



Expression Profile of Feed Efficiency Related Genes in High and Low Feed-efficient Colored Synthetic Male Line (CSML) Broiler Chickens

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ABSTRACT

Background: In commercial poultry production, where feed expenses account for about 70% of total costs, improving feed efficiency is crucial for profitability and sustainability. CSML exhibits a multi-Colored plumage and brown eggshells, closely resembling native desi birds, making it highly desirable for crossbreeding to improve indigenous poultry.

Methods: A total of 100 CSML chicks were reared individually from 2 to 12 weeks of age to assess Feed Conversion Ratio (FCR), Residual Feed Intake (RFI), Residual body weight Gain (RG) and Residual Intake and Body Weight Gain (RIG). Based on these traits, birds were divided into high feed efficient (HFE) and low feed efficient (LFE) groups, with HFE representing the top 20% and LFE the bottom 20%.

Result: HFE birds showed significantly better performance, gaining 7.87% body weight gain, consuming 9.96% less feed, achieved 17.25% better FCR and demonstrating improved RFI (179.86%), RG (220.03%) and RIG (182.50%) compared to LFE birds. Gene expression analysis *via* real-time qPCR revealed upregulation of SGLT1 (nutrient transporter) and GHRL (growth-related) genes and down regulation of CDx (gut development) genes in HFE birds, suggesting their role in feed efficiency. The study concludes that HFE CSML birds exhibit enhanced feed efficiency traits than LFE group, which can be used for production of high feed efficient hybrids.

Key words: Broiler chickens, CDx, CSML, Feed efficiency, GHRL, Residual feed intake, SGLT1.

INTRODUCTION

Feed costs alone represent over 70% of total production expenses in poultry farming, underscoring the critical importance of improving feed efficiency (Akintunde *et al.*, 2024; Dinani *et al.*, 2021). Efficient feed utilization is pivotal in reducing production costs, environmental impact and resource use (Rashid *et al.*, 2020). Feed conversion ratio (FCR), defined as the amount of feed consumed per unit of body weight gain, remains a widely used metric for feed efficiency, where lower values signify higher efficiency (Singh *et al.*, 2018). An alternative metric, Residual Feed Intake (RFI), introduced by Koch *et al.* (1963), accounts for the difference between observed and predicted feed intake, adjusted for maintenance and production. RFI is favored for its linear properties, avoiding biases associated with ratio-based measures. Berry and Crowley (2012) proposed residual intake and body weight gain (RIG), a novel trait combining feed consumption and growth performance, offering a comprehensive measure of feed efficiency.

The Colored Synthetic Male Line (CSML) is a specialized parent line of colored broilers developed in 1995 under the All India Coordinated Research Programme (AICRP) at the ICAR-Central Avian Research Institute (CARI), Izzatnagar, India, a premier institute in poultry science. The CSML was established from a synthetic base population developed during 1989-1990. The origins of this population date back to 1972, when Cornish, Plymouth Rock and New Hampshire breeds were included in a genetic improvement programme. This programme initially

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focused on improving the 10th week body weight through mass selection, which was later refined to emphasize body weights primarily at the 8th and then to 6th week of age (CARI Annual Report-2017). From 2001, selection of these lines focused for higher 5th week body weight. So, as a consequence of long-term selection (near about 40 years), the population has reached the plateau for production with reduced genetic variability. Hence, the current study was carried out on the same population with the prime objective to improve the FCR for development of feed efficient line.

The CSML is notable for its exceptional growth performance, feed efficiency and adaptability. CSML exhibits

a multi-colored plumage and brown eggshells, closely resembling native desi birds, making it highly desirable for crossbreeding to develop indigenous poultry. The mixed-sex average body weight of CSML at 5 weeks is 1222.63 g, with an FCR of 1.61 and reaches 1623.7 g by six weeks. Farmers highly favor CSML due to its rapid growth, superior feed efficiency and robust immunocompetence, which also command higher market prices. Studies by Thapa (2018) and Vispute (2021) highlight the superiority of CSML birds, with respect to broiler traits, immunological attributes and suitability for rural farming systems, further emphasizing its potential in genetic improvement programs.

Advances in selective breeding and dietary optimization have significantly enhanced feed efficiency, enabling increased production with reduced feed requirements (Yi *et al.*, 2015). Improvements in FCR by 0.20-0.25 units can markedly lower production costs and enhance profitability. Molecular regulation of feed efficiency involves neural signals, mitochondrial function, hormonal pathways and nitrogen recycling. Gene expression studies indicate that upregulation of genes associated with efficient cellular energy production contributes to improved feed efficiency and muscle development (Bottje *et al.*, 2012). Integrating genetic, molecular and management approaches to optimize feed efficiency is crucial for sustainable poultry production to meet the growing demand for eggs and meat in India. The current study aimed to integrate genetic basis of RFI with feed efficiency genes like CDX, SGLT1 and GHRL in Chicken.

MATERIALS AND METHODS

Population structure and experimental design

The study was conducted at the ICAR- Central Avian Research Institute, Izatnagar, under the approval and guidelines of the Institutional Animal Ethics Committee. It involved 100 CSML broiler parent chicks of the 21st generation, sourced from CARI's germplasm unit. The chicks were reared under Completely Randomized Design for a 12-week period (April-June, 2024).

From 0 to 2 weeks, chicks were group-fed on deep litter, then housed individually in metal cages from 3 to 12 weeks of age. Two experimental diets-chicks (0-4 weeks) and grower (5-12 weeks) were formulated according to ICAR (Mandal, 2013) standards and essential micronutrients were incorporated to ensure balanced nutrition. A measured quantity of feed was offered daily in mash form, with care taken to minimize feed wastage. Daily feed intake and leftover feed were recorded to determine feed consumption, while body weight was measured biweekly using a digital weighing scale (± 0.10 g). The body weight gain (BWG) and weekly feed consumption were then determined.

Feed efficiency traits were calculated at 2-week intervals (2-4 weeks, 4-6 weeks, 6-8 weeks, 8-10 weeks and 10-12 weeks) as well as for the overall study period

(2-12 weeks). The FCR, RFI and Residual Body Weight Gain (RG) and RIG was calculated as follows.

$$FCR = \frac{\text{Total feed consumption (kg)}}{\text{Body weight gain (kg)}}$$

RFI: The difference between the actual and expected feed intake was referred to as residual feed intake (based on regression). The RFI was calculated using linear regression, as suggested by Yi *et al.* (2015) for poultry, using the equations below:

$$RFI = TFI - \{b_0 + b_1 MMW + b_2 BWG\}$$

Where,

TFI= Total feed intake.

MMW= Metabolic mid weight=

$$[(\text{Body weight at start} + \text{Body weight at end})/2]^{0.75}$$

BWG= Body weight gain.

b0 , b1 , b2= Regression coefficients.

$$RG = WG - \{b_0 + b_3 MMW + b_4 TFI\}$$

Where,

WG= Weight gain.

TFI= Total feed intake.

MMW= Metabolic Mid Weight= $[(\text{Body weight at start} + \text{Body weight at end})/2]^{0.75}$.

b0= Intercept.

b3= Partial regression coefficients of MMW.

b4= Partial regression coefficient of TFI.

Residual intake and body weight gain (RIG)

It is a linear combination of RFI and RG.

$$RIG = RG - RFI$$

On the basis of the overall performance of all four feed efficiency traits (FCR, RFI, RG and RIG), birds were ranked and divided into two categories: low and high feed efficiency. Six birds from each category were selected for gene expression analysis.

Gene expression analysis

At 12 weeks of age, 200 mg jejunum tissue samples were aseptically collected and preserved in RNA Later (QIAGEN). RNA was extracted using the TRIzol method and quantified with a NanoDrop spectrophotometer. cDNA was synthesized using the HiGemoMB kit (Hi-Media). Gene-specific oligonucleotide primers are listed in Table 1. Real-time PCR was conducted on a Bio-Rad CFX96 system to assess the expression of feed efficiency-related genes, using GAPDH as the reference. Relative gene expression was analyzed with REST 2009 software.

RESULTS AND DISCUSSION

Body weight

The data on BWG, FI and FCR, RFI, RG, RIG of colored broiler parent during 2-12 weeks was presented in the Table 2. Body weights in this study ranged from 150-296 g, 388-966 g, 877-1588 g, 923-1609 g, 1540-2134 g and

1619–2856 g at 2, 4, 6, 8, 10 and 12 weeks, respectively. Similar trends were reported by Ekka *et al.* (2016), with mean weights of 221.44±24.18 g, 663.42±36.50 g, 1359.89±84.19 g and 1993.55±35.72 g at 2, 4, 6 and 8 weeks in CSML birds. However, the weights observed in the present study were lower than those reported by Vispute (2021) and Thapa (2018) at all corresponding weeks of age. Yousefi *et al.* (2024) also reported higher weights in Cobb 500 birds-422.4 g, 1501.1 g and 2718.7 g at 2, 4 and 6 weeks. Notably, the 4th week body weight in this study exceeded that of Arbor Acres (586 g) and Hubbard Classic (508 g) but was comparable to Cobb 500 (730 g) as reported by Nassar *et al.* (2019).

Body weight gain (BWG)

The BWG recorded at 2-week intervals showed that the average BWG during the 0-4-week period was higher than the 317 g reported by Thanabalan and Kiarie (2022), but the 5-12-week BWG was lower than their 1837 g value for the 5-19-week growing phase. Nassar *et al.* (2019) also reported higher BWG during weeks 3-4 in Broiler crossbreds. Compared to our study, Prakash *et al.* (2017) observed slightly higher BWG in weeks 4 and 5 (239 g and 328 g, respectively), while Ekka *et al.* (2016) reported higher BWG in CSML birds during weeks 2-4 (441.98 g), 4-6 (696.47 g) and 6-8 (633.66 g). Yousefi *et al.* (2024) noted substantially greater BWG during weeks 2-4 and 4-6 (1178.74 g and 1217.75 g) in Cobb 500 birds. Similarly, Thapa (2018) reported higher BWG during the 2-4 (572.58 g) and 4-6 week (732.18 g) periods. The cumulative BWG from 0-6 weeks in this study (1025.12 g) was also lower than Vispute (2021) 1266.91 g. These comparisons

suggest relatively lower growth performance during the early growth phase in the present study.

Feed intake

Yousefi *et al.* (2024) reported feed intake between 900-1400 g in optimized broiler strains, consistent with the 942.91 g recorded at week 6 in this study. Compared to Vispute (2021), who observed 435.48 g (0-3 weeks) and 831.43 g (3-6 weeks), the current study recorded higher intake during weeks 2-4 (961.29 g) and 4-6 (1560.91 g), indicating better early-stage feed intake in CSML birds. At week 8, feed intake peaked at 1738.52 g, exceeding the 1250-1450 g range reported by Nassar *et al.*, (2019), but slightly below the 1650 g noted by Ekka *et al.*, (2016) for CSML birds. Thanabalan and Kiarie (2022) reported intake between 1000-1600 g in broiler breeders, aligning with the present findings, though the sharp peak at week 8 was not observed.

Feed efficiency traits

During the 2-4 and 4-6-week periods, birds showed relatively efficient FCRs of 2.32 and 2.60, which significantly deteriorated in later stages, rising to 5.16, 3.98 and 4.77 during the 6-8, 8-10 and 10-12 week period respectively. Prakash *et al.*, (2017) reported a similar FCR of 2.32 in week 5, while Ekka *et al.* (2016) noted a more gradual increase from 1.75 (week 1) to 3.22 (week 8), contrasting with the abrupt spike in this study. Final FCRs reported by Yousefi *et al.* (2024) for Ross 308, Cobb 500 and Arian (1.67-2.2) and those by Thapa (2018) and Vispute (2021) (ranging from 1.45 to 1.95) were all better than values observed here. Overall, the current study revealed better and more variable FCRs, particularly in later stages,

Table 1: Oligonucleotide primers of genes used in the current study.

Genes	Primer	Annealing temp. (°C)	Product size (bp)
CDX	F- CTCGGACTTCGCCAGCTACC R- GGTGCCGTATGAGTGCCAGTC	56	124
SGLT1	F- TATTATCCTGCTTGCTAT R- CATTTCATATACTTCTCCAT	49	173
Ghrelin	F- CCTTGGGACAGAACTGCTC R- CACCAATTTCAAAAGGAACG	40	203
GAPDH	F- CCGTCCTCTCTGGCAAAGTCC R- AGCCCCAGCCTTCTCCATG	58	166

Table 2: Production performance of CSML birds at different ages (Mean±SE).

Parameters	2-4 week	4-6 week	6-8 week	8-10 week	10-12 week	SEM	Level of significance
Body weight gain	421.26 ^c ±9.16	603.86 ^a ±8.54	233.86 ^a ±11.07	453.57 ^b ±9.08	357.83 ^d ±8.77	6.80	0.000
Feed intake	961.29 ^c ±15.87	1560.91 ^b ±22.75	942.91 ^c ±24.01	1738.52 ^a ±13.38	1561.07 ^b ±12.25	16.90	0.000
FCR	2.32 ^c ±0.03	2.60 ^c ±0.03	5.16 ^a ±0.41	3.98 ^b ±0.08	4.77 ^a ±0.21	0.11	0.000
RFI	-0.04 ^b ±7.88	-0.00 ^b ±12.77	0.00 ^b ±12.12	0.00 ^b ±10.31	904.65 ^a ±12.63	16.81	0.000
RG	-0.13 ^a ±2.87	0.01 ^a ±6.20	0.00 ^a ±6.83	-0.00 ^a ±7.36	-904.65 ^b ±10.36	16.35	0.000
RIG	-0.09 ^a ±9.09	0.00 ^a ±17.24	0.00 ^a ±17.80	-0.00 ^a ±15.77	-1809.30 ^b ±21.48	32.94	0.000

^{ab} Means bearing different superscripts within a row differ significantly (P<0.05).

indicating lower feed efficiency likely influenced by genetic, environmental and managerial factors.

On the basis of data on FI, BWG and MMW for 2-12 weeks the RFI and RG were calculated using coefficient of regression equation (Supplementary Table 1 and 2). Significantly ($P < 0.05$) better RFI was observed during the 2-4 weeks period and it declines as age progresses. The RG reported during 10-12 were better than other periods. There is no significant difference in RG, RIG reported in the current study. Significantly better ($P < 0.05$) RIG was observed in 10-12-week period.

Ranking of CSML birds

Based on overall data (2-12 weeks) for FCR, RFI, RG and RIG, the birds were ranked and grouped into high feed efficient (top 20%) and low feed efficient (bottom 20%) using a combined index (Supplementary Table 3 and 4).

Production traits of selected birds during 2-12 weeks

In this study, HFE CSML birds showed higher body weight gain, lower feed intake and better FCR than LFE birds (Table 3), consistent with findings by Willems *et al.* (2013); Zampiga *et al.* (2021) attributed lower feed intake in efficient birds to better digestive efficiency and nutrient allocation for improved growth rate. As noted by Havenstein *et al.* (1994), FCR tends to worsen with age due to rising maintenance energy needs. Birds with low RFI values had significantly lower feed intake and better FCR than high RFI birds, making RFI a valuable selection trait. Selecting for low RFI not only reduces feed costs but also minimizes nitrogen waste and environmental impact (Bezerra *et al.*, 2013; Fathi *et al.*, 2021). When grouped by RG, high-efficiency birds gained more weight with less feed. Similarly, birds with high RIG values showed both lower feed intake and higher weight gain, highlighting the advantage of combining RFI and RG. RIG closely represents the ideal of low feed intake with high growth and has

moderate correlations with body weight gain. However, excessive emphasis on growth in selection indices may negatively affect conformation traits like hip and leg structure, as well as footpad and breast skin health potentially impairing mobility and welfare.

Expression of feed efficiency-related genes in CSML birds

Differential expression analysis of growth-related genes in intestinal tissue of High and Low Feed Efficient (HFE and LFE) CSML birds revealed significant variations (Table 4).

SGLT1 is a primary mediator of glucose absorption in the small intestine (Hediger and Rhoads, 1994) and its higher expression indicates better assimilation efficiency. In this study, SGLT1 was upregulated in high feed efficiency (HFE) CSML birds, aligning with findings by Bottje *et al.* (2012); Bottje and Carstens (2009) and Mott *et al.* (2008), who linked elevated SGLT1 expression to improved nutrient uptake and growth.

Ghrelin plays a role in regulating growth hormone (GH) release (Nagaya *et al.*, 2001) and exists in two molecular forms-acyl ghrelin (active) and des-acyl ghrelin (inactive)-in both gastric tissues and circulation (Ariyasu *et al.*, 2001). Ghrelin has been identified in several species, including mammals and birds (Wada *et al.*, 2003). Ghrelin-immunoreactive cells are found in parts of the chicken hypothalamus and administration of human ghrelin has been shown to elevate plasma GH levels in young chickens (Ahmed and Harvey, 2002). Ghrelin's active form can suppress feed intake (Saito *et al.*, 2002), consistent with our findings and previous studies showing a genetic link between RFI and feed intake (Prakash *et al.*, 2017).

The CDx gene, which regulates intestinal development and maintenance (Geyra *et al.*, 2002), showed lower expression in HFE birds. Similarly, Shinde *et al.* (2018) observed reduced CDx gene expression in chicks deprived of feed for 6, 24 and 36 hours, suggesting that intestinal stress diminishes CDx expression. This may indicate a more

Supplementary Table 1: RFI estimation using the coefficient of regression equation.

Regression model	Unstandardized coefficients		Standardized coefficients beta	t value	Significance level
	B	Std. error			
Constant	-90.765	905.716		-0.100	0.920
Metabolic mid weight (MMW)	35.228	11.147	0.808	3.160	0.002
Body weight gain (BWG)	-0.158	0.760	-0.053	-0.208	0.835

Predictors: Constant, MMW and BWG, Dependent variable: Total feed intake.

$RFI = TFI - (-90.765 + 35.228 \times MMW + (-0.158) \times BWG)$

Supplementary Table 2: RG estimation using the coefficient of regression equation.

Regression model	Unstandardized coefficients		Standardized coefficients beta	t value	Significance level
	B	Std. error			
Constant	-892.98	79.283		-11.263	0.000
Metabolic mid weight (MMW)	14.26	0.579	0.973	24.635	0.000
Total feed intake (TFI)	-0.003	0.013	-0.008	-0.208	0.835

Predictors: Constant, MMW and TFI, Dependent variable: BWG.

$RG = BWG - (-892.978 + 14.265 \times MMW + (-0.003) \times TFI)$

Supplementary Table 3: Ranking of selected top 20% of CSML birds having HFE based on FCR, RFI, RG and RIG.

High feed efficient group										
Wing band	FCR	FCR rank	RFI	RFI rank	RG	RG rank	RIG	RIG rank	Combined rank value	Combined rank
D-1393	2.93	3	-682.50	3	46.21	21	728.70	3	27.75	1
D-1407	2.93	2	-464.63	13	50.80	20	515.43	12	38.00	2
D-1370	2.95	4	-576.53	9	42.70	24	619.23	5	38.25	3
D-1384	2.92	1	-910.23	1	22.41	40	932.63	1	42.25	4
D-1497	3.15	23	-550.92	10	64.24	11	615.16	6	45.50	5
D-1381	3.09	10	-471.55	12	31.37	35	502.92	13	60.25	6
D-1353	3.08	9	-94.21	42	92.75	3	186.96	37	63.25	7
D-1468	3.14	21	-326.90	24	54.48	16	381.38	18	65.50	8
D-1359	3.16	24	-543.72	11	35.98	30	579.69	8	67.00	9
D-1414	3.07	8	-353.02	21	31.43	34	384.45	17	67.25	10
D-1420	3.09	12	-317.89	25	38.89	27	356.77	20	69.00	11
D-1373	3.06	7	-369.81	18	22.05	41	391.85	15	69.75	12
D-1365	3.12	19	-252.72	31	54.28	17	307.00	25	73.25	13
D-1460	3.12	17	-49.94	48	119.59	1	169.53	39	75.75	14
D-1465	3.04	6	-664.10	5	-25.26	64	638.84	4	76.00	15
D-1440	3.19	29	-185.77	36	65.52	10	251.29	29	82.25	16
D-1363	2.99	5	-837.55	2	-34.25	77	803.29	2	84.50	17
D-1434	3.16	26	-362.51	20	27.76	37	390.27	16	87.00	18
D-1372	3.11	16	65.73	59	100.33	2	34.61	47	88.75	19
D-1432	3.12	18	-291.96	28	27.55	38	319.51	23	89.75	20

Note: When the same combined rank value occurs, importance is given to FCR.

Supplementary Table 4: Ranking of selected bottom 20% of CSML birds having LFE based on FCR, RFI, RG and RIG.

Low feed efficient group										
Wing band	FCR	FCR rank	RFI	RFI rank	RG	RG rank	RIG	RIG rank	Combined rank value	Combined rank
D-1463	3.59	84	173.36	70	-31.82	73	-205.18	74	245.50	81
D-1486	3.59	85	198.00	72	-30.59	70	-228.59	76	246.00	82
D-1489	4.05	102	439.39	87	28.90	36	-410.49	84	246.00	83
D-1389	3.47	72	143.26	68	-56.76	89	-200.02	72	247.00	84
D-1447	3.93	101	1102.50	102	39.26	26	-1063.24	102	254.50	85
D-1377	3.43	66	418.16	85	-41.80	82	-459.96	87	254.75	86
D-1395	3.51	76	527.44	91	-30.28	69	-557.72	91	258.75	87
D-1404	3.60	87	778.01	100	7.21	47	-770.80	100	259.00	88
D-1466	3.70	91	258.59	79	-38.40	80	-296.98	79	269.75	89
D-1360	3.59	86	648.89	98	-24.76	63	-673.65	97	271.25	90
D-1474	3.86	98	615.50	94	-11.57	57	-627.07	94	272.50	91
D-1397	3.90	100	644.98	97	3.57	52	-641.41	95	272.75	92
D-1437	3.67	89	439.15	86	-46.58	86	-485.73	88	283.00	93
D-1369	3.84	97	930.02	101	-16.89	60	-946.91	101	283.25	94
D-1475	3.73	94	722.56	99	-28.19	68	-750.75	99	285.75	95
D-1441	3.71	93	205.04	73	-120.91	100	-325.95	81	286.25	96
D-1415	3.68	90	477.79	90	-48.01	87	-525.80	90	289.50	97
D-1362	3.73	95	618.11	95	-36.25	79	-654.36	96	293.00	98
D-1487	3.78	96	408.88	84	-103.45	99	-512.33	89	301.25	99
D-1355	3.88	99	569.18	92	-136.46	101	-705.64	98	316.50	100

Note: When the same combined rank value occurs, importance is given to FC.

Table 3: Production performance of selected colored broiler parent (HFE and LFE) during 2-12 weeks.

Parameters	HFE	LFE	SEM	Level of significance
Body weight gain	2166.45±46.14	1995.90±48.42	35.72	0.015*
Feed intake	6647.40±133.78	7382.90±135.40	110.87	0.000**
FCR	3.07±0.01	3.71±0.04	0.05	0.000**
RFI	-412.03±56.45	515.93±57.10	90.01	0.000**
RG	43.44±8.14	-36.19±9.88	8.98	0.000**
RIG	455.48±50.58	-552.13±53.00	88.40	0.000**

Values are Mean±SE based on 20% of the population selected.

^{ab} Means bearing different superscripts within a row differ significantly (P<0.05).

Table 4: Differential expression of growth associated genes in the intestine tissue of HFE and LFE group of CSML birds.

Gene	Type	Groups	Reaction efficiency	Relative fold expression	P (H1)	Level of significance
GAPDH	REF		1	1		
CDx	TRG	HFE	1	0.607±0.30	0.013	*
		LFE	1	61.29±36.09		
SGLT1	TRG	HFE	1	2.90±0.98	0.027	*
		LFE	1	0.34±0.09		
GHRELIN	TRG	HFE	1	304.09±113.28	0.032	*
		LFE	1	21.43±12.12		

* Correlation is significant at the 0.05 level.

efficient gastrointestinal system or reduced reliance on CDx due to optimized gut function or microbiota in HFE birds.

CONCLUSION

Based on findings of the study it is confirmed that selecting parent birds based on a combination of multiple feed efficiency traits-such as RFI, FCR, RG and RIG is more efficient, both in terms of growth performance and economic viability, compared to selection based on a single trait alone. High feed-efficient CSML birds consistently exhibited enhanced feed efficiency traits, including lower feed intake, better FCR and higher body weight gain, leading to substantial cost savings in poultry production. Integrating these feed efficiency traits with molecular data, such as the up regulation of nutrient absorption-related genes and growth regulation genes provides a more extensive approach for improving selection and breeding strategies. This multi-trait selection approach not only optimizes growth performance but also reduces feed costs and environmental hazard by minimizing nitrogenous waste. Future research should explore genetic polymorphisms and gut microbiome influences the advance cost-effective and sustainable poultry production.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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