Chemical analysis and Angiotensin I-converting enzyme inhibitory activity of enzymatic hydrolysates derived from meat of goat-kids with supplemental selenium

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Abstract

Background: Selenium (Se) 's effects on animal health are well-documented due to its antioxidant and immune system regulatory properties. However, there is still a lack of

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scientific evidence regarding the impact of Se on muscle tissue. Se supplementation in ruminants enhances the antioxidant activity of myocytes and increases Angiotensin-converting enzyme (ACE) inhibitory activity. Generating bioactive peptides derived from meat could hinder the production of angiotensin II, a key player in the development of cardiovascular diseases.

Methods. Forty-five suckling goat kids were randomized into one of three groups: (1) CG: group without Se supplemented in the diet; (2) GSS: group with a single injectable dose subcutaneously of sodium selenite (Na_2SeO_3) at a dose of 0.25 mg/kg of body weight; (3) GSM: group with an oral administration of selenomethionine (SeMe) at a dose of 0.3 mg/kg of body weight). The effect of both sources of Se was evaluated on the proximate composition of meat and liver and the angiotensin I-converting enzyme inhibitory activity of meat-derived enzymatic hydrolysates.

Results. The kids' goat meat from the GSM group had a higher protein content ($p<0.05$). The fat content gradually increased over time in the GSM treatment, approximately doubling (from 1.77 to 3.68). The degree of hydrolysis of the meat samples decreased ($p<0.05$) in the treatments supplemented with Se (GSS and GSM). Conversely, the degree of hydrolysis significantly increased ($p<0.05$) over time in treatments with Se (GSS and GSM). At two hours, the electrophoretic patterns of the enzymatic hydrolysates revealed a molecular weight between 23.44 and 27.5 kDa, with the most intense bands. At 21 days post-slaughter, a greater degree of hydrolysis was observed in the Se-supplemented treatments (GSS and GSM) compared to the control group (CG). The meat protein content and the rate of ACE inhibition after hydrolysis improved (50% and 2%, $p < 0.05$) with GSM at seven days post-slaughter. Following hydrolysis, the IC50 of the selenium-supplemented groups decreased ($p <0.05$) for both the amount of CAE and IC50 values.

Conclusion. This is the first report describing the ACE inhibitory activity of bioactive peptides derived from goat kid meat with supplements. These results indicate the presence of ACE in goat meat; however, the percentage of ACE inhibition after hydrolysis was only enhanced with selenomethionine dosing at 7 days post-slaughter. The study's novelty reveals that supplemented selenium synergizes with ACE in goat meat. It is essential to continue these studies to identify specific bioactive peptides, antioxidant activities, and goat meat's biological and functional value, considering it a functional food that can prevent metabolic diseases and serve as a healthy alternative for the human population.

Introduction

Cardiovascular diseases associated with angiotensin II occur due to the activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitochondrial dysfunction,

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inflammation, and the reduction of endogenous antioxidant enzymes (Koju et al., 2019). Nitric oxide synthase (NOS) is another source of O_2 in which the substrate arginine or the cofactor tetrahydrobiopterin is limited. The uncoupling of NOS occurs due to pathological conditions associated with angiotensin II, such as hypertension, atherosclerosis, and diabetes. (Loperena & Harrison, 2016). Sources of $O_2^{\cdot}$ are eliminated in the cell by the enzymatic action of superoxide dismutase (SOD) that form H_2O_2 and oxygen, while other antioxidant enzymes, such as catalase and glutathione peroxidase, intervene to eliminate H_2O_2. Under normal physiological conditions, the rate of reactive oxygen species (ROS) generation is balanced by the rate of elimination through endogenous and dietary antioxidants such as SOD, catalase, thioredoxin, glutathione, and vitamins. Deregulation of angiotensin II signaling induces an increase in ROS, causing hypertension and cardiovascular dysfunction. (Jin & Kang, 2024). Several cardiovascular studies suggest that selenium (Se) and tocopherol reduce the risk of cardiovascular diseases, such as coronary heart disease. (Ju et al., 2017); NADPH is essential in regenerating antioxidant components known as reduced glutathione. Various pathways aid in the regeneration of NADPH, with the pentose phosphate pathway being the primary one. Some studies have shown that individuals with hypertension produce more reactive oxygen species and have an impaired antioxidant defense system, which increases oxidative stress. Antioxidants inhibit oxidation reactions, minimizing damage and the production of free radicals; this antioxidant deficiency initiates the onset of cardiovascular diseases (Wang & Kang, 2020).

Se is an essential trace element required for optimal human health, and it is incorporated into selenocysteine and selenomethionine, which are necessary for the synthesis of selenoproteins; these participate in oxidative stress, inflammation and immunity (Adadi, Barakova, Muravyov, & Krivoshapkina, 2019). Se deficiency is a serious problem in more than 500 million people in the world (Dinh et al., 2018); it is associated with multiple cardiovascular diseases, including myocardial infarction, heart failure, coronary heart disease, and atherosclerosis (Shimada , Alfulaij & Seale, 2021). Some epidemiological studies in humans indicate a positive association between low blood Se concentration and hypertension, known as Keshan disease. (Huang , Xie , Song & Zhang, 2022). The symptoms of the disease are cured with Se supplementation (Liao , Yan , Huang & Li, 2024). The effect of Se on human health is channeled with Glutathione peroxidase (GPx) activities to control blood pressure. Se is the main component of GPx; GPx activity reduces lipid peroxidation, atherosclerotic plaque formation, and platelet aggregation (Mansour et al., 2017). Se intake improves GPx activity and protects against hypertension and myocardial infarction (Handy & Loscalzo, 2022). Hypertension affects the antioxidant defense

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system and is partly a consequence of some dietary factors such as high sodium, fat, and refined carbohydrate content. (Lei et al., 2023). It has been reported that Se supplementation is used in aquatic animals. (Kohshahi, Sourinejad, Sarkheil, & Johari, 2019; Mansour et al., 2017) and poultry (Khajeh Bami, Afsharmanesh, Espahbodi, & Esmaeilzadeh, 2022; Zhang et al., 2020) Improves the growth, survival, and the activity of some antioxidant enzymes. However, other studies assess the effects of supplementary Se on the growth performance and antioxidant status in goats (Bano et al., 2019; Mojapelo & Lehloenya, 2019). In this context, since meat is a food high in protein, it is reasonable to consider it a good source of bioactive peptides (Ashaolu, Le, & Suttikhana, 2023). Diverse bioactivities, such as antioxidant, antihypertensive, antithrombotic, and antidiabetic effects, have been reported in peptides derived from fish, bovine, chicken, pork, and duck meats (Bezerra et al., 2019; Li et al., 2020; Maky & Zendo, 2021; Verma, Chatli, Mehta, & Kumar, 2018).

This study hypothesizes that adequate or high levels of Se in muscle tissue may enhance the antioxidant activity of myocytes and increase Angiotensin-converting enzyme (ACE) inhibitory activity. This could prevent the production of angiotensin II, a key player in the development of cardiovascular diseases. Importantly, this study is the first to investigate the ACE inhibitory activity of enzymatic hydrolysates derived from kids-goat meat supplemented with different Se sources. The findings of this study could significantly contribute to our understanding of the role of Se and ACE. Additionally, the effect of these supplements on the chemical composition of the meat and liver tissue was also evaluated, providing valuable insights into the potential health benefits of Se supplementation.

Materials & Methods

Animals

The study was conducted using 45 kid goats of the Pastoreña breed (average weight of 4.6 ± 1.14 kg, average age of 30 days) obtained from Oaxaca, Mexico. The animals were transported to the Colegio de Postgraduados, Montecillo campus (Texcoco, State of Mexico, Mexico) under comfortable environmental conditions, specificallypreciselyre of 18.5 °C and relative humidity of 60%. The kid goats were fed exclusively on goat milk *ad libitum*. All procedures performed with the animals in this study were approved by the Animal Ethics Committee of the Colegio de Postgraduados (approval reference code: 12013008) and by the national guidelines for animal care under NOM-033-ZOO-1995.

Experimental treatments

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After a 7-day adaptation period prior to begbeforehe experiment, the animals were randomly assigned to three groups (n=15 for each group) using a straightforward randomization method based on their weight. The experimental treatments were organized as follows: (1) CG: group without selenium (Se) supplementation; (2) GSS: group receiving a single subcutaneous injectable dose of sodium selenite (Na_2SeO_3) at 0.25 mg/kg of body weight; (3) GSM: group receiving oral selenomethionine (SeMe) at 0.3 mg/kg of body weight.

Meat samples

For each experimental treatment (i.e., CG, GSS, and GSM), five animals were subjected to fasting for 14 h and moved to the COLPOS slaughterhouse, 500 m away. The animal euthanasia was performed with a non-penetrating captive bolt stunner (CASH® Small Animal Tool), producing immediate insensibility. It was slaughtered in appropriate conditions according to NOM-033-ZOO-1995 at 7, 14, and 21 days. From each sacrificed animal, samples of *Biceps femoris* (BF) muscles were cut from the left side of the carcasses after 4 hours post-mortem. The meat samples were collected in Ziploc bags identified by treatment and kid number and frozen at –18 °C until analysis.

Proximate Composition Analyses of Meat

The chemical analysis of the meat was determined following the official methods of the AOAC (Association of Official Analytical Chemists). (Horwitz & Latimer, 2005). In this sense, the moisture (AOAC Method 934.01) was determined by weight loss after drying the sample at 100-110 °C for 18 h; protein (AOAC Method 2001.11) by the micro Kjeldahl method by digesting the sample with sulfuric acid and then distillation with sodium hydroxide using a nitrogen-to-protein conversion factor value of 6.25; fat (AOAC Method 920.39) was determined by the continuous extraction method in a Goldfish system using petroleum ether as a solvent; and ashes (AOAC method 942.05) by incineration at 600 °C until total loss of organic matter. All sample meat was made in duplicate. In addition, the chemical composition of the liver (i.e., protein, fat, moisture, and collagen contents) was measured using near-infrared spectroscopy (FoodScan™ Lab, Foss, Sweden) according to Anderson et al. (2007). Methods.

Preparation of enzymatic hydrolysates derived from the meat of kids-goat

Enzymatic hydrolysates from the meat of kids' goats were obtained, according to Escudero Fernández (2010). First, protein concentrates were extracted by freeze-drying meat samples from kids' goats. Briefly, the samples were freeze-dried for 48 hours in a Labconco FreeZone 6 Freeze Dryer (Labconco Corp., Kansas City, MO) at -51 ºC under a vacuum pressure of 0.120

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mBar. Afterward, the freeze-dried samples were ground in an electric coffee grinder (Hamilton Beach®, China) for 30 seconds to obtain dry powders. Subsequently, the dry powders were defatted with petroleum ether, and the defatted materials were stored at -20ºC until hydrolysis. Next, 4 grams of each dry powder sample were resuspended in 43 mL of distilled water, and the pH was adjusted to 2.0 with 0.1 M HCl. These suspensions were heated at 37 ºC and hydrolyzed for two hours with pepsin using an enzyme-to-substrate ratio of 1:100. Hydrolysis was terminated by heating to 95 °C for 10 minutes.

Degree of hydrolysis

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (hot), was determined using the pH-stat method. (Adler-Nissen, 1986) and calculated using the following equation:

$$DH(\%) = \frac{h}{h_{tot}} = \left[\frac{\beta \times Nb}{\alpha \times M_p \times h_{tot}}\right] \times 100$$

Where β and Nb refer to the amount of NaOH used during the proteolysis of the substrate and its normality, respectively; α represents the average degree of dissociation of the a-NH_2 groups in the protein substrate; M_p is the mass (g) of the protein; and that denotes the total number of peptide bonds in the protein substrate. The values of h_{tot}=7.6 and α=1, and the pH and temperature were regulated according to the suggestion of Nielsen, Petersen, and Dambmann (2001).

Determination of protein content

The protein content of enzymatic hydrolysates derived from the meat of goat kids was estimated using the Bradford method. (Bradford, 1976)A standard curve was constructed using bovine serum albumin (0.75, 0.5, 0.25, 0.125, 0.062 mg/mL).

Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Tricine-SDS-PAGE)

The method was modified for gel electrophoresis. Hydrolysate samples (10 µL) were mixed with 25 µL of 4x buffer (TruPAGE 4x PCG3009, Sigma Aldrich) and 65 µL of distilled water. The resulting solution was maintained at 70 ºC for 10 minutes, followed by cooling with ice water. A Mighty Small II SE 250 (Hoeffer, EE. UU.) system with TruPAGE (PCG2012-10EA; Sigma Aldrich) gels (gradient 4-20%) was utilized. Molecular weight markers with the following molecular weights were included as a reference: 23.4, 30.1, 43.6, 52.4, 63, 70.7, 79.4,

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100, 123, 147.9, 162.1, 177.8, and 282 kDa. Electrophoresis was conducted at 25 mA (120 V) for 60 minutes. A staining solution containing Coomassie Brilliant Blue R-250 (Biorad 1610400) was applied to stain the gels for 30 minutes, followed by de-staining with 50% ethanol.

Angiotensin-Converting Enzyme Inhibitory Activity

With some modifications, ACE inhibitory activity was evaluated as described by Hayakari, Kondo, and Izumi (1978). Samples (40 µL) were combined with 20 µL of angiotensin-converting enzyme (100 mU) and incubated for 5 min at 37 ºC. Next, 100 µL of the substrate, Hippuryl-His-Leu (HHL) 0.3% (w/v), and the reaction was carried out for 45 min at 37 °C. After that, 360 µL of 2,4,6-trichloro-s-triazine dissolved in dioxane and 720 µL of potassium phosphate buffer (0.2 M, pH 8.3) were added to terminate the reaction. Later, the resulting solution was centrifugated (10,000 g, 10 min), and the absorbance of the supernatant was recorded at 382 nm (Multiskan Go, Thermo Scientific).

$$\text{ACE inhibition } (\% = \frac{(A - B)}{(A - C)} x\ 100$$

Where: A denotes absorbance in the presence of ACE and the sample; B indicates the absorbance of the control, and C refers to the absorbance of the reaction blank.

The IC_{50} (ACA in kDa) before and after hydrolysis was calculated with a regression equation using the following formula: y=mx+b

Statistical analysis

The data were statistically analyzed as a complete randomized design with a factorial arrangement of 3×3 (samples days x treatments), using the PRO MIXED model procedure performed on SAS software version 9.0. The factors were the experimental treatment supplements with Se (i.e., CG, GSS, and GSM) and the slaughter time (i.e., 7, 14, and 21 d). The terms of the model were the experimental treatment, slaughter time (repeated measures), and the interaction of both. The means of each factor were compared using the Tukey test, and differences were considered significant when $p < 0.05$. The following statistical model was used:

$$Y_{ijk} = \mu + T_i + S_j + \varepsilon_{ijk}$$

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Where: Y_{ijk} = variable response; μ = mean; Ti = effect of experimental treatment; Sj = effect of slaughter time; and $\mathcal{E}_{ijk}$ = random error.

Results

Chemical Composition of Meat

The physicochemical composition of meat derived from kids fed goat diets with Se is shown in Table 1. Our results indicated no effects of the experimental treatment and time factors ($p>0.05$) on the moisture content of the meat samples; thus, their values did not vary between treatments and slaughter days. In contrast, the kids-goat meat from the GSM group exhibited a higher protein content ($p<0.05$) than the three slaughter times and other experimental groups. At 21 days of slaughter, the GSM treatment had approximately 5% more ($p<0.05$) protein compared to the group without Se supplementation (CG). Conversely, the fat content gradually increased over time in the GSM treatment, which rose approximately 2-fold (from 1.77 to 3.68). Similarly, the fat content increased about 1.5 times at 21 days in the GSM treatment compared to the CG treatment. In contrast, the ash content in both CG and GSS treatments decreased significantly over time ($p<0.05$), while in the GSM treatment, a significant increase ($p<0.05$) was observed over time.

Table 1.

Chemical Composition of Liver

The contents of moisture, protein, fat, and collagen of the liver-derived from kids-goat-fed diets with Se are shown in Table 2. For the treatments CG and GSM, the moisture content increased significantly ($p<0.05$) over time, increasing 2.03% and 1.13%, respectively, from 7 to 21 d of slaughter. While for the treatment GSS, the moisture content decreased significantly ($p<0.05$) at 21 d compared to the values of 7 and 14 d. Similarly, the protein content of GSS increased significantly ($p<0.05$) over time, while for the treatments, CG and GSM had a contrary behavior, decreasing significantly ($p<0.05$) from 7 to 21 d of slaughter. The fat content had the same behavior as protein content; the treatment GSS with more fat (*ca.* 40%) at 21 d of slaughter. In contrast, the treatments with Se (GSS and GSM) had more collagen at 7 and 14 d of slaughter, while on the contrary, at 21 d, the CG had more collagen compared to those groups supplemented with Se.

Table 2.

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Degree of hydrolysis

The meat percentage of ACE inhibition before hydrolysis improved at 7d of slaughter (12.1%, $p<0.05$) with GSM and at 14 (54%, $p<0.05$) and 21d (49%, $p<0.05$) of slaughter with GSS and GSM, respectively. The experimental treatment and time factors ($p<0.05$) affected the degree of hydrolysis of the meat samples hydrolysates. It was observed that at 14 d, the degree of hydrolysis decreased ($p<0.05$) in the treatments supplemented with Se (GSS and GSM), while at 21 d of slaughter, had a contrary behavior, with the treatments GSS and GSM having major ($p<0.05$) degree of hydrolysis compared to the group without Se supplementation (CG). Particularly, it was observed that the degree of hydrolysis increased ($p<0.05$) significantly over time in those treatments with Se (GSS and GSM).

Protein content

Overall, both before and after hydrolysis, the protein content significantly decreased ($p<0.05$) over time in all treatments (5). Similarly, at 21 days post-slaughter, the treatments supplemented with Se (GSS and GSM) exhibited higher ($p<0.05$) protein content compared to the group without Se supplementation (CG). Generally, the protein content in samples prior to hydrolysis was greater than that after hydrolysis (Table 3).

Tricine -SDS-PAGE electrophoresis

The electrophoretic patterns of the enzymatic hydrolysates at zero hours and two hours of hydrolysis obtained from the enzymatic hydrolysates derived from the meat of kid goats supplemented with Se. It can be observed that the native protein patterns at zero hours of hydrolysis of meat from kid goats supplemented with Se were very similar across all treatments. These non-hydrolyzed proteins exhibited a molecular weight between 37.1 and 213.7 kDa. In contrast, the electrophoretic patterns of the enzymatic hydrolysates at two hours showed a molecular weight range of 23.44 to 27.5 kDa, with the bands exhibiting greater intensity. At 21 days post-slaughter, a higher degree of hydrolysis was observed in the treatments supplemented with Se (GSS and GSM) compared to the group without Se supplementation (CG) (Figure 1). Notably, it was observed that the degree of hydrolysis increased significantly ($p<0.05$) over time in the treatments with Se (GSS and GSM).

Figure 1.

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ACE inhibitory activity of enzymatic hydrolysates derived from meat samples

Overall, the ACE inhibitory activity of samples after hydrolysis was higher than that of samples before hydrolysis (Table 3). The ACE inhibitory activity values after hydrolysis were 4 to 6 times greater than those before hydrolysis. Following hydrolysis, the ACE inhibition percentage for all treatments significantly increased over time ($p < 0.05$). Notably, the meat protein content and the rate of ACE inhibition after hydrolysis improved (by 50% and 2%, $p < 0.05$) with GSM at 7 days post-slaughter. The IC50 values are determined by the molecular weight of ACE before and after hydrolysis. After hydrolysis, the IC50 values of the selenium-supplemented groups decreased ($p < 0.05$), along with the amount of CAE (Table 3).

Table 3

Discussion

Chemical Analysis of Meat and Liver

The physicochemical composition of meat derived from kid-goat-fed diets with Se showed no effects of the experimental treatment and time factors on moisture content. Thus, Se supplementation did not influence the moisture content of the meat samples, which were between 72.5-79.2%. Similar observations in moisture contents were reported in Indian kids-goat meat with values of 72.4-75.3% (Das & Rajkumar, 2010), Brazilian kids-goat (75.9-76.9%)(Freitas et al., 2011), and kids-goat originating from East Africa (70.7%) (Shija et al., 2013). In a related study, the supplementation of organic Se (Se yeast) at the dose of 0.3 mg/kg diet showed 51.8% moisture in 4-month kids-goat, which was below compared to the data of our study. The protein content in meat samples was increased by the supplementation of Se, which could be a result of the hypertrophic effect of Se in the muscles of goats. (Parveen Samo et al., 2018; Rannem et al., 1995). In addition, it has been reported that supplementation with minerals such as Se in the diet influences growth and nutrient digestibility rates (Parveen Samo et al., 2018). For example, Del Razo-Rodriguez et al. (2013) reported that the total tract digestibility of lambs was increased because of Se supplementation at a dose of 0.2-0.6 mg/kg. On the other hand, kids-goat meat contains a low-fat content clow fat to other meats such as beef or pork (>10% fat) (Park et al., 2018). In the literature on goat meat, the fat content was reported between 2.2-4.05% (De Palo, Maggiolino, Centoducati, & Tateo, 2015; Ivanović et al., 2020), consistent with our findings. Our data suggest that Se supplementation increased the fat *ca.* 43% at 21 d of slaughter compared to 7 d of slaughter. In a related study, similar results were obtained by Parveen Samo et al. (2018), showing a 37% fat increase in the group supplemented with Se compared to the group without it. Se supplementation has been

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519 reported to alter lipid metabolism by decreasing cholesterol deposition in muscle, possibly by
520 changing the ratio between reduced glutathione and oxidized glutathione (Netto et al., 2014).
521 This may suggest meat with desirable characteristics. For example, it has been reported that
522 the meat's taste, tenderness, and succulence are influenced by muscle collagen content and
523 other factors like pH, sarcomere length, streaky fat, and the degree of muscle protein
524 degradation (Erasmus, Muller, & Hoffman, 2017; Martínez Marín, 2008).
525
526 As mentioned above, our findings suggest that supplementation improves certain chemical
527 components of goat meat from kids, providing desirable quality attributes for
528 commercialization. To the best of our knowledge, this is the first study reporting the effect of
529 selenium (Se) diets on the chemical composition of liver derived from kids' goats, which can
530 serve as reference values for future research. Overall, there was no general effect over time of
531 Se on the liver's chemical composition. However, it was noted that the experimental treatment
532 GSS, a group of kids' goats supplemented with Se through a subcutaneous injectable dose,
533 increased their protein, fat, and collagen content compared to the group without Se
534 supplementation (CG) or the group administered Se orally. This indicates that the route of Se
535 administration can influence its effect on liver composition. For instance, organic Se is
536 considered more bioavailable than inorganic forms (Amoako, Uden, & Tyson, 2009). However,
537 further studies are needed to validate this observation.
538
539 **Degree of hydrolysis, protein content, and SDS-PAGE analysis**
540 In our study, the degree of hydrolysis was influenced by the Se supplementation and the time
541 of slaughter. This parameter indicates the efficiency of the hydrolysis of meat proteins to
542 produce bioactive peptides (Bahari, Saari, Salim, & Ashari, 2020), so a higher degree of
543 hydrolysis may imply an increase in both the number and size of peptides, which can enhance
544 their bioactivities (Upata et al., 2022). Therefore, selenium positively affects the degree of
545 hydrolysis of meat, although no prior studies have been found on the impact of selenium on
546 the hydrolysis of animal products. Concurrently, there is evidence of increased activity of
547 certain hydrolytic enzymes in plants due to selenium's influence (Zeid, Gharib, Ghazi, &
548 Ahmed, 2019); a similar or alternative mechanism could occur in meat. In contrast, the protein
549 content decreased in all treatments after hydrolysis, consistent with previous reports on fish
550 (Wisuthiphaet & Kongruang, 2015) and chicken (Yuliatmo, Fitriyanto, & Bachruddin, 2017)
551 protein hydrolysates. According to these authors, larger proteins were converted into smaller
552 peptides and amino acids after hydrolysis, while the methods used to quantify proteins in
553 hydrolysates (i.e., Bradford, Lowry) can only detect proteins with a molecular weight greater

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Deleted: some…ertain chemical components of goat meat from kids-goat… providing desirable quality attributes for their …ommercialization. On the other hand, to…o the best of our knowledge, this is the first study reporting the effect of selenium (Se) diets with Se …n the chemical composition of liver derived from kids-goat…ids' goats, which can be used…erve as reference values for future research. Overall, there was no generalized…eneral effect over time of Se on the liver's chemical composition of the liver.… However, it was observed…oted that the experimental treatment GSS, a group of kids-goat with…ids' goats supplemented with Se through an… subcutaneous injectable dose subcutaneously… increased their content of …rotein, fat, and collagen in comparison…ontent compared to the group without Se supplementation (CG) or to …he group with Se administered Se orally. Considering this,…his indicates that the route of Se administration route …an influence its effect on liver composition. For example … [2]

Deleted: value of the …egree of hydrolysis can suggest a higher …ay imply an increase in both the number and size of peptides, which can improve…nhance their bioactivities (Upata et al., 2022). Thus, Se…herefore, selenium positively influences…ffects the degree of hydrolysis of meat, although no previous…rior studies have been found on the effect…mpact of Se…elenium on the hydrolysis of animal products. At the same time…oncurrently, there is evidence of the increases …ncreased activity of some…ertain hydrolytic enzyme activity …nzymes in plants by the impact of Se…ue to selenium's influence (Zeid, Gharib, Ghazi, & Ahmed, 2019); a similar or another pathway…lternative mechanism could occur in meat. In contrast, the protein content decreased in all treatments after the …ydrolysis process, which is according to… consistent with previous reports on fish (Wisuthiphaet & Kongruang, 2015) and chicken (Yuliatmo, Fitriyanto, & Bachruddin, 2017) protein hydrolysates. According to these authors, after hydrolysis, large…arger proteins were converted into smaller peptides and amino acids, … [3]

than 3000 Da. SDS-PAGE confirmed both observations regarding the degree of hydrolysis and protein content; following hydrolysis, larger proteins were degraded into smaller peptides and amino acids. Based on the results, a greater degree of hydrolysis may indicate that the meat proteins were further broken down into multiple polypeptide chains, small peptides, and amino acids. Similar results on SDS-PAGE were reported in hydrolysates of Korean native cattle (Lee & Hur, 2017) and spent hen meat (Kumar et al., 2021).

ACE inhibitory activity

Angiotensin-I converting enzyme (ACE) is related to controlling blood pressure and electrolyte homeostasis by converting angiotensin I into potent vasoconstrictor angiotensin II. (Ahmad et al., 2023). Thus, the inhibition of ACE can help to regulate the blood pressure in hypertensive persons and the treatment of various cardiovascular diseases such as heart failure, myocardial infarction, diabetic nephropathy, or renal dysfunction. (Ktari et al., 2014). Although current medical treatments are very effective (e.g., captopril, enalapril, alacepril, or lisinopril), they showed several side effects (inflammatory response, dry cough, taste disturbance, or angioneurotic edema in some patients. (Ambigaipalan, Al-Khalifa, & Shahidi, 2015). Our data indicated that ACE inhibitory activity increased five-fold over time in all treatments, particularly in groups with Se supplementation. All hydrolysates demonstrated ACE inhibitory activity greater than 90%, more significant this study shows that kid-goat meat hydrolysates can inhibit ACE activity. The ACE inhibitory capacity of these peptides may be attributed to their distinct amino acid compositions and hydrophobicity. Scientific literature suggests that hydrophobic amino acid residues, such as leucine, valine, alanine, tryptophan, tyrosine, proline, and phenylalanine, preferentially bind to the catalytic sites of ACE. Therefore, these peptides can act as strong competitive ACE inhibitors. (Jung et al., 2006; Wu, Liao, & Udenigwe, 2017). However, further studies are necessary to characterize the peptides responsible for the observed bioactivities. In particular, peptidomics and mechanistic studies are needed to elucidate amino acid sequences and structure-activity relationships, and in silico docking modeling studies are needed to observe enzyme–peptide interactions, such as ACE-peptides.

Conclusions

This is the first report describing the ACE inhibitory activity of bioactive peptides derived from Se-supplemented goat meat. Selenomethionine dosing for 21 days improved the total

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protein content of the meat by 36%. On the other hand, the percentage of ACE inhibition in the meat before hydrolysis was enhanced at 7 days of slaughter with selenomethionine and 14 and 21 days of slaughter with the application of both selenium sources, respectively. These results indicate the presence of ACE in goat meat; however, the percentage of ACE inhibition after hydrolysis was only improved with selenomethionine dosing at 7 days of slaughter. The lack of ACE recovery in the other two slaughter periods was likely due to a lack of precision in the purification technique. The study's novelty indicates that supplemented selenium had a synergism with ACE in goat meat. It is necessary to continue these studies to identify specific bioactive peptides, antioxidant activities, and goat meat's biological and functional value, considering it a functional food that can prevent metabolic diseases and be a healthy alternative for the human population.

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