicle weight at 20 weeks is perhaps a temporary physiological response to dietary mushroom constituents, perhaps due to alterations in endocrine signalling or nutrient partitioning, but this does not seem to have any permanent negative effect on reproductive development.

The feed form and the inclusion of mushrooms did not have any significant interaction effect on any of the reproductive parameters at 16, 20 or 24 weeks. This means that the effect of these two dietary factors is independent and additive and not synergistic. The absence of the interactive effects simplifies the interpretation of the main effects and means that the feed form and *Pleurotus ostreatus* supplementation can be altered separately, and does not show unexpected combined effects.

The longitudinal reproductive development of the 16, 20 and 24 week study was enlightening on the ontogenesis of the reproductive tract in the Harco Black pullets. The maturation of the reproductive organs could not be quantified at 16 weeks in all the treatment groups, which was a confirmation that the sexual maturation had not yet been triggered at the age of 16. The earliest indications of reproductive development might be seen at 20 weeks, including the length of oviducts, which could be measured, follicle count, and follicle weight, but the ovaries had not developed yet. The 24-week mark marked the total observation of the reproductive development and measurement of all the parameters was possible among the treatment groups. This maturity curve is in tandem with the natural maturation cycle of commercial-type pullets that are of an egg type and which normally sexually mature between 18 to 22 weeks of age. The first six weeks should be provided with pellets or crumbled feed to stimulate feeding and growth, and mash feed should be provided later, as recommended by Alabi *et al*. (2025), because the nutritional requirements of pullets change to meet their reproductive requirements.

The commercial rearing programmes of pullets apply to the results of this work because the reproductive organs of the pellet-fed birds developed better after 24 weeks of rearing, and thus, pelletizing the layers' feed is a good way of developing the ovarian formation. Nevertheless, producers must take into account the possible trade-offs, such as the previous finding by Gous and Morris (2001) that feeding on pellets can delay age at first egg but will enhance body weight gain. The absence of any sustained positive effects of *Pleurotus ostreatus* supplementation at 1 g/kg indicates that increased levels of inclusion or alternative mushroom preparations (e.g., spent mushroom substrate or fermented products) could be needed to exert the positive effects observed by Törős *et al*. (2024). Further studies are needed to examine the dose response network and prolonged supplementation time to comprehensively uncover the potential of oyster mushrooms as a feed supplement to improve the reproductive performance of laying hens.

Conclusion

The results of this study's data reveal that pelletized feed significantly improved reproductive organ development in pullets, particularly in terms of ovary number and ovary weight at maturity. Dietary inclusion of oyster mushroom

(*Pleurotus ostreatus*) at 1 g/kg had minimal effect on reproductive parameters, although it influenced follicle weight at an earlier stage. Further research regarding oyster mushroom inclusion at greater levels should be conducted.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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