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Research Article

Comparative Serum Amino Acid Profile of Rajasri and Commercial Layer Birds by High Throughput Liquid Chromatography Mass Spectrophotometry

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Abstract

Rajasri is a backyard dual purpose (meat and egg) desi bird acts an important source of livelihood for rural people. Raising of these birds can improve food security and economic status of below poverty line families in India, while commercial layer birds are raised mainly for egg production in India. Commercial layer bird produces more eggs about double in quantity compared to Rajasri bird, but the meat of Rajasri is more delicious and believed to have nutritional values. However, no data is available on the significance of the amino acids in meat and egg quality. To understand the aminoacids present in serum during egg laying. A study was conducted to evaluate the comparative serum amino acid profile on 28 week's old Rajasri and commercial layer birds. Birds were divided into 3 groups and each group contain 9 birds. Serum samples collected from 9 birds pooled to 3 in each group, respectively. Later serum was separated by centrifugation at 2000xg for 10 minutes. Thereafter, the serum samples were processed and subjected to LC/MS/MS analysis using an API 3000 triple quadrupole mass spectrometry system and results were analyzed by using Prism10 Statistical software. Higher concentration of alanine, glutamine, threonine, serine and histidine were observed in Rajasri birds compared to commercial layer birds. Based on earlier the studies the observed amino acids play a specific role in modulating metabolic pathways, antioxidant system and enzymatic processes, which can potentially influence meat production and quality.

Keywords: Rajasri; Commercial Layer; Amino Acids; LC-MS; Meat Quality

Introduction

Poultry production is one of the prospective opportunities to accomplish quick and sustainable production of superior protein to meet the growing mandate for animal protein [1]. Rajasri a dual-purpose bird, developed for backyard farming has a great potential due to its versatile capacity to adapt in various climatic conditions [2,3]. These birds are exclusively raised in the backyards, spread across all categories of households [4]. The physical characteristics of the bird include medium sized with long shanks and colourful plumage resembling indigenous birds with laying capacity of 160-180 eggs per annum [5]. They have higher immunity levels compared to commercial layers and can be easily raised in village environments [6]. They will be ready for marketing in a short period of time and will also get a good market price. Compared to the meat quality characteristics of broilers, significantly higher colour and flavour scores in Rajasri meat was reported in a work done by [7]. Hence, along with egg production, meat of Rajasri breed has high demand on account of its suitability in preparation of processed meat products retaining the quality without any adverse effects on meat quality.

Nutritive value of animal products plays a significant role in modulating immune response and has been a focal point to explore the same in optimizing human nutrition. Amino acids are the building blocks of proteins that have both structural and metabolic functions in humans and other animals. In mammals and birds proteinogenic amino acids include alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, and valine [8]. Humans and other animals have dietary requirements for amino acids that are synthesizable de novo in animal cells [9]. Layer birds have physiological requirement for amino acids to synthesize body, egg proteins and non-protein compounds like serotonin, adrenaline, nitric oxide, glutathione, and carnitine. The diet of the layers should be formulated as per the ideal amino acid requirement given by NRC (1994). Although there is accurate supplementation of the amino acids in the diet but still there are differences across the different breeds with respect to egg characteristics as multiple factors like genetic and environmental affect the same. With this background, the present study was designed to understand and compare two popular layer breeds reared in AP and Telangana, Rajasri and commercial layers. It was hypothesized that difference in their amino acid profiling might be responsible for the superior quality of egg and meat in Rajasri birds. Amino acid content in the serum which could be utilized for synthesis of both body and egg protein might be rationale for the superior quality of Rajasri eggs with higher nutritional value as compared to commercial layers. There has been little insight into the comparative serum amino acid profile through LC/MS/MS analysis of Rajasri birds to commercial layers chickens. Hence, in the present study the serum amino acid profile of both the layer breeds has been explored, compared and analyzed.

Methodology

Experimental design

The experimental birds comprised of 9 Rajasri and 9 commercial layers (Figure 2), each aging ~28 weeks age divided into 3 groups and each group contain 9 birds maintained at Livestock farm complex of N.T.R College of Veterinary Science, Gannavaram (Figure 1). The birds were provided with wheat-based diet in the form of wet –mash (feed moistened by water) @ 150g feed per day and ad libitum water. Clean drinking water was provided daily and necessary health care measures were adapted. Routine managemental practices were adapted for all the birds as per standard practices of farm.

Blood collection

2 ml of blood was collected from birds were divided into 3 groups and each group contain 9 birds. Serum samples collected from 9 birds pooled to 3 in each group respectively in clot activators by puncturing the wing vein under aseptic conditions. Blood collected was kept for 4 hours at room temperature for serum extraction. Following the extraction, serum was centrifuged at 2000 g for10 minutes followed by aliquoting in 2ml falcon tubes for further processing.

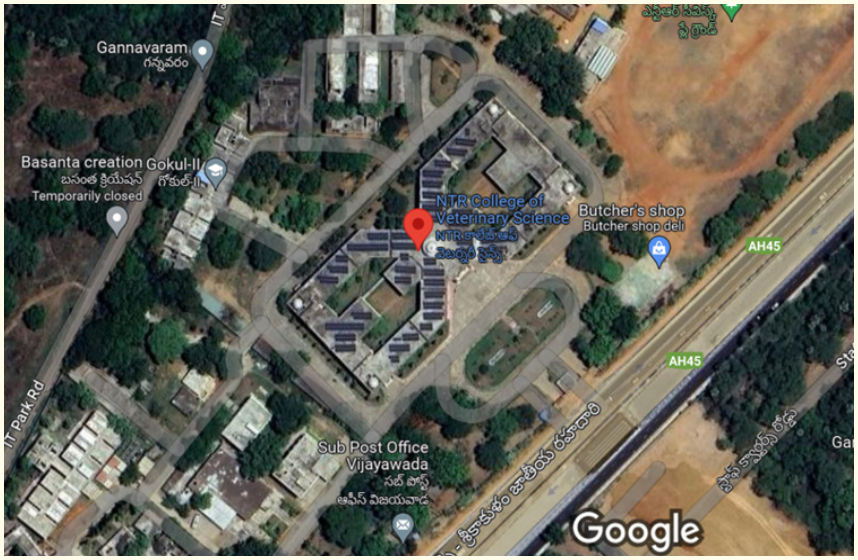


Figure 1: Place of research done: NTR College of Veterinary Science, Gannavaram, NTR District, Andhra Pradesh.

Figure 2: a) Rajasri, b) White leghorn.

LC/MS/MS sample preparation

A mixture of methanol (850 µL) and internal standard (50 µL) was added to 100µL of plasma, serum, or urine in a 1.5mL polypropylene microfuge tube. Internal standards were present at a final concentration of 50 µmol/L with the exception of D3-MET and D2-tyrosine (TYR) which were present at 10 and 25 µmol/L, respectively. The mixture was vortexed, allowed to stand for 10min, then centrifuged for 3min at 10000g and 400µL of methanolic extract was dried completely under nitrogen. Then, 100 µL of 3N HCl/n-butanol was added to the sample residue and incubated for 7.5 min at 60°C in a capped borosilicate vial. Following butylation, the mixture was dried completely under nitrogen, reconstituted in 250µL of mobile phase (20% acetonitrile, 0.1% formic acid), and transferred to a borosilicate autosampler vial for injection.

LC/MS/MS analysis

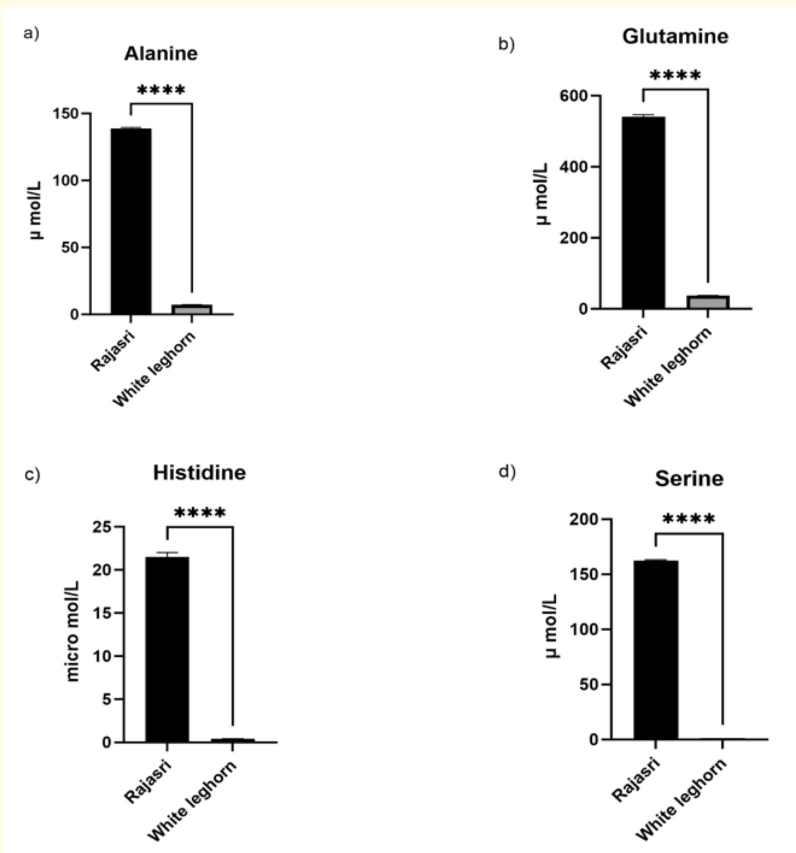
MS/MS analysis was carried out using an API 3000 triple quadrupole mass spectrometry system (Applied Biosystems, Foster City, CA, USA]. Flow was split 1:4 into an electrospray ionization (ESI) source operated at 325°C in positive mode at 2000 V, nebulizer gas at 14 mTorr, curtain gas at 12 mTorr and collision gas at 10 mTorr. Data were acquired in multiple reaction monitoring (MRM) mode using Analyst software (version 1.4.2). Optimal fragmentation patterns were determined for each amino acid at a concentration of approximately 10 µM. The mass spectrometer was tuned using polypropylene glycol to unit mass resolution (±0.7 amu). Sample introduction (10µL) was accomplished using a 1100 HPLC system (Agilent, Santa Clara, CA, USA) equipped with autosampler. Samples were introduced into an isocratic flow of 20% acetonitrile, 0.1% formic acid at 1mL/min. Chromatography was carried out using a C8, 4.612.5 mm5 µm guard column and a 4.6150 mm5 µm mm resolving column (Zorbax Eclipse XDB-C8, Agilent). Time between injections was 22min. Carryover was assessed with a sample blank following triplicate analysis of the 1000 µmol/L sample and was <1 µmol/L for all amino acids.

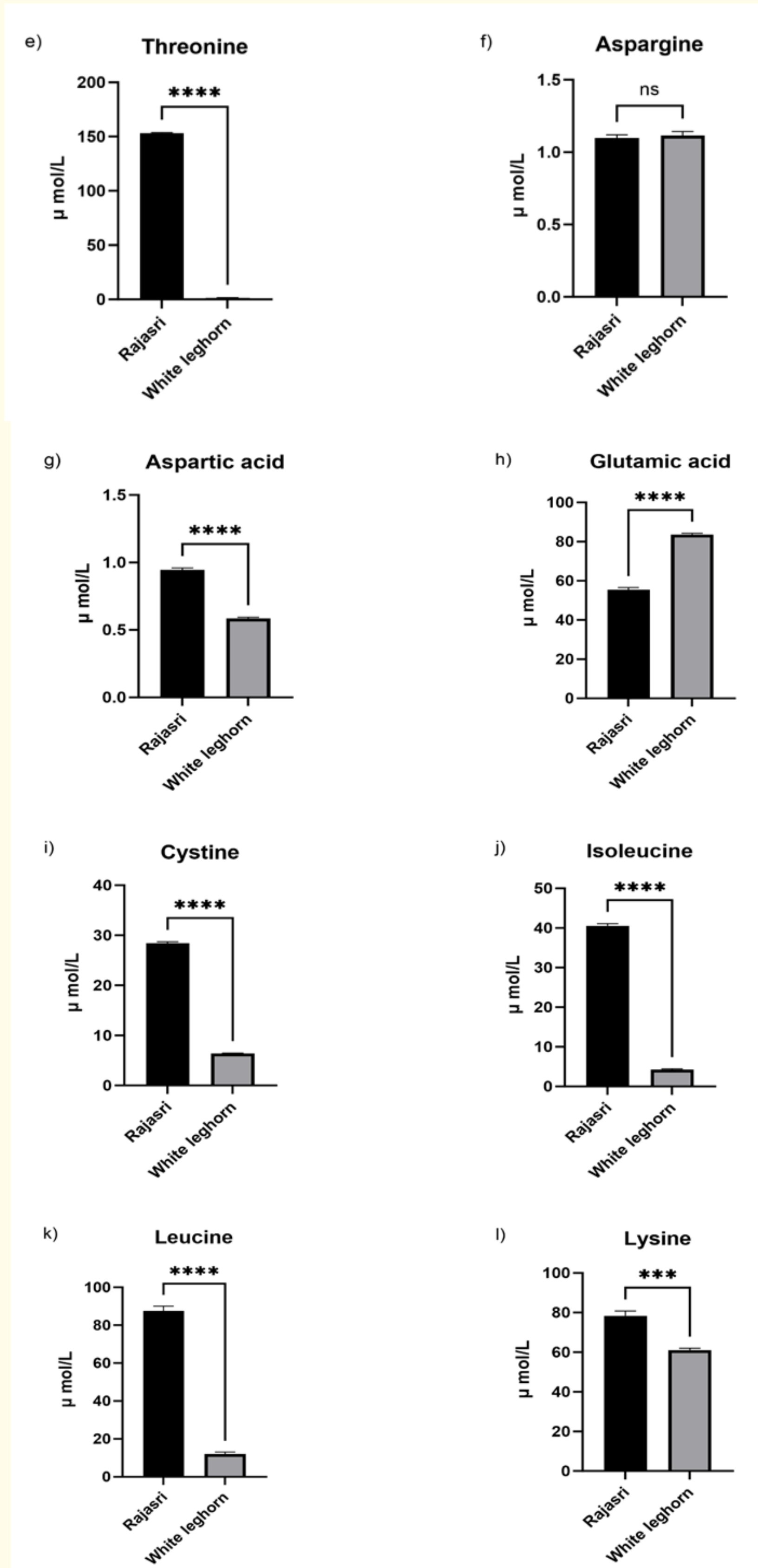
Statistical analysis

Unpaired t-Test was performed by using Graph pad prism version 10 at various significance levels ($P < 0.001$ and $P < 0.05$).

Results and Discussion

The pooled serum samples from both the groups were analyzed by LC/MS/MS technique and the Figure 3&4 and Table 1 as shown below indicated the comparative amino acid analysis between Rajasri and White leg horn. The results indicated that out of 20 amino acids profiled which included alanine, aspartic acid, cystine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine were found to be significantly higher ($p < 0.0001$) in Rajasri than white leg horn. While the concentration of arginine, aspargine and lysine were found be non- significant in both the groups.




e)
Threonine
****
200
150
100
50
0
μ mol/L
Rajasri
White leghorn
f)
Aspargine
ns
1.5
1.0
0.5
0.0
μ mol/L
Rajasri
White leghorn
g)
Aspartic acid
****
1.5
1.0
0.5
0.0
μ mol/L
Rajasri
White leghorn
h)
Glutamic acid
****
100
80
60
40
20
0
μ mol/L
Rajasri
White leghorn
i)
Cystine
****
40
30
20
10
0
μ mol/L
Rajasri
White leghorn
j)
Isoleucine
****
50
40
30
20
10
0
μ mol/L
Rajasri
White leghorn
k)
Leucine
****
100
80
60
40
20
0
μ mol/L
Rajasri
White leghorn
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Lysine
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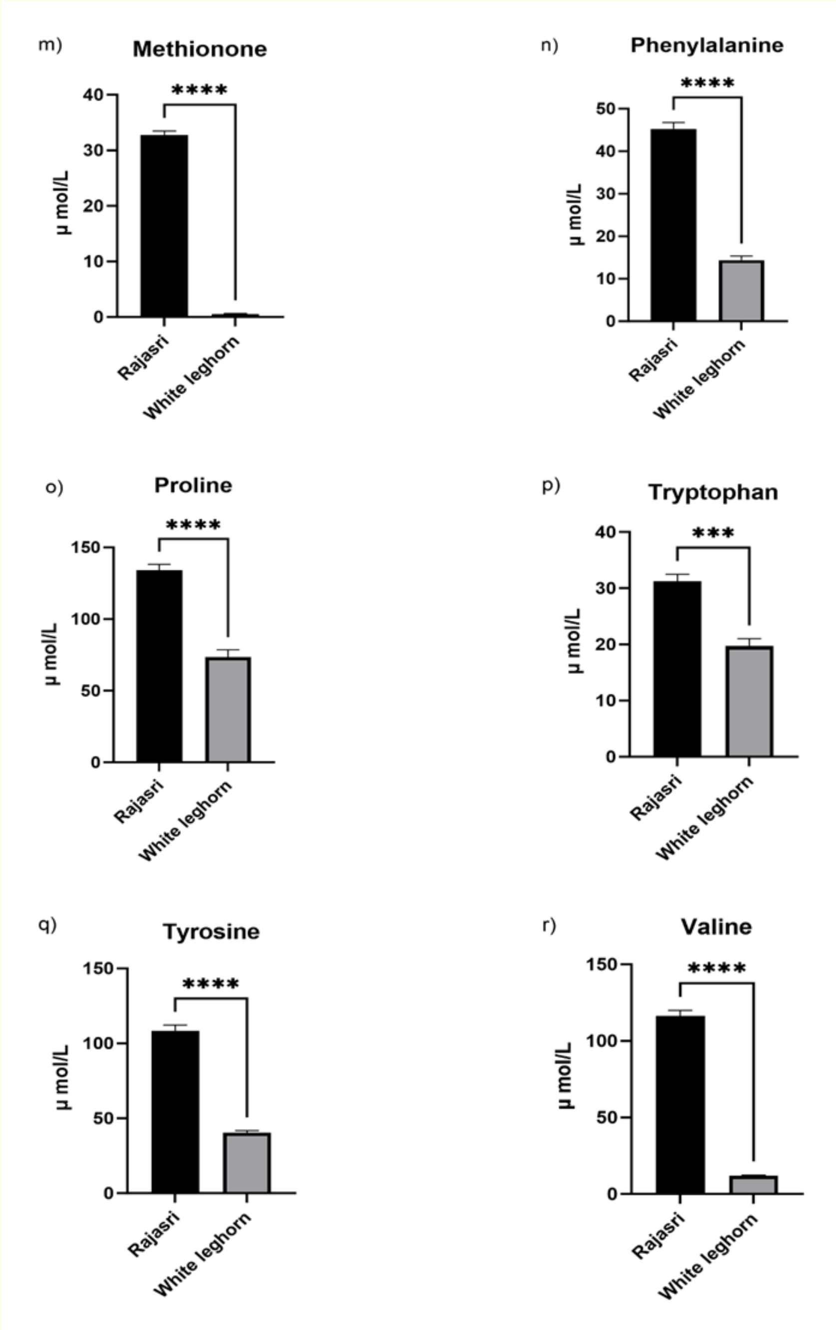
Rajasri
White leghorn


m)
Methionone
****
40
30
20
10
0
μ mol/L
Rajasri
White leghorn
n)
Phenylalanine
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50
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10
0
μ mol/L
Rajasri
White leghorn
o)
Proline
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150
100
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0
μ mol/L
Rajasri
White leghorn
p)
Tryptophan
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0
μ mol/L
Rajasri
White leghorn
q)
Tyrosine
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150
100
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μ mol/L
Rajasri
White leghorn
r)
Valine
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150
100
50
0
μ mol/L
Rajasri
White leghorn

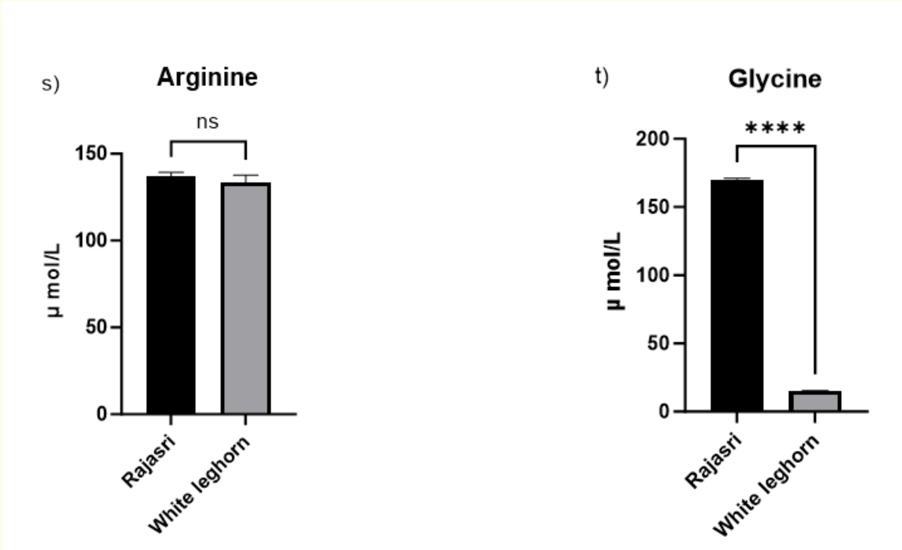

Figure 3: The graphs a), b), c), d), e), f), g), h), i), j), k), l), m), n), o), p), q), r), s), t) represents serum amino acid profile between Rajasri and White leghorn birds.

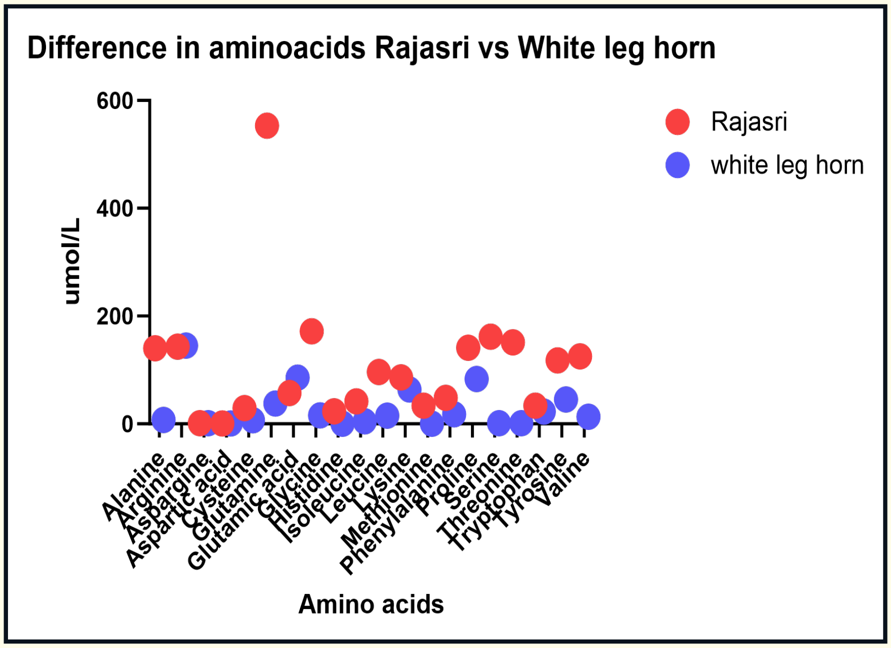

Figure 4: Bubble chart of amino acids between Rajasri and White leghorn.

Aminoacids	Rajasri bird value IN µ mol/L			Commercial layer bird value IN µ mol/L		
Alanine	140.49	138	139	7.25	6.9	6.5
Arginine	143.03	140.99	135.89	145.05	134.98	138
Aspargine	1.14	1.08	1.16	1.02	1	1.09
Aspartic acid	0.98	0.91	0.92	0.61	0.56	0.58
Cystine	29.18	28.9	28.2	6.58	6.4	6.32
Glutamine	553.47	550.12	546.21	37.68	38.14	36.52
Glutamic acid	57.18	55.2	51.3	85.99	84.23	83.15
Glycine	171.75	172.1	170	15.59	16.13	14.6
Histidine	23.38	20.11	21	0.29	0.35	0.4
Isoleucine	41.94	40.9	41.24	4.76	4.11	3.98
Leucine	96.02	89.21	80.4	15.32	11.31	12.9
Lysine	86.06	80.4	78.9	63.78	60.99	61.88
Methionone	33.86	32.12	34.99	0.28	0.12	0.19
Phenylalanine	48.43	47.99	45.89	17.56	14.88	12.5
Proline	141.29	140.81	120.98	83.34	80.9	78.12
Serine	161.82	162.31	165.02	1.4	1.48	1.52
Threonine	151.37	155.12	153.18	1.48	1.5	1.42
Tryptophan	33.88	30.9	26.79	22.54	21.88	20.98
Tyrosine	117.52	100.96	98.97	45.32	40.98	36.87
Valine	125.39	120.99	118.98	13.37	10.92	11.21

Table 1: Amino acid profile between Rajasri and white leghorn.

Branched chain amino acids like isoleucine and valine are important in maintaining gut immunity, antioxidant capacity, and critical metabolic processes [10-12]. They are also thought to be crucial in egg production due to their role in regulation of fatty acid metabolism in the liver [13,14]. As a consequence of this function, the lipoprotein production could be rate-limiting for egg yolk formation [15]. Isoleucine is also essential for growth, optimum egg mass, and egg production [16-18]. The results of the present study indicated higher leucine, isoleucine, valine concentration in the serum of Rajasri birds compared to white leg horn which might support our hypothesis indicating the superior quality of Rajasri eggs to white leg horn. In a study on laying hens it was reported that valine is said to be the next limiting amino acids after methionine, lysine, tryptophan, and threonine [19]. Tryptophan has been reported to be active in regulation of immune response in animals [20]. The products of tryptophan metabolism like indoleamine 2,3-dioxygenase, kynurenine, quinolinic acid, and melatonin, might improve immunity and induce anti-inflammatory response [21]. Few other metabolites also play a role in inhibition of T lymphocyte proliferation, elevation of immunoglobulin levels and promotion of antigen-presenting organization in tissues [15]. Tryptophan has also been reported to affect the feeding behaviour of the animals as it is a precursor of the neurotransmitter serotonin [22]. This also supports the results of the present study where Rajasri has higher Tryptophan concentration than leg horn. Lysine content is important as it is used as the basis for setting the requirements for all other amino acids in the ideal amino acid profile [23]. Higher lysine diet was believed to drop egg production in broiler breeders as reported [24]. In the present study, there is no significant difference in lysine content which indicated that both the breeds had ideal amino acid profile. Methionine besides being an important amino acid for protein synthesis also has antioxidant functions [25]. It acts as a precursor of cysteine and glutathione in the transsulfuration pathway [26] and through methionine sulfoxide reduction action [27]. Arginine is associated with metabolically important molecules such as creatine, nitric oxide, glutamate, polyamines, proline, and glutamine [28]. Arginine has also been reported to influence bone development with formation of collagen and connective tissue [29,30]. It also stimulates growth hormone release which influences epiphyseal growth plate [31,32].

Elevated arginine levels in Japanese quails supplemented with zinc and l –arginine were reported [33] and also have been shown to alleviate oxidative stress, stimulate protein synthesis through activation of mTOR pathway [34], improve antioxidant capacity, and humoral and cell-mediated immunity [35]. Methionine improves serum SOD activity [36] and improves antioxidant defence through increased GSH and reduces oxidative stress [37]. Threonine is the third limiting amino acid and is essential for protein synthesis and maintenance in the body [38]. Threonine, like several other amino acids, is immediately utilized by the small intestine and is not accessible to extra intestinal tissues [39,40]. Threonine is a particularly important necessary ingredient because, as compared to other amino acids, it has the highest metabolism in the portal-drained viscera [13]. Threonine is an essential amino acid critical for maintaining intestinal integrity and functionality, with over 30% of intestinal proteins comprising Threonine. This amino acid is extensively metabolized in the portal-drained viscera and is a major component of mucin, accounting for up to 11% of its amino acid composition, underscoring its significant role in gut health. The synthesis of mucins, which are vital glycoproteins secreted by goblet cells to form a protective mucus layer, relies heavily on threonine, given that it constitutes approximately 30% of mucin's amino acid composition . This high threonine content is particularly important for the O-linked glycosylation critical for mucin structure and function, which involves the attachment of glycans to the hydroxyl oxygen of threonine and serine residues. The intestinal epithelium is shielded from mechanical, chemical, and bacterial insults by this mucin-rich mucus layer, which also contributes to an unstirred water layer that limits direct contact between luminal antigens and the epithelium [41]. Consequently, an increased demand for threonine for mucin synthesis is observed during periods of immune system stimulation, thereby increasing the dietary threonine requirements for protein deposition and maintenance. Moreover, threonine supplementation has been shown to increase villus height, goblet cell density, and the expression of tight junction proteins, all contributing to enhanced gut barrier function and overall intestinal health. This vital amino acid is often limited in broiler diets, highlighting the necessity of optimizing its supplemental levels to ensure robust growth and intestinal fitness, especially when animals experience gut challenges from microbial invasions or environmental stressors [41]. In the present study, though there was no significant difference of arginine between the groups but still their concentration indicated the immune status and thereby competency of these breeds in better productivity in A.P poultry industry.

Conclusion

Production animals are often subjected to periods of stress which includes heat stress and intestinal pathogens like coccidiosis, which might compromise intestinal integrity and barrier function thereby productivity [37]. The results of the present study explored the serum amino acid profile of Rajasri and White leg horn with comparison among the different amino acid content. Although the results indicated both the breeds had optimum content of different amino acids but significantly higher levels of few of the amino acids indicated better immunity, disease resistance and antioxidant status thereby signifying the superior productivity of Rajasri breed in comparison to white leg horn. However, as this is the first study so far with respect to the serum amino acid profiling in Rajasri and White leg horn there is a need to validate through further study on egg characteristics. This could probably explore the effect of serum amino acid content on egg productivity in both the breeds thereby the superior quality of Rajasri eggs could be established.

Highlights

- Raising of Rajasri birds can improve food security and economic status of below poverty line families in India, while commercial layer birds are raised mainly for egg production in India.
- Commercial layer bird produces more eggs about double in quantity compared to Rajasri bird, but the meat of Rajasri is more delicious and believed to have nutritional values.

- To understand the aminoacids present in serum during egg laying. A Study was conducted to evaluate the comparative serum amino acid profile on 28 weeks old Rajasri and commercial layer birds.
- Thereafter, the serum samples were processed and subjected to LC/MS/MS analysis using an API 3000 triple quadrupole mass spectrometry system and results were analyzed by using Prism10 Statistical software
- Higher concentration of alanine, glutamine, threonine, serine and histidine were observed in Rajasri birds compared to commercial layer birds.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Naveen Swaroop identified problem, synthesized hypothesis and designed study based on gaps and applicability, and executed work and performed data analysis and written manuscript. Ashok Chandra A, helped in designing study and execution of work, Susmitha T helped in designing study and execution of work, Vasantha designed study and execution of work, Srikanth designed study and execution of work, Aswani kumar, identified problem, synthesized hypothesis and designed study based on gaps and applicability.

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