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Dietary inclusion of golden apple snail egg powder as a natural astaxanthin source modulates MUC2 gene expression without affecting performance or organ weight in laying pullets

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Abstract

Golden apple snail eggs (GASE) is a potential natural source of astaxanthin, a carotenoid with notable antioxidant properties. However, the presence of antinutrients within GASE raises concerns regarding their effective utilisation in animal diets. This study evaluated the impact of dietary GASE on growth, internal organs (pancreas, liver), and genes for intestinal integrity (*MUC2*) and amino acid transport (*SLC7A2*, *ASCT1*) in laying chicken pullets. A total of one hundred and forty-four 12-week-old laying chicken pullets were assigned to a completely randomised design with 4 treatments and 6 replication. The treatments consisted of a basal diet based on broken rice (B), the basal diet supplemented with 20 g/kg GASE (L), the basal diet supplemented with 40 g/kg GASE (H), and a control diet based on maize without GASE (M). Following a 28-day experimental period, measurements of pullet growth performance, pancreas and liver weights, and jejunal histomorphometry revealed no significant differences among the dietary groups ($p>0.05$). The dietary inclusion of GASE led to a suppression of *MUC2* gene expression ($p<0.05$). Conversely, the expression levels of the amino acid transporter genes, *SLC7A2* and *ASCT1*, remained unaffected by the GASE supplementation ($p>0.05$). These findings suggest GASE powder is a safe feed ingredient for laying chickens at the tested levels in this short-term study. Further research is needed to fully understand astaxanthin's potential protective effects on the intestine and other organs, as well as the long-term effects of dietary GASE.

Keywords: golden apple snail eggs, natural, astaxanthin, laying chickens

Highlight

- Dietary GASE suppressed the MUC2 gene expression in laying chicken pullet, but did not affect ASCT1 and SLC7A2 genes.
- Dietary addition of GASE up to 40 g/kg neither impair growth performance, pancreas, liver, nor jejunal morphometry of laying chicken pullet.
- GASE can be used as a natural astxanthin source for laying chicken.

Introduction

Pomacea canaliculata is a freshwater snail originating from South America that has become recognised as a globally significant invasive species, notably in Asian agricultural systems (Naylor 1996). Its polyphagous feeding habits encompass a broad spectrum of plant matter, and reproduction is characterised by the deposition of distinctive pink egg clutches above the waterline (Cowie 2002; Carlsson et al. 2004). The species' rapid reproductive capacity and voracious consumption, particularly of juvenile rice crops, have resulted in considerable agricultural losses and ecological disturbances within invaded habitats, solidifying its status as a major pest in numerous wetland ecosystems (Joshi 2007; Hayes et al. 2008; Constantine et al. 2023). Various strategies, including manual removal, chemical and biochemical treatments, and the introduction of natural predators, have been implemented to mitigate the detrimental effects of this snail in paddy fields (Panda et al. 2021; Azmi et al. 2022); however, the infestation demonstrates persistence.

Despite these negative attributes, the golden apple snail eggs (GASE) contain astaxanthin (AX), a carotenoid molecule with the formula $C_{40}H_{52}O_4$, characterised by two hydroxyl and two carbonyl functional groups. Its extensive system of 13 conjugated double bonds results in its orange to deep-red pigmentation (Ambati et al. 2014). Due to its distinctive molecular structure, AX exhibits strong antioxidant properties and offers health advantages such as reducing inflammation, improving skin, and enhancing eye health (Cao et al. 2023). Dreon et al. (2004) reported that GASE contains 72 nmol of carotenoids per gram, mainly astaxanthin in its free (40%), monoester (24%), and diester (35%) forms. The application of AX has seen a recent surge, extending beyond its use as a feed additive in poultry and aquaculture to include foods, medicinal and

cosmetic applications. Consequently, the exploration of novel sources, especially natural AX, will become essential (Nishida et al. 2023).

Limited scientific literature indicates that the addition of 50 mg/kg dietary AX extracted from fresh GASE enhanced the red skin colour of fancy carp to a level comparable with that of a synthetic source (Boonyapakdee et al. 2015). One report indicated that the incorporation of GASE powder (up to 15%) increased skin pigmentation as well as the antioxidant defence system (superoxide dismutase; SOD) in blood parrot fish (Yang et al. 2016). In Arab chickens, egg yolk colour (average yolk colour fan score) was significantly increased from 6.96 to 2.23, while total carotenoid content increased from 6.00 to 14.40 mg/g by including 12% of GASE powder in their diet (Nusantoro et al. 2020).

Alongside AX content, GASE contains ovorubin, which is the major protein found in the perivitelline fluid of the eggs of *Pomacea canaliculata* (Dreon et al. 2003). This fluid also comprises carbohydrates, lipids, carotenoid pigments, and a proteinase inhibitor, contributing to the biochemical defences of snail eggs against predation inhibitor (Dreon et al. 2010). Oral administration of perivitelline fluid to mice led to significant morphological changes in the small intestinal epithelium (shorter, wider, and fused villi), which consequently diminished the absorptive surface area, particularly in the proximal region (Giglio et al. 2018). Toxicity tests in bullfrogs indicated that the intraperitoneal injection of 200 µl of 170 mg/Kg GASE extract (a dose 50 times higher than the LD_{50}) was not lethal. However, observations at 24 hours revealed inflammation and structural alterations in the frog's small intestine which may alter bullfrog physiology, limiting their ability to absorb egg nutrients (Brola et al. 2020).

Ovorubin is classified as a Kunitz-type trypsin inhibitor. The presence of anti-nutritional agents, such as trypsin inhibitor, reduce the breakdown of proteins into

amino acids, thus decreasing their availability for absorption. Pancreatic enlargement in chickens showed a linear increase in response to dietary trypsin inhibitor activity (TIA) after the TIA levels exceeded a threshold of 1.4 mg/g in the diet (Hoffmann et al. 2019). This morphological change represents a compensatory response aimed at increasing the production of digestive enzymes to counteract the inhibited trypsin activity (Kuenz et al. 2022). In addition, trypsin inhibitors affect the expression of several genes (MUC2, SLC7A2, and ASCT1) in chicken intestine, which can impair gut integrity and nutrients transport (Aderibigbe et al. 2020).

To our knowledge, there have been no previous reports that assess the effect of GASE on laying chickens' performance and gene expression. To explore the potential utilisation of GASE as a natural AX source, we examined the effect of GASE on growth performance, internal organs and the expression of genes related to intestinal integrity and amino acid transport in laying chicken pullets.

Material and methods

Animal ethics

All the experimental procedures followed the standard regulations of animal welfare and ethics of Institut Biosains, Universitas Brawijaya (Approval certificate No: 049/KEP-UB/2024).

GASE Preparation

The GASE were collected from the stems and leaves of rice plants, grasses, and the walls of agricultural irrigation channels in Jember, Indonesia. Debris and soil were removed before the GASE were dried in an oven at 60 °C. The GASE were then cooled to room temperature and subsequently powdered using a mini-grinder. From 1000 g

fresh, cleaned GASE, the process yielded 206.12 – 222.04 g of powder, representing a yield of 20.61–22.20%. The proximate analysis (in duplicate) of the GASE powder resulted in a dry matter content of 95.77%, crude protein 20.32%, ether extract 6.43%, and ash 59.59%. In addition, HPLC analysis (in duplicate) of the GASE powder determined an astaxanthin content of 26.62 ppm.

Pullets management, diets, and experimental design

One hundred and forty-four laying chicken pullets (ISA Brown; 11 weeks old) were purchased from a local breeder. The poultry house was an open system with ventilation and equipped with fans. The room temperature was 25–30 °C and the relative humidity was approximately 60%. The lighting programme was 16L:8D. The pullets were reared in individual galvanised battery cages (length: 60 cm, width: 40 cm, height: 50 cm), arranged in three tiers per stack. All pullets had free access to feed and drinking water.

The pullets were assigned to a completely randomised design, consisting of four treatment groups, each with six replicates and six pullets per replicate. The diet was formulated to meet or exceed the nutrient requirements of laying chickens from the National Standardization Agency of Indonesia (BSN 2024). The basal diet was formulated based on broken rice, and a positive control of a pigmented diet was formulated based on maize, a common source of yellow pigment in laying chicken feeds (Table 1). Prior to the experimental treatments, all pullets were fed the basal diet for two weeks. Following this period, the treatment groups were provided with the following diets: B (basal diet, no GASE), L (basal diet + 20 g/kg GASE), H (basal diet + 40 g/kg GASE), and M (maize-based feed, no GASE). The GASE was in the form of powder. The total AX content of diets B, L, H, and M was 0.82, 1.42, 1.95, and 2.64 ppm, respectively. No medication was administered, and there was no pullet mortality throughout the feeding trial.

Growth Performance measurement

All pullets were individually weighed at the beginning (day 1) and end (day 28) of the experiment. Throughout the experimental period, no mortality occurred. Growth performance, in terms of average body weight gain (BWG) and average feed intake (FI), was recorded and calculated cumulatively. The feed conversion ratio (FCR) was determined by dividing the total feed intake by the total body weight gain.

Sampling procedures

At the end of the experiment, one pullet per replicate was randomly selected and weighed. The pullets were then slaughtered by cervical dislocation. The pancreas and liver were removed, rinsed with saline solution, and then weighed. The jejunum was removed, and a 1.5 cm segment was excised from the mid-jejunum for histological morphometry.

Pancreas, liver, and jejunal morphometry

Values for internal organs (pancreas and liver) are expressed as wet weight and organ indice as percentage of live body weight. Mid-jejunal segments were collected from one bird per replicate with median body weight, flushed with saline, and fixed in 10% neutral buffered formalin. Samples were subsequently dehydrated with ethanol and embedded in paraffin wax. Sections of 5 µm were stained with haematoxylin and eosin. Villus height and crypt depth were measured from four complete, vertically oriented villi per slide, and the villus height to crypt depth ratio was calculated. Histological sections were assessed using standard light microscopy. Intestinal villus lengths and areas were quantified via histomorphometric analysis using NIH ImageJ 1.53c software.

Gene expression

To examine gene expression, total RNA was isolated from the jejunum tissue using a Tissue Total Mini Kit (Geneaid, Taiwan), following their guidelines. The quality of the extracted RNA was checked using a spectrophotometer, and only high-quality samples (with an A_{260}/A_{280} ratio of 1.8 or higher) were used to create cDNA with a Toyobo ReverTra Ace kit (Japan). The specific primer sequences used for amplification in this study are listed in Table 2. The amounts of messenger RNA for the genes were measured using a real-time PCR system from Toyobo (with SYBR Green) and a CFX384 instrument. The PCR setup involved denaturation, annealing, and extension temperatures of 95 °C for 10 seconds, 50 °C for 30 seconds, and 60 °C for 15 seconds, respectively. Subsequently, the samples were analysed in triplicate, and amplification was carried out for 40 cycles. The relative levels of *MUC2*, *SLC7A2*, and *ASCT1* mRNAs, compared to a reference gene (*GAPDH*), were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical analysis

Following confirmation that the data met the assumptions of normality and homogeneity of variance, a one-way analysis of variance (ANOVA) was performed using SPSS 23. Where significant differences were identified among groups, Tukey's test was used for post-hoc comparisons. Results were considered significant at $p < 0.05$. The data are presented as the mean ± standard error of the means (SEM).

Results

Growth performance

The growth performance parameters of laying chicken pullets fed experimental feeds

are presented in Table 3. Feed intake was significantly influenced by the dietary treatments ($p < 0.01$). The highest feed intake was observed in laying chicken pullets fed the maize-based diet (M), registering 91.34 g/day. The pullets fed diets B, L, and H (ranging from 80.07 to 83.64 g/day) statistically showed similar feed intake. Final body weight and body weight gain were not affected by the dietary treatments ($p > 0.05$). The average final body weight of the chickens ranged from 1579.82 to 1636.80 g, while the body weight gain ranged from 480.03 to 539.12 g. The B, L, H, and M pulltes displayed the same feed conversion ratio, ranging from 5.75 to 6.30 ($p > 0.05$).

Pancreas and liver weight

The weights of the pancreas and liver in laying pullets, as affected by the GASE treatments and the maize-based diet, are presented in Table 4. Pancreas weight ranged from 2.40 to 2.82 g (0.14 – 0.17 %), while liver weight ranged from 29.26 to 38.41 g (1.75 – 2.29 %). No significant differences among groups were obsereved for pancreas and liver weights ($p > 0.05$).

Jejunal histomorphometry

Table 5 illustrates the effect of dietary GASE addition and maize feed on the jejunal histomorphometry. Villous height, crypt depth, and the ratio of villus height to crypt depth were not affected by the experimental diets ($p > 0.05$).

Gene expression

The effect of GASE and maize-based feed on the expression of the MUC2, SLC7A2, and ASCT1 genes is presented in Figure 1. The pullets fed maize based-feed (M) showed the highest *MUC2* gene expression ($p < 0.05$). The pullets fed B and L diets showed comparable *MUC2* gene expression. However, the addition of 40 g/kg GASE

powder (treatment H) downregulated *MUC2* gene expression in laying chicken pullets. The expression of *SLC7A2* and *ASCT1* genes was not influenced by dietary treatment ($p > 0.05$).

Dicussion

The diets utilised in this study were formulated to be isoenergetic and isonitrogenous. The principal difference between the diets was the basal feed ingredient; B, L, and H diets were based on broken rice, whereas M diet was based on maize. Consequently, the highest feed intake observed in chickens fed with M diet was attributed to the inclusion of maize. This observation aligns with the explanation by Klopfenstein *et al.* (2013), who noted that maize is a superior feedstuff for poultry, exhibiting greater palatability compared to other grains such as wheat and millet. A study replacing yellow maize with millet in broiler starters demonstrated higher consumption of maize (44.43 g/day *vs*. 41.70 g/day) compared to millet (Bulus et al. 2014). Similarly, Zhang *et al.* (2021) reported a decrease in feed intake of broiler when maize-based diets were replaced with rice, broken rice, and rice bran at equivalent energy and protein levels.

Daily ingestion of purified ovorubin (100 µl) from fresh *P. canaliculata* eggs decreased feed intake and growth in rats during the initial 3 days. However, this effect subsided after prolonged ovorubin feeding, potentially due to the animals' adaptation to the protease inhibitor (Dreon et al. 2010). Previous data indicate that when poultry are fed diets containing elevated concentrations of trypsin inhibitor, both their growth and feed intake are negatively affected (Evans et al. 2021; Kuenz et al. 2022) Interestingly, the feed intake of laying chicken pullets fed with a diet supplemented with GASE was comparable to that of those on the basal diet. This suggests that the powdered form of GASE did not negatively impact the pullets' appetite.

The growth performance of the chickens in this study was comparable to the findings reported by Al-Harthi (2014). That study used brown marine algae (a pigment source rich in fucoxanthin) as a dietary supplement for laying pullets, which showed similar performance between 14 and 20 weeks of age. Supplementation with xanthophyll at doses of 20 and 40 mg/kg had no effect on the growth of laying hens aged 1–21 days (Gao et al. 2013). The addition of lutein, another carotenoid, to the feed also did not affect the growth of White Leghorn laying hens aged 12–30 days (Meriwether et al. 2010).

In contrast to these findings, the addition of 120 and 240 mg/kg of β-carotene was able to increase the growth of laying hens (Hy-Line) aged 1 to 21 days. Similarly, male Hy-Line chickens supplemented with β-carotene at a dose of 60 mg/kg showed higher body weight gain than chickens fed a control diet (Hui et al. 2020). Furthermore, the inclusion of β-carotene combined with several herbs (turmeric and ginger) increased the growth of chickens (1–21 days) compared to the control (Gong et al. 2020). These discrepancies suggest that the growth response of chickens to carotenoids is influenced by genetics, age, sex, and the specific carotenoid species itself.

One aspect examined in this study was whether GASE is safe for use in laying hens. To this end, this research assessed the weight of the pancreas, liver, and the morphometry of jejunum as indicators. As a response to the inhibition of the digestive process by antitrypsin, the size of the pancreas will increase (undergo hypertrophy) to enhance the secretion of digestive enzymes (Kuenz et al. 2022). Previously, morphological changes in intestine and liver tissue that impaired metabolism due to antinutrients were observed by Ortiz et al. (1994) and Emiola et al. (2007). In the present research, the data for pancreas and liver weight showed no significant difference. This suggests that the dietary inclusion of GASE powder at levels up to 40

g/kg did not induce any morphometric impairment in either of these organs in laying chicken pullets during this developmental period.

Paiva et al. (2014) elucidated that longer villi are indicative of a greater surface area and enhanced absorption capacity, and *vice versa*. Crypt depth can be associated with cell turnover, potentially as a response to epithelial cell damage, inflammation, and sloughing. A greater villus-to-crypt ratio is associated with improved intestinal function as it correlates with the balance between villus and crypt (Gilani et al. 2021). The reported ranges for villus length, crypt depth, and villus-to-crypt ratio in healthy and normal 40-week-old ISA Brown laying hens are 1.06–1.48 mm, 0.18–0.26 mm, and 5.10–5.51, respectively (Anas et al. 2024). During the grower phase, laying hens have a jejunal villus length of 0.93 mm, a crypt depth of 0.15 mm, and a ratio of 6.08 (Souza et al. 2014). Evaluation of the villus and crypt data and comparison with the previous literature indicates that the use of GASE in this experiment did not induce abnormalities in the jejunum of the laying hen pullets.

Another aspect examined in this researh was the gene expression. *MUC2* gene serves as a nutrigenomic marker for gut integrity in chickens. Specifically, the expression levels of *MUC2*, which codes for a mucin protein, are associated with the health and function of the intestinal barrier (Ayalew et al. 2025). The dynamics of the *MUC2* gene within the body are influenced by various factors, including the physiological condition of the body (due to disease or infection), age, and diet. Murai et al. (2018) observed that MUC2 gene expression in chicken jejunum was comparable among the groups of corn, polished rice, brown rice, and paddy rice.

The findings of this research indicate that chickens fed a maize-based diet exhibited higher *MUC2* gene expression compared to those on broken rice-based diets. This observation aligns with existing literature suggesting a link between dietary fibre

and *MUC2* expression. Hao et al. (2022) have observed that dietary fibre intake upregulates *MUC2* expression, promoting mucin secretion. Maize is known to typically possess a greater fibre content, originating from its outer layers, whereas broken rice generally contains less fibre. Therefore, the higher *MUC2* expression in the maize group could be attributed to the greater fibre content of maize compared to broken rice.

The inclusion of this GASE (up to 40 g/kg) in the diet of laying pullets resulted in a suppression of *MUC2* gene expression, consistent with the known action of trypsin inhibitors (Aderibigbe et al. 2020). Reduction in MUC2 expression could lead to a thinner mucus or less effective mucus layer, potentially increasing susceptibiity to pathogenic cahllenges (Proszkowiec-Weglarz et al. 2020; Liu et al. 2020). However, downregulation of MUC in this experiment did not translate into the expected broader pathological changes. Specifically, the supplementation did not affect the structure of the intestinal villi and crypts of the birds. While the *MUC2* gene, a gut integrity marker, was indeed downregulated, this did not manifest in growth performance detriments within the scope of this research. The inclusion of GASE in powdered form neither significantly impact the weight of the pancreas and liver, nor did it induce the expected impairment of the intestinal villi and crypt architecture. Furthermore, the expression of genes encoding amino acid transport (*SLC7A2* and *ASCT1*) remained unchanged. Thus, processed GASE appears promise as a potentially safe feed component for laying pullets at the tested inclusion levels.

The apparent absence of widespread negative effects of GASE in this research could potentially be attributed to several factors. Dietary GAZE addition level was insufficient at the tested level to elicit significant negative effect of growth performance, internal organs, and some gene expression. Next to astaxanthin, GASE is a notable source of ovorubin (28.4 mg/g, Dreon et al. 2003), a Kunitz-type trypsin inhibitor, with

concentrations comparable to those found in raw soya beans (around 27.9 mg/g trypsin inhibitor, Wedekind et al. 2020) with 19.7 mg/g activity (Han et al. 1991). It is established that trypsin inhibitor activity below 4.0 mg/g could not induce pancreatic hypertrophy and damage the intestinal architecture (Clarke and Wiseman 2007). As trypsin inhibitor is generally considered heat-labile (Avilés-Gaxiola et al. 2018), the 60°C heating process in the preparation was likely effective in mitigating the anti-nutritional properties of GASE in this experiment. Additionally, the AX present in the GASE powder possibly could have interacted with ovorubin, potentially modulating or buffering its effects on the pancreas and intestinal morphology due to its antioxidant and antiinflammatory properties. The duration of the experiment might also have been a contributing factor, being sufficient to influence gene expression but not long enough for macroscopic or microscopic tissue alterations to become apparent.

GASE inclusion increases the amount of dietary astaxanthin, a valuable antioxidant. However, as Dansou et al. (2021) observed, the potential benefits of this compound were diminished in laying hens fed a high dose (213.4 mg/kg) of astaxanthin. Additionally, the prolonged exposure to GASE's anti-nutritional agents may pose a significant risks of chronic health issues and organ damage, which requires further investigation.

Conclusion

In summary, this research has shown that incorporating golden apple snail egg (GASE) powder into the diet of laying chicken pullets (at levels up to 40 g/kg) did not negatively impact their growth performance, the weights of the pancreas and liver, or the structure of the jejunum. Although GASE supplementation resulted in reduced expression of the *MUC2* gene, an indicator of gut integrity, the expression of amino acid transporter genes (*SLC7A2* and *ASCT1*) were not altered. At the levels tested, GASE powder

appears to be a safe dietary component for laying chicken pullets. Nevertheless, further investigation is needed to explore the potential protective effects of astaxanthin on the intestine and other organs, as well as the consequences of higher inclusion levels in diets and longer-term dietary exposure to GASE on these variables.

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Declaration of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

Data are available from the corresponding author upon rea-sonable request.

Table 1. Ingredients and dietary composition of basal and maize based diets used in this experiment.

Ingredients (g/kg, as fed basis)	Basal diet	Maize based diet
Rice, broken	500	–
Corn	–	500
Layer concentrate[1]	250	250
Rice bran	125	125
Wheat pollard	60	60
Dicalcium phosphate	20	20
CaCO3	30	30
Premix[2]	3	3
Salt	2	2
Lysin	9	9
Freetox[3]	1	1
Analysed composition (%)		
Dry matter	90.63	90.68
Crude protein	17.56	17.86
Crude fat	8.69	4.26
Crude fibre	6.11	6.82
Abu	15.81	17.98
Gross energy (MJ/kg)	15.44	16.02

[1]The layer concentrate contained a minimum of 35% crude protein.
[2]The premix povrived per kg diet: vitamin A 3600 IU, vitamin D 600 IU, vitamin E 2.4 IU, vitamin K3 0.6 mg, vitamin B1 0.6 mg, vitamin B6 0.15 mg, vitamin B12 3.6 ug, vitamin C 7.5 mg, calciumD=panthotenate 1.8 mg, niacin 12 mg, cholin chlorde 3 mg, lysin 9 mg, methionine 9 mg, manganese 36 mg, iron 6 mg, iodine 0.06 mg, zinc 30 mg, cobalt 0.06 mg, copper 1.2 mg, santoquin 3 mg.
[3]A mycotoxin binder contains hydrated sodium calcium aluminosilicate.

Table 2. Primer sequence of reference and gene of interest used in this study.

Gene	Primer sequence (5' – 3')	Product size (bp)	Gen ID
Reference			
GAPDH	Forward: TCC TAG GAT ACA CAG Reverse: CGG TTG CTA TAT CCA	151	NM_204305
Gene of interest			
MUC2	Forward: GCT ACA GGA TCT GCC Reverse: AAT GGG CCC TCT GAG	152	XM_421035
SLC7A2	Forward: TGC TCG CGT TCC CAA Reverse: GGC CCA CAG TTC ACC	67	NM_001199102.1
ASCT1	Forward: TTG GCC GGG AAG GAG Reverse:AGA CCA TAG TTG CCT	63	XM_001232899.4

GAPDH: glyceraldehyde-3-phosphate dehydrogenase, *MUC2*: mucin 2, *SLC7A2*: solute carrier family 7 member 2, *ASCT1*: alanine serine cysteine threonine transporter 1.

Table 3. Effect of different addition level of GASE and maize based feed on growth peformance traits of laying chicken pullets.

Parameters	Treatment				SEM	*p*-value
	B	L	H	M		
IBW (g)	1101.23	1112.58	1091.20	1097.68	8.673	0.867
FI (g/d)	83.59[a]	80.07[a]	83.64[a]	91.34[b]	1.079	<0.001
FBW (g)	1613.98	1582.62	1579.82	1636.80	14.137	0.452
BWG (g/period)	512.75	480.03	488.62	539.12	6.437	0.503
FCR	5.75	6.30	6.06	5.98	0.180	0.781

a–b Means in the same column within similar row followed by different letters are significantly different at $p < 0.05$. B: basal diet (formulated based of rice, without GASE powder). L: basal diet + 20 g/kg GASE powder. H: basal diet + 40 g/kg GASE powder. M: maized based feed without GASE powder. IBW: initial body weight. FI: feed intake. FBW: final body weight. BWG: body weight gain. FCR: feed conversion ratio.

Table 4. Pancreas and liver weight of laying chicken pullets fed diets with different GASE powder addition and maize based feed.

Parameter	Treatment				SEM	*p*-value
	B	L	H	J		
Pancreas						
Weight (g)	2.40	2.52	2.77	2.82	0.078	0.170
Indice (%)	0.14	0.15	0.16	0.17	0.004	0.146
Liver						
Weight (g)	30.55	31.85	38.41	29.26	1.299	0.076
Indice (%)	1.82	1.90	2.29	1.75	0.018	0.078

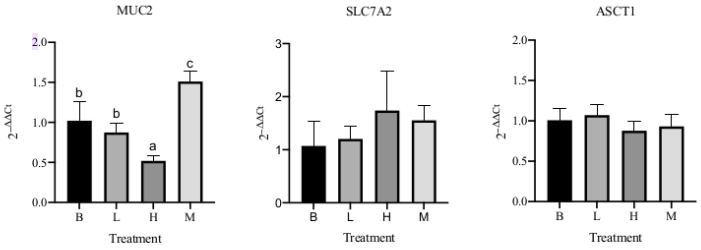
B: basal diet (based on broken rice, without GASE powder). L: basal diet + 20 g/kg GASE powder. H: basal diet + 40 g/kg GASE powder. M: based on maize, without GASE powder.

Table 5. Jejunal morphometry of laying chicken pullets fed diets with different level of GASE powder addition and maize based feed.

Parameter	Treatment				SEM	*p*-value
	B	L	H	J		
Villi height (μm)	1470	1200	1380	1280	45.594	0.425
Crypts depth (μm)	230	250	300	240	11.800	0.397
Villi to crypts ratio	6.60	5.16	4.81	5.36	0.290	0.073

B: basal diet (based on broken rice, without GASE powder). L: basal diet + 20 g/kg GASE powder. H: basal diet + 40 g/kg GASE powder. M: based on maize, without GASE powder.

Figure1. Jejunal gene expression of gut integrity (*MUC2*) and amino acid transporters (*SLC7A2* and *ASCT1*) in laying chicken pullets fed diets with different GASE powder additions and a maize-based feed. B: basal diet (based on broken rice, without GASE powder). L: basal diet + 20 g/kg GASE powder. H: basal diet + 40 g/kg GASE powder. M: maize-based feed without GASE powder. Values are means, and error bars indicate standard deviation. Letters above bars denote significant differences at $p < 0.05$.



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