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Original Article

Determination of the Relationship of Serum Amino Acid Profile with Sex and Body Weight in Healthy Geese by Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT

The aim of this study is to determine the serum amino acid profile using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS / MS) in healthy male and female geese of the same age that were raised in similar care and feeding conditions. The animal material of the study consisted of a total of 41 geese, 18 male, and 23 female of the same age (9 months). After a 12h fasting period of the geese, blood samples were taken from V. subcutanaeulnaris into tubes without anticoagulant. After separating the serums, the samples were preserved at -20 ^{O} C degrees until Methylglutaryl (Met-Glu), Valine (Val), Leucyl-Isoleucine (Leu-Ile), Methionine (Met), Phenylalanine (Phe), Argininosuccinate (ASA), Tyrosine (Tyr), Aspartic acid (Asp), Alanine (Ala), Arginine (Arg), Citrulline (Cit), Glycine (Gly), Ornithine (Orn), Glutamic acid (Glu) were analyzed. The Val, Asp, Arg, Cit, Gly, and Orn levels of male geese were higher compared to female geese in the research (p<0.05). It was determined that Asp, Arg, Cit, and Gly levels increased as the body weights of the geese increased (p<0.05). It was also determined that the effect of Gender x Body Weight interaction on Val, Cit, and Orn levels was significant (p<0.05). As a result; it has been concluded that the serum amino acid profile of healthy geese can vary according to gender and live weight, and more studies are needed to elucidate the reasons for these changes.

INTRODUCTION

Protein molecules are polymers of amino acid (AA) residues, linearly linked by peptide bonds. Although there are more than 500 naturally occurring amino acids (AAs) (Wagner & Musso, 1983) only 20 of these are found in plant and animal proteins. These 20 amino acids classified as Proteinogenic amino acids (Wu *et al.*, 2016) are; alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine (Lewis, 2001; NRC, 2012). Beyond being the building blocks for protein synthesis, different AAs have different biochemical functions (Hou & Wu, 2017).

The skeletal muscle, which is the main reservoir of protein or AAs in the body, is a dynamic tissue. Protein molecules in muscle mass constantly go through a natural intracellular life process called the protein cycle, where old or damaged proteins are broken down and new proteins are synthesized (Wu, 2013; Liao *et al.*, 2015). Alongside muscle cells, all other living cells in an animal's body have an intracellular protein cycle. The intracellular fluids must take in and remove free AAs through the extracellular fluids and blood circulation, based on the metabolic state of the cells, for this cycle to continue. Free AAs present in plasma in different concentrations are intermediates of the whole-body protein

cycle and nutrient metabolism, and play very important roles during the protein transfer process (Abumrad & Miller, 1983; Liao *et al.*, 2018).

Comparison of reference plasma AA concentrations (at starvation) with current plasma AA concentrations (after feed intake) can provide important information about the bioavailability of dietary AAs (Wu, 2013). The profiles of digestible or bioavailable AAs in a diet, especially for those that cannot be synthesized in animal cells, are the most important factors determining the nutritional value of dietary proteins or the efficiency of dietary protein for metabolic us (Hou *et al.*, 2015; Hou *et al.*, 2016). Studies have shown that the plasma free amino acid (PFAA) profile can be used as an effective biomarker in the detection of lifestyle-related diseases (Wang *et al.*, 2011; Würtz *et al.*, 2012; McCormack *et al.*, 2013; Würtz *et al.*, 2013).

The analysis of amino acids is thought to play an extremely important role in detecting disorders called aminoacidopathies. Today, various analytical methods such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) are used for amino acid analysis (Uaariyapanichkul *et al.*, 2018). Using liquid chromatography-tandem mass spectrometry (LC-MS / MS), each sample can be analyzed quickly within 2 minutes. Furthermore, multiple analytes can be tested by using a small amount of blood sample. In this method, the false positive rate is about 0.05%, and the accuracy is more than 90% (Jones & Bennett, 2002; Fingerhut *et al.*, 2014).

In this study, the aim is to determine the serum amino acid profile using Liquid Chromatography-Tandem Mass Spectrometry in healthy male and female geese of the same age that were raised in similar care and feeding conditions.

MATERIAL AND METHODS

The animal material of the study consisted of a total of 41 geese, 18 male, and 23 female of the same age (9 months). The geese were clinically healthy, had similar care and feeding conditions, and were raised in the Bozova district of Şanlıurfa province (Figure 1). After a 12hfasting period of the geese, blood samples were taken from V. subcutaneaulnaris into tubes without anticoagulant. After separating the serums, the samples were preserved at -20 ºC degrees until Methylglutaryl (Met-Glu), Valine (Val), Leucyl-Isoleucine (Leu-Ile), Methionine (Met), Phenylalanine (Phe), Argininosuccinate (ASA), Tyrosine (Tyr), Aspartic acid (Asp), Alanine (Ala), Arginine (Arg), Citrulline (Cit), Glycine (Gly), Ornithine (Orn), Glutamic acid (Glu) were analyzed.

Ethical Approval

Ethical approval for this study was obtained from the Harran University Local Ethics Committee for Animal Experiments (Decision number: 2020/006/15).

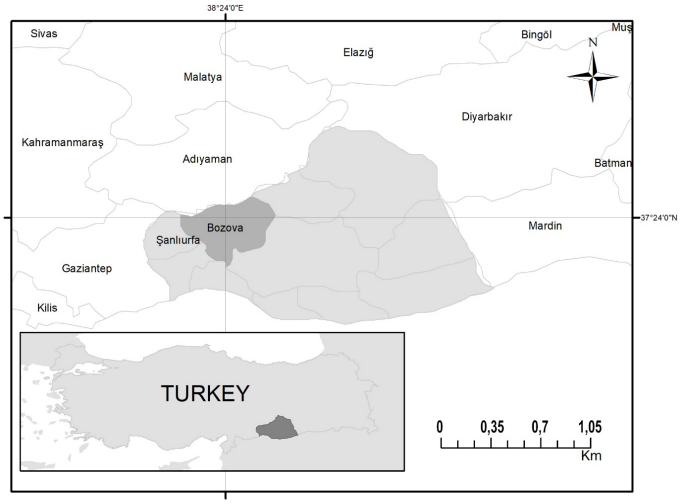

Figure 1 – The map of Şanlıurfa province, in which the study was performed.

Tandem Mass Spectrometry

Each sample was extracted by dispensing 300 lL of an extraction solution consisting of a mixture of methanol and an aqueous solution of 3 mmol/L hydrate hydrazine, at approximate relative volume/ volume ratios of 66.6 % and 33.3%, respectively. In the extract solution, stable heavy isotope analogues of several amino acids were used for internal standards. Samples obtained from the extract were injected into an LCMS-8040 device (Shimadzu Corporation, Japan). The percentage of each analyte was defined compared to a standard including each analyte. The standard concentrations for amino acids were in the range of 500–2500 lmol/L.

Statistical Analysis

The data were analyzed with a factorial model of the general linear model procedure using SPSS software (SPSS version 23.0; IBM Corp., Armonk, NY, USA). The interaction of live body weight and sex on Met-Glu, Val, Leu-Ile, Met, Phe, ASA, Tyr, Asp, Ala, Arg, Cit, Gly, Orn, and Glu parameters were determined using the PROC GLM procedure.

$Y_{ijk} = \mu + LBW_i + G_j + (LBW \times G)_{ij} + e_{ijk}$

Where: Y_{ijk} is the response variable (MethylGlutaryl, Val, Leu_Ile, Met, Phe, ASA, Tyr, Asp, Ala, Arg, Cit, Gly,

Orn, and Glu); μ is the overall mean common to all observation; LBW_i is the fixed effect of live weight (i = 4); G_j is the fixed effect of gender (j = 2), $(LBW \times G)_{ij}$ is the first-order interaction and e_{ijk} is the random residual error. Statistical significance was set at $p \leq 0.05$. Post hoc tests were performed, using Duncan's Multiple Range Test.

RESULTS

The serum amino acid profiles of male and female geese with different body weights are given in Table 1. The Val, Asp, Arg, Cit, Gly, and Orn levels of male geese were higher compared to female geese's in the research ($p<0.05$). It was determined that Asp, Arg, Cit, and Gly levels increased as the body weights of the geese increased ($p<0.05$).

It was also determined that the effect of Gender x Body Weight interaction on Val, Cit, and Orn levels was significant ($p<0.05$). Another finding is that the highest value in terms of Val, Cit, and Orn levels was in the male geese group of 3.5 kg and above. The lowest values were determined in the male geese of 2.60-2.89 kg group is for valine, in the female geese of 2.90-3.19 kg group is for Cit, and in the female geese of 3.50 kg and above group is for Orn. It was determined that valine, Cit, and Orn values increased as the bodyweight increased in males (except for the 3.20-3.49kg group), while the increase in body weight in females had fluctuating values.

It was also determined that body weight, sex, and Gender x Body Weight interaction did not affect Leu_Ile, Met, Phe, ASA, Tyr, Ala, and Glu values ($p>0.05$).

DISCUSSION

Amino acids have different biochemical functions beyond serving as building blocks in protein synthesis (Hou & Wu, 2017). Protein molecules in muscle mass constantly go through a normal intracellular life process called the protein cycle, old or damaged proteins are broken down and new proteins are synthesized during this process (Wu, 2013; Liao *et al.*, 2015). Comparison of available plasma AA concentrations (after feed intake) with

Table 1 – Serum amino acid profile in healthy female and male geese.

Group	Live Weight	MethylGlutaryl	Val	Leu_Ile	Met	Phe	ASA	Tyr	Asp	Ala	Arg	Cit	Gly	Orn	Glu
Female	2.60-2.89 kg	0.026	688.046^{b}	594.058	99.524	240.167	0.134	350.888	53.586	856.876	584.850	20.615^{b}	516.589	71.148^{b}	377.949
	2.90-3.19 kg	0.036	543.255^{b}	520.050	83.183	212.190	0.123	338.138	57.357	886.922	556.889	18.713^{b}	455.339	73.812^{b}	403.390
	3.20-3.49 kg	0.024	739.939^{b}	644.409	106.171	252.574	0.151	436.404	89.408	912.831	742.523	32.319^{b}	583.513	92.775^{b}	491.425
	3.50 and up	0.032	567.142^{b}	449.197	103.673	226.322	0.223	302.849	78.005	1139.163	663.458	28.710^{b}	539.705	55.349^{b}	461.349
Male	2.60-2.89 kg	0.029	538.893^{b}	459.221	80.570	190.552	0.167	250.794	61.872	833.117	471.728	19.419^{b}	432.047	60.371^{b}	360.998
	2.90-3.19 kg	0.022	879.632^{ab}	767.354	114.217	238.584	0.246	361.950	105.728	1119.585	878.893	36.087^{b}	677.651	127.601^{b}	578.488
	3.20-3.49 kg	0.029	864.675^{ab}	700.142	111.921	260.440	0.214	403.945	100.093	1087.599	814.880	29.300^{b}	673.005	97.636^{b}	476.669
	3.50 and up	0.022	1215.308^{a}	828.127	170.642	382.404	0.280	548.537	170.278	1190.852	1384.971	71.233^{a}	1099.938	239.608^{a}	633.131
SEM		0.009	133.027	95.801	15.947	40.755	0.063	66.827	22.230	155.074	149.058	7.252	107.736	29.672	82.267
Live Weight															
2.60-2.89 kg		0.027	613.470	526.639	90.047	215.360	0.150	300.841	57.729^{b}	844.997	528.289^{b}	20.017^{b}	474.318^{b}	65.760	369.473
2.90-3.19 kg		0.029	711.443	643.702	98.700	225.387	0.185	350.044	81.542^{ab}	1003.253	717.891^{ab}	27.400^{b}	566.495^{b}	100.707	490.939
3.20-3.49 kg		0.026	802.307	672.275	109.046	256.507	0.183	420.175	94.751^{ab}	1000.215	778.702^{ab}	30.810^{b}	628.259^{ab}	95.206	484.047
3.50 and up		0.027	891.225	638.662	137.158	304.363	0.251	425.693	124.141^{a}	1165.007	1024.215^{a}	49.971^{a}	819.821^{a}	147.479	547.240
SEM		0.006	94.064	67.742	11.276	28.818	0.045	47.253	15.719	109.654	105.400	5.128	76.181	20.981	58.171
Gender															
Female		0.029	634.595	551.928	98.137	232.813	0.158	357.070	69.589	948.948	636.930	25.089	523.787	73.271	433.528
Male		0.025	874.627	688.711	119.338	267.995	0.227	391.306	109.493	1057.788	887.618	39.010	720.660	131.304	512.321
SEM		0.005	68.098	49.041	8.164	20.863	0.032	34.209	11.380	79.384	76.304	3.712	55.151	15.189	42.113
Source of Variation															
Gender		0.556	0.020	0.060	0.079	0.245	0.145	0.486	0.021	0.342	0.029	0.014	0.019	0.012	0.198
Live Weight		0.988	0.188	0.275	0.069	0.216	0.537	0.134	0.048	0.266	0.028	0.008	0.042	0.104	0.151
Gender * Live Weight		0.646	0.048	0.062	0.091	0.163	0.897	0.144	0.302	0.789	0.081	0.034	0.064	0.035	0.469

reference plasma AA concentrations (at fasting) can provide important information about the bioavailability of dietary AAs (Wu, 2013). For this purpose; several studies have been conducted to determine limiting AAs in rats (McLaughlan & Illman, 1967), pigs (Mitchell *et al.*, 1968), and poultry (Fernández-Fígares *et al.*, 1997) diets, and on the AA requirements of these animals. Profiles of AAs that cannot be synthesized in animal cells are the most important factor determining the nutritional value and efficiency of dietary proteins (Hou *et al.*, 2015; Hou *et al.*, 2016).

Generally; an animal's plasma AA concentrations increase in proportion to the levels of the respective AAs after dietary intake of a protein-rich feed. For example, there is a linear relationship between dietary level and plasma concentration of AAs such as leucine, isoleucine, or valine (Johnson & Anderson, 1982). Adibi & Mercer (1973) found that in humans, total free AA concentrations in blood plasma increased significantly after a protein-rich meal. However, these simple correlations are not always correct, and the plasma concentrations of most AAs are not linearly related to dietary intakes. The relationship between plasma and dietary AA concentrations may be influenced by interactions or antagonism between structurally related AAs competing for intestinal absorption (Matthews, 2000). Liu *et al.* (2019) reported that body weight gain of modern broilers is more sensitive to amino acid concentrations.

Davey *et al.* (1973) performed a study in pigs to determine the effect of dietary protein level, animal age, and sampling time on plasma concentrations of baseline amino acids. Their results showed that the concentrations of valine, isoleucine and leucine in the plasma of pigs fed a 20% crude protein diet were significantly higher than those fed a 13% crude protein diet, while the opposite was found for threonine. No difference was found for methionine, phenylalanine, lysine, histidine, arginine.

Gender is an important determinant of body composition and energy expenditure in humans. Protein oxidation has been reported to be lower in women than in men in the basal postprandial state (Volpi *et al.*, 1998). The gender differences in protein metabolism are of great interest since Gonadotropic hormones (Follicle-stimulating hormone-FSH and Luteinizing hormone-LH) are known to affect metabolism (Jensen *et al.*, 1994). Bancel *et al.* (1994) showed that plasma concentrations of valine, leucine, isoleucine, proline, glutamine, glutamate, and phenylalanine are higher in men.

In this study, serum Val, Asp, Arg, Cit, Gly, and Orn levels of male geese were higher than females'. This can be explained by gender-related differences in protein metabolism. In a study investigating serum amino acid concentrations in aging men and women, men have been reported to have higher concentrations of essential amino acids and branched-chain amino acids compared to women (Pitkänen *et al.*, 2003).

Bancel *et al.* (1994) reported that concentrations of total plasma amino acids, citrulline, cysteine, histidine, glutamine, glutamate, lysine, ornithine, and phenylalanine, were higher in the blood plasma of elderly individuals (80-100 years) than younger adults (20 -25 years). Pitkänen *et al.* (2003) reported that significant interactions between sex and age were observed in 7 of 22 single amino acids.

It was also determined that the effect of Gender x Body Weight interaction on Val, Cit, and Orn levels was significant ($p<0.05$). The small intestine is the main source of circulating citrulline, which is synthesized from proline and then converted to arginine (Dillon *et al.*, 1999). In addition, citrulline has a role in the formation of urea (Di Pasquale, 1997).

As a result; it has been concluded that the serum amino acid profile of healthy geese can vary according to gender and live weight, and more studies are needed to elucidate the reasons for these changes.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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