

# The sarcoplasmic protein profile of breast muscle in Turkeys in response to different dietary ratios of limiting amino acids and *Clostridium perfringens*-induced inflammation

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**ABSTRACT** In this study, the effects of the Arginine/Lysine (**Arg/Lys**) ratio in low- and high-methionine (**Met**) diets on the sarcoplasmic protein profile of breast muscles from turkeys reared under optimal or challenge (*Clostridium perfringens* infection) conditions were determined. One-day-old Hybrid Converter female turkey poults (216 in total) obtained from a commercial hatchery on hatching day, and on the basis of their average initial body weight were randomly allocated to 12 pens (4 m<sup>2</sup> each; 2.0 m × 2.0 m) containing litter bedding and were reared over a 42-day experimental period. Diets with high levels of Lys contained approximately 1.80% and 1.65% Lys and were offered in two successive feeding periods (days 1–28 and days 29–42). The supplemental levels of Lys were consistent with the nutritional specifications for birds at their respective ages as established in the Management Guidelines for Raising Commercial Turkeys. The experiment was based on a

completely randomized 3 × 2 × 2 factorial design with three levels of Arg (90%, 100% and 110%) relative to the content of dietary Met (30 or 45%) and without (–) or with (+) *C. perfringens* challenge at 34, 36, or 37 d of age. Meat samples were investigated in terms of pH, color, and sarcoplasmic protein profile. The experimental factors did not influence meat quality but the dietary Arg content affected meat color. The sarcoplasmic protein profile was influenced by all studied factors, and glycolytic enzymes were the most abundant. This study evidenced strong association between the challenge conditions and the involvement of glycolytic enzymes in cell metabolism, particularly in inflammatory processes, and DNA replication and maintenance in turkeys. The results showed an effect of *C. perfringens* infection and feeding with different doses of Arg and Met may lead to significant consequences in cell metabolism.

**Key words:** poultry, amino acids, arginine, methionine, glycolytic enzymes

2022 Poultry Science 101:102195

<https://doi.org/10.1016/j.psj.2022.102195>

## INTRODUCTION

Poultry meat is valued for its high protein content, lower fat content and resulting low caloric content, a favorable ratio of unsaturated to saturated fatty acids, and high levels of n–3 polyunsaturated fatty acids (**PUFAs**) compared with other types of meat. Meat quality is affected by antemortem and postmortem processing. In this context, stress factors play key roles by

influencing the quality attributes, technological effects and processing suitability of the meat. Poor quality meat is unacceptable to consumers and is therefore not marketed ([Baracho et al., 2006](#)). Adequate nutrition plays an important role in the growth and development of birds and affects poultry meat quality ([Jankowski et al., 2020](#)). For optimized production and sustainable health, poultry require sufficient levels of necessary body-accessible nutrients. However, most feeds commonly given to farm animals are deficient in some nutrients, and therefore, dietary supplements are needed ([Shabani et al., 2019](#)). The nutritional value and/or quality of dietary proteins used in poultry feed formulations vary: amino acid availability to the bird is an important measure of protein quality. Total dietary protein in feed consists of heterogeneous mixtures of

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Received April 28, 2022.

Accepted September 16, 2022.

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different proteins, which are digested at different rates, creating variations in the rates of different amino acid uptake in the gut. Moreover, nutrient levels are not solely based on proteins, complicating dietary analyses; for example, although the chemical compositions of proteins are indistinguishable, the various linkages of proteins with carbohydrates, lipids and other proteins influence the interactions and composition of the diet, thus affecting the digestibility of dietary proteins.

Castro and Kim (2020) claimed that certain amino acids, such as arginine (**Arg**), methionine (**Met**), and cysteine, are precursors for other essential molecules involved in immune defense, the antioxidant system, cell signaling, and gene expression, and therefore, they affect animal growth and development. Because poultry are subjected to stressful conditions throughout the rearing period, amino acids, including their secondary metabolites, can be fed to benefit the general health of birds. For example, Ognik et al. (2020) postulated that the use of optimal levels and proportions of lysine (**Lys**), Arg, and Met in compound feeds leads to their optimal exploitation in the growth of contemporary turkey hybrids raised for slaughter and reduces metabolic disorders. Wu et al. (2014), Liao et al. (2015), and Castro and Kim (2020) emphasized that Arg supplementation modulates musculoskeletal health development, reduces fat accretion, and improves the antioxidant system. Moreover, Met can improve bone development and exhibits the potential to mitigate the negative effects of heat stress. Understanding how these amino acids can ameliorate stress may provide novel insights into their use in nutritional strategies to improve bird health (Castro and Kim, 2020). Notably, Lys, Met, and Arg limit the biological value of protein in cereal- and soybean meal-based diets fed to turkeys (NRC, 1994), but the optimal dietary inclusion rates and ratios are hotly debated (Zhang et al., 2017; Jankowski et al., 2020). In particular, the metabolism of essential amino acids as well as birds' responses to dietary ratios differs and largely depends on the rearing conditions (optimal or challenge) of the birds (Konieczka et al., 2021). In this study, to determine the effect of different Arg-to-Lys ratios in diets with either low or high Met levels on the sarcoplasmic protein profile of breast muscle, birds reared in optimal (with no challenge) or challenge (*Clostridium perfringens* infection) conditions were evaluated. Although the pathogenic mechanisms of this pathogen are poorly understood in poultry (He et al., 2022), we chose this challenge-model based on the previous reports, in which their pathogenesis has been linked to the ability of *C. perfringens* to disrupt collagen, which is the main structural component of connective tissue (Wade et al., 2015). This was also evidenced in our recent study on the chicken model (Konieczka et al., 2020). In our very recent study, we also found that the diets of increased Arg, Lys, and Met fed to turkeys over the first 4-wk period of growth and exposed to different challenge factors (including *C. perfringens* challenge) increased significantly the transcripts levels not only of tight junction proteins genes but also selected genes

encoding nutrient transporters (Konieczka et al., 2022). Because both of these gene-groups are engaged in processes related to the maintenance of homeostasis in whole biological system (Castro and Kim 2020), we speculated that by targeting them, it may be an effective intervention to manage the initial stage of inflammation in the host, which could in turn affect metabolic processes contributing to meat features associated with quality in turkeys. Because the metabolism of key elements, including peptides, fatty acids, and amino acids in the host depends on gut barrier functions to a high extent (Bortoluzzi et al., 2018), we assumed that different ratio of essential amino acids, would be effective in supporting gut integrity and thereby shall affect meat features in challenge conditions. The study was based on the hypothesis that bacterial infection and feed with different Arg-to-Lys ratios and either low or high Met levels influence the sarcoplasmic protein profile (especially glycolytic enzymes) in breast muscle and thus may have significant effects on muscle cell metabolism.

## MATERIALS AND METHODS

### Ethics Statement

The study protocol and all procedures, including the number of birds used in this study, were evaluated and approved by the Ethics Committee at the University of Warmia and Mazury, Olsztyn, Poland (Resolution no. 82/2017), and the birds were maintained under guidelines comparable to those laid down by EU Directive 2010/63/EU.

### Birds and Housing

One-day-old Hybrid Converter female turkey poults (216 in total) were obtained from a commercial hatchery (Grelavi in Ketrzyn, NE Poland) on hatching day, and on the basis of their average initial body weight, these birds were randomly allocated to 12 pens; 6 pens of 18 birds for unchallenged birds and 6 pens of 18 birds for challenged birds (the area of each pen was 4 m<sup>2</sup>; 2.0 m × 2.0 m) containing litter bedding and were reared over a 42-d experimental period. The birds subjected to *C. perfringens* challenge were maintained in the same room, but were separated from uninfected birds to prevent cross-contamination. The temperature, humidity and lighting programs were the same for all pens located in the experimental room, following the standard recommendations in the Management Practices for Hybrid Converter Turkeys (Aviagen Turkeys, 2016).

### Experimental Design and Diets

The detailed feeding program and composition of the experimental diets were previously reported by Ognik et al. (2020). Briefly, the birds received ad libitum access to experimental diets, which were formulated to be isocaloric and meet or exceed the turkeys' nutritional

**Table 1.** Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis) fed to turkeys (unchallenged and challenged) at 1–4 and 5–6 wk of age.<sup>1</sup>

Item	1–4 wk	5–6 wk
<b>Ingredients</b>		
Wheat	43.98	47.72
Maize	10.00	10.00
Soybean meal, 48% CP	28.77	26.54
Rapeseed meal	3.00	3.00
Potato protein	5.00	2.96
Soybean oil	0.95	2.85
Maize gluten meal	3.50	3.00
Sodium bicarbonate	0.20	0.20
Sodium chloride	0.15	0.16
Limestone	2.07	1.87
Monocalcium phosphate	1.94	1.55
L-Threonine	0.09	0.10
Choline chloride	0.10	0.10
Vitamin-mineral premix <sup>2</sup>	0.25	0.25
<b>Calculated nutrient content</b>		
Metabolizable energy, kcal/kg	2820	2950
Crude protein	27.0	24.5
Arginine	1.58	1.44
Lysine	1.36	1.19
Methionine	0.44	0.39
Met + Cys	0.91	0.83
Threonine	1.02	1.01
Calcium	1.30	1.15
Available phosphorus	0.70	0.60

<sup>1</sup>Modified from Ognik et al., (2020).

<sup>2</sup>Provided per kg diet (feeding periods: weeks 0–4 and 5–6): mg: retinol 3.78 and 3.38, cholecalciferol 0.13 and 0.12,  $\alpha$ -tocopheryl acetate 100 and 90, vit. K3 5.8 and 5.6, thiamine 5.4 and 4.7, riboflavin 8.4 and 7.5, pyridoxine 6.4 and 5.6, cobalamin 0.032 and 0.028, biotin 0.32 and 0.28, pantothenic acid 28 and 24, nicotinic acid 84 and 75, folic acid 3.2 and 2.8, Fe 64 and 60, Mn 120 and 112, Zn 110 and 103, Cu 23 and 19, I 3.2 and 2.8, Se 0.30 and 0.28, respectively.

needs (Table 1). Each of six treatment was administered to one pen of 18 birds in unchallenged birds and to respective one pen of 18 birds in challenged birds. Diets with high levels of Lys contained approximately 1.80% and 1.65% Lys and were offered in two successive feeding periods (days 1–28 and days 29–42). The supplemental levels of Lys were consistent with the nutritional specifications for birds at their respective ages as established in the Management Guidelines for Raising Commercial Turkeys (Aviagen Turkeys, 2016). The experiment was based on a completely randomized 3 × 2 × 2 factorial design with three levels of Arg (90%, 100%, and 110%) relative to the content of dietary Met (30 or 45%) and without (–) or with (+) *C. perfringens* challenge. To attain specific amino acid levels in the diets, the amino acid concentrations in the basal diet and supplements were analytically determined, and on this basis, the final concentrations were adjusted and analytically confirmed.

### *C. perfringens* Challenge

All of the birds from challenged group (18 turkeys per pen) were subjected to challenge. The birds were challenged at 34, 36, and 37 d of age with *C. perfringens* inoculum. A 1 mL dose of bacterial inoculum cultured overnight in brain heart infusion medium (Sigma Aldrich) containing approximately

10<sup>8</sup> CFU/mL *C. perfringens* was given to birds *per os*. Before *C. perfringens* challenge, a coccidia vaccine containing different *Eimeria* species (Laboratorios HIPRA S.A., Spain) was administered to 31- and 34-day-old turkeys to create a favorable gut environment for *C. perfringens* colonization.

### Sample Collection

A total of six 42-day-old turkeys from each treatment group were weighted (individually) and sacrificed by cervical dislocation, and the right breast muscles (*pectoralis major*) were collected, placed in separate polystyrene bags and transported under cold conditions to the laboratory of Warsaw University of Life Sciences for analyses (n = 6/group was considered for each analysis in this study). Then, the samples were chilled for 24 h at 4°C. The next day, the quality parameters of the meat samples were evaluated in the laboratory. The samples for extraction of muscle protein were packed in closed Ziploc bags and frozen at –80°C. The samples were stored for up to one month for further analyses.

### Meat Quality Measurement

The meat pH value was directly measured 48 h (pH 48) after slaughter with a WTW 330i pH meter (Weilheim, Germany) equipped with special electrodes (SenTixSP Number 103645); three measurements were taken. The electrode was calibrated using WTW buffers with pH values of 4.01 (No. 108706) and 7.00 (No. 108708), consistent with the guidelines established by the National Institute of Standards and Technology/Polish Biochemical Society (NIST/PTB).

Meat color was measured 48 h postmortem on the basis of the CIE L\*a\*b\* system using a CR310 Minolta Chroma Meter (Osaka, Japan) with a D65 light source at an 8° standard observer angle and 8 mm aperture. The loin chops (2 cm long) were cut and allowed to bloom without a cover at 4°C for 1 h prior to color measurement (performed in triplicate).

### Profile of the Sarcoplasmic Proteins

SDS–PAGE was performed to determine the sarcoplasmic protein profile following the method of Bollag and Edelstein (1999) using a Bio–Rad apparatus in the Laboratory of the Department of Biochemistry in the Faculty of Agriculture and Biology in the Warsaw University of Life Sciences. The concentration of the protein was determined following the Bradford<sup>14</sup> procedure. A sample of 150 mg meat was homogenized at 13,500 rpm with 600  $\mu$ L of 0.003 M phosphate buffer at pH 7, for 1 min. The homogenate was centrifuged at 10,000 × g for 3 min. The supernatant containing sarcoplasmic proteins was aliquoted and frozen at –80°C for further analysis. Proteins were dissolved 1/1 (v/v) in buffer containing Tris-HCl, pH 6.8; 8 M urea; 2 M thio-urea; 0.375 M  $\beta$ -mercaptoethanol; 3% SDS; and 0.05%

blue bromophenol. The mixture was then heated at 95°C for 3 min. Then, 15 µg of the protein with glycerol (at a 10% final concentration) were loaded into each well of a 12% acrylamide Tris-HCl separating gel with a 5% stacking gel. Electrophoresis separation was carried out at room temperature for 1 h at 75 V and then for 8 h at 150 V. The gels were stained with Coomassie Brilliant Blue R250. Image analysis and quantification were performed using GelScan v.1.5 software (Kucharczyk TE, Poland). Thermo Scientific protein markers (Thermo Scientific Part No. 26616, 26617, 26618, Page Ruler Pre-stained Protein) were used as references for molecular weight determination.

## Statistical Analyses

The data were analyzed with Statistica version 13 software (TIBCO Software Inc, 2017). The basic descriptive statistics (mean and standard error) were calculated. The normality of the data distribution was determined by the Shapiro–Wilk test. The effects of three levels of Arg (90%, 100%, and 110%) relative to the content of dietary Met (30 or 45%) and *C. perfringens* infection (–, +) were evaluated using three-way ANOVA (3 × 2 × 2 factorial design). The significance of differences between means was calculated based on a least significant differences test (LSD). The significance levels were determined based on values of  $P < 0.05$  and  $< 0.01$ . A Principal component analysis (PCA)-based multivariate analysis was performed to gain a better understanding of the total variability and characteristics of all experimental groups with respect to the sarcoplasmic protein profile.

## RESULTS AND DISCUSSION

Body weight (BW) response; BW response of 42-day-old turkeys is presented in Table 2. Overall, different levels of Arg and infection significantly affected BW at the end of the trial. Birds fed diet with Arg 110% had higher BW than that of Arg 90%, whereas Arg 100% did not differ from the other treatments ( $P = 0.004$ ). Applied challenge resulted in significant BW depression ( $P = 0.016$ ). A significant interactions were found; Arg × Met ( $P = 0.014$ ) as well as Arg × Met × infection ( $P = 0.036$ ). Although, a 2-way interaction is in line

**Table 2.** Body weight response to different dietary arginine (Arg) and methionine (Met) levels in unchallenged (–) and challenged (+) turkeys at 42 day of age.

Trait	Body weight, kg
Arg level, % (A)	
90	2.33 <sup>a</sup>
100	2.43 <sup>ab</sup>
110	2.50 <sup>b</sup>
Met level, % (B)	
30	2.41
45	2.44
Infection (C) <sup>1</sup>	
–	2.47 <sup>a</sup>
+	2.37 <sup>b</sup>
SEM	0.023
P-value	
Arg	0.004
Met	0.521
Interactions	0.016
A × B	0.014
A × C	0.133
B × C	0.871
A × B × C	0.036

<sup>ab</sup>Mean values followed by different superscript letters are significantly different at the  $P \leq 0.05$  level.

<sup>1</sup>Birds were challenged at 34, 36, and 37 d of age with *C. perfringens* bacteria.

with the other indices response associated with interactions reported herein (opposite action between Arg 90% vs. Arg 110% in most cases), the 3-way interactions can not be logically explained. Nevertheless, it is interesting finding indicating that the most pronounced effect can be expected comparing low and high Arg dietary levels, which justified manipulations in Arg level in turkeys rations.

The data related to the meat quality evaluations are summarized in Table 3 according to the dietary treatment and effect of *C. perfringens* infection. Notably, the average pH and color parameter values presented in Table 3 were similar to the results reported by Jankowski et al. (2020). The analysis of variance did not indicate a significant effect for any of the examined factors, except for the effect of the Arg content on the a\* color parameter (Table 3). The results presented in Table 3 indicate that as the Arg level increased, the redness of the meat also increased. This result suggests that an increase in the proportion of Arg may change muscle metabolism and increase the muscle pigment (myoglobin) proportion or decrease muscle bleeding. Jankowski et al. (2020) stated that Arg is a precursor in the synthesis of certain metabolic components, including

**Table 3.** The effects of *Clostridium perfringens* infection and dietary Arg/Met-Lys ratios on meat quality characteristics in turkeys.

Trait	Effect of <i>C. perfringens</i> infection			Effect of dietary Arg/Met-Lys ratios						SEM	
	–	+	P	Arg level (%)			P	Met level (%)			
				90	100	110		30	45		
pH	5.68	5.69	ns	5.70	5.68	5.68	ns	5.68	5.68	ns	0.01
L*	50.31	50.64	ns	49.90	50.85	50.67	ns	50.73	50.22	ns	0.25
a*	–0.31	–0.46	ns	–0.60 <sup>a</sup>	–0.54 <sup>a</sup>	–0.02 <sup>b</sup>	0.05	–0.41	–0.37	ns	0.10
b*	6.18	5.83	ns	5.88	5.87	6.26	ns	6.05	5.96	ns	0.19

Explanation: Arg, arginine; Lys, lysine; Met, methionine; ns, non significant; 0.05 – differences significant at  $P < 0.05$ ; without (–) or with (+) *C. perfringens* challenge at 34, 36, or 37 d of age.

<sup>a,b</sup>Mean values followed by different capital superscript letters are significantly different at the  $P \leq 0.05$  level.

nitric oxide, which promotes blood flow and the supply of oxygen, glycogen, amino acids, creatine, and other essential nutrients in muscle. The results with respect to pH and color parameters were in agreement with the results of Zampiga et al. (2019) and Jankowski et al. (2020), who did not report an effect of increasing Arg levels in feed on meat quality parameters, such as pH and color, in chickens or turkeys. The test results presented in Table 3, which show no effect of Arg or Met level in the feed on the final pH of the meat, were consistent with the results reported by Ognik et al. (2021), who indicated no effect of feed with different proportions of Arg or Met on the blood glucose levels in turkeys. Other authors observed a significant effect of increasing the amount of Met added to feed on the quality of chicken and turkey meat. Gardzielewska et al. (2005), Wang et al. (2009), and Drazbo et al. (2015) showed the effect of Met added to the diet of turkeys on the pH value and lightness ( $L^*$ ), redness ( $a^*$ ) and other meat quality traits.

The results of a variance analysis showed a significant effect of all studied factors, infection by *C. perfringens* and Arg as well as Met level, on the sarcoplasmic protein profile (Table 4). Significant double and triple interaction effects among the above mentioned main factors were also found in the profile of sarcoplasmic proteins analyzed in the samples obtained from muscle tissue (Supplementary Tables S1–S4). The protein profile analysis showed that glycolytic enzymes critical to post slaughter muscle glycolysis were the primary proteins identified on the basis of SDS–PAGE (Figure 1). Di Luca et al. (2011) stated that muscle exudate provides valuable information about the pathways and processes underlying the postmortem aging period. The proportions of the total amount of individual enzymes in this study were similar to those in the profile of the natural drip loss sample taken from chicken breast muscle by Bowker et al. (2016). In the present study, the

predominant enzymes were enolase (EN), glyceraldehyde phosphate dehydrogenase (GAPDH)/lactate dehydrogenase (LDH), creatine kinase (CK)/phosphoglycerate kinase (PGAK), and aldolase (ALD). Significantly lower amounts of phosphorylase b/phosphorylase b kinase (PHb/PHbK), phosphoglucomutase (PGM), phosphoglycerate mutase (PGAM), triosephosphate isomerase (TPI), and myokinase (MK) were also found. Tasoniero et al. (2020) showed a slightly different profile of sarcoplasmic proteins in broiler breast. They found the highest content of GAPDH, ALD, EN, CK/PGAK (from 20 to 12%) and while the average amount for PGM-PK-PGI, LDH and GP (from 8.4 to 6%) and the lowest for PGAM, TPI (from 4.6 to 3.4%). The results of this study showed that *C. perfringens* infection significantly increased the PGM, EN, CK/PGAK, ALD, and TPI contents led to significant decreases in PGAM and MK levels (Table 4). These results indicate that in the context of infection and inflammation, the courses of glycolysis and glycolysis may differ from those of uninfected tissue. Kvidera et al. (2017) stated that an activated immune system consumes more glucose and proteins (e.g., for antibody synthesis). According to Marino et al. (2014), the differences in sarcoplasmic protein profiles depend on changes that occur during aging and postmortem degradation. Wang et al. (2017) showed that heat stress treatment increases the activity of pyruvate kinase (PK). Also, Fu et al. (2014) showed a significant influence of stress on the level of glycolytic enzymes such as: Glucose-6-phosphate isomerase, 6-Phosphofructokinase, Glyceraldehyde-3-phosphate dehydrogenase, Phosphoglycerate mutase, Pyruvate kinase, Lactate dehydrogenase, Creatine kinase. Some studies have shown that nutritional factors can significantly affect the sarcoplasmic protein profile and glycolytic enzyme abundance (Shibata et al., 2009; Przybylski et al., 2017; Watanabe et al., 2020). The study of

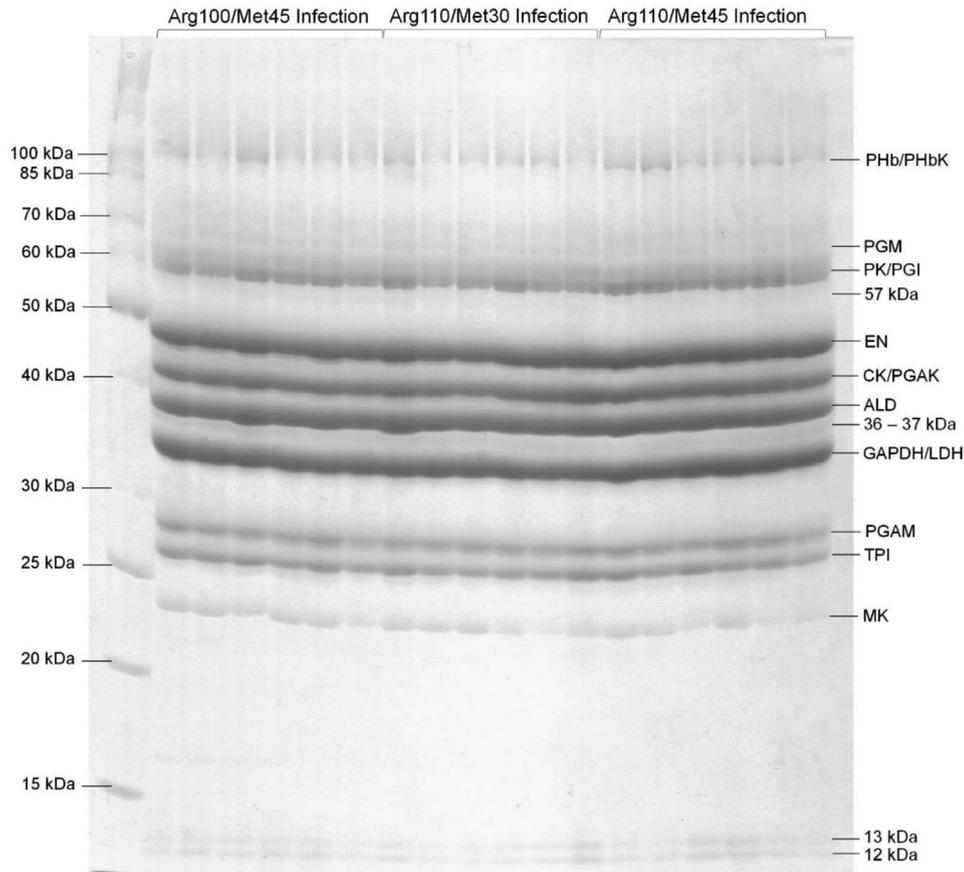
**Table 4.** The effects of *Clostridium perfringens* infection and dietary Arg/Met-Lys ratios on the sarcoplasmic protein profile of breast muscle exudate in turkeys.

Trait	Effect of <i>C. perfringens</i> infection			Effect of dietary Arg/Met-Lys ratios							
	-	+	p	Arg level (%)			p	Met level (%)		p	SEM
				90	100	110		30	45		
PHb/PHbK	6.63	6.28	ns	5.72 <sup>a</sup>	6.93 <sup>b</sup>	6.71 <sup>b</sup>	0.05	6.39	6.52	ns	0.27
PGM	6.93 <sup>a</sup>	7.31 <sup>b</sup>	0.05	7.22 <sup>ab</sup>	7.34 <sup>a</sup>	6.7b <sup>b</sup>	0.05	7.35 <sup>a</sup>	6.88 <sup>b</sup>	0.05	0.13
PK/PGI	10.19	10.45	ns	9.87 <sup>a</sup>	10.17 <sup>a</sup>	10.92 <sup>b</sup>	0.05	10.02 <sup>a</sup>	10.62 <sup>b</sup>	0.05	0.11
57 kDa	2.05 <sup>A</sup>	1.48 <sup>B</sup>	0.01	2.36 <sup>a</sup>	1.83 <sup>b</sup>	1.09 <sup>c</sup>	0.05	2.13 <sup>a</sup>	1.39 <sup>b</sup>	0.05	0.10
EN	15.81 <sup>A</sup>	16.81 <sup>B</sup>	0.01	12.01 <sup>a</sup>	11.54 <sup>b</sup>	11.96 <sup>a</sup>	0.05	12.13 <sup>a</sup>	11.54 <sup>b</sup>	0.05	0.12
CK/PGAK	11.67 <sup>a</sup>	12.01 <sup>b</sup>	0.05	11.93 <sup>a</sup>	12.10 <sup>a</sup>	11.89 <sup>a</sup>	0.05	11.19 <sup>b</sup>	12.58 <sup>c</sup>	0.05	0.09
ALD	11.64 <sup>A</sup>	12.33 <sup>B</sup>	0.01	11.90	1.86	12.19	ns	11.74 <sup>A</sup>	12.23 <sup>B</sup>	0.01	0.13
37-36 kDa	2.26 <sup>A</sup>	1.43 <sup>B</sup>	0.01	2.36 <sup>a</sup>	1.69 <sup>b</sup>	1.48 <sup>b</sup>	0.05	2.06 <sup>A</sup>	1.36 <sup>B</sup>	0.01	0.10
GAPDH/LDH	14.72	14.45	ns	14.65 <sup>b</sup>	15.72 <sup>a</sup>	14.32 <sup>b</sup>	0.05	14.24 <sup>b</sup>	14.51 <sup>b</sup>	0.05	0.10
PGAM	7.70 <sup>A</sup>	7.14 <sup>B</sup>	0.01	7.55	7.48	7.21	ns	7.32	7.72	ns	0.09
TPI	5.90 <sup>a</sup>	6.20 <sup>b</sup>	0.05	5.63 <sup>a</sup>	6.13 <sup>b</sup>	6.39 <sup>b</sup>	0.05	5.94	6.16	ns	0.08
MK	2.57 <sup>A</sup>	1.91 <sup>B</sup>	0.01	2.06 <sup>a</sup>	2.49 <sup>b</sup>	2.16 <sup>ab</sup>	0.05	2.29	2.18	ns	0.08
13 kDa	0.93 <sup>a</sup>	1.07 <sup>b</sup>	0.05	0.83 <sup>a</sup>	1.05 <sup>b</sup>	1.11 <sup>b</sup>	0.05	0.97	1.03	ns	0.04
12 kDa	1.00 <sup>A</sup>	1.16 <sup>B</sup>	0.01	1.05	1.08	1.10	ns	1.10	1.05	ns	0.04

Explanation: Arg, arginine; Lys, lysine; Met, methionine; ns, non significant; without (–) or with (+) *C. perfringens* challenge at 34, 36, or 37 d of age; 0.05 – differences significant at  $P < 0.05$ ; 0.01 – differences significant at  $P < 0.01$ .

<sup>a,b</sup>Mean values followed by different capital superscript letters are significantly different at the  $P \leq 0.05$  level.

<sup>A,B</sup>Mean values followed by different capital superscript letters are significantly different at the  $P \leq 0.01$ .



**Figure 1.** SDS-PAGE analysis of soluble sarcoplasmic proteins taken from the *pectoralis* muscle in turkeys. The left lane corresponds to a molecular weight scale. Abbreviations: PHb/PHbK, phosphorylase b/phosphorylase b kinase; PGM, phosphoglucomutase; PK/PGI, pyruvate kinase/phosphoglucose isomerase; EN, enolase; CK/PGAK, creatine kinase/phosphoglycerate kinase; ALD, aldolase; GAPDH/LDH, glyceraldehyde-3-phosphate dehydrogenase/lactate dehydrogenase, PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; and MK, myokinase.

Watanabe et al. (2020) showed that reduction in dietary lysine (from 100% to 90% recommended requirement) upregulated 22 metabolites (among them, inter alia, related to glycolytic transformations) and 24 were down-regulated. In turn, studies by Zhai et al. (2012) showed that the level of methionine in the ration of broiler chickens significantly affects a number of metabolic pathways, including may influence gluconeogenesis and suggest that sarcoplasmic hypertrophy predominated over myofibrillar hypertrophy. The conclusions of the aforementioned studies are consistent with the results presented in Table 4, which show a significant influence of Arg and Met level on the sarcoplasmic protein profile. Increasing the level of Arg in the diet significantly influenced the linear increase in PHb/PHbK, PK/PGI, and TPI in the natural drip loss sample (Table 4). However, a different outcome was obtained for PGM, EN, GAPDH/LDH, and MK; that is, in general, an increase in Arg levels from 90 to 100% increased the levels of these enzymes, but when the Arg was increased to 110%, the levels of these enzymes decreased (Table 4). Despite significant differences between the food groups (Arg level) in the glycolytic enzyme profile of the natural drip loss sample, no significant differences were noted in the final pH values or the L\* and b\* color parameters (Table 4). These outcomes show that, despite the

different enzymatic profiles of individual groups, no significant differences in the extent of postslaughter glycolysis or *postmortem* changes were observed in muscle tissue. These results are consistent with those of the previously cited study by Jankowski et al. (2020). According to Malinowska (1999) and Przybylski et al. (2016), the range of postmortem glycolysis and quality of meat depend not only on enzymatic activity but also on the number and reciprocity of the relationships between enzymes and the substrate concentrations.

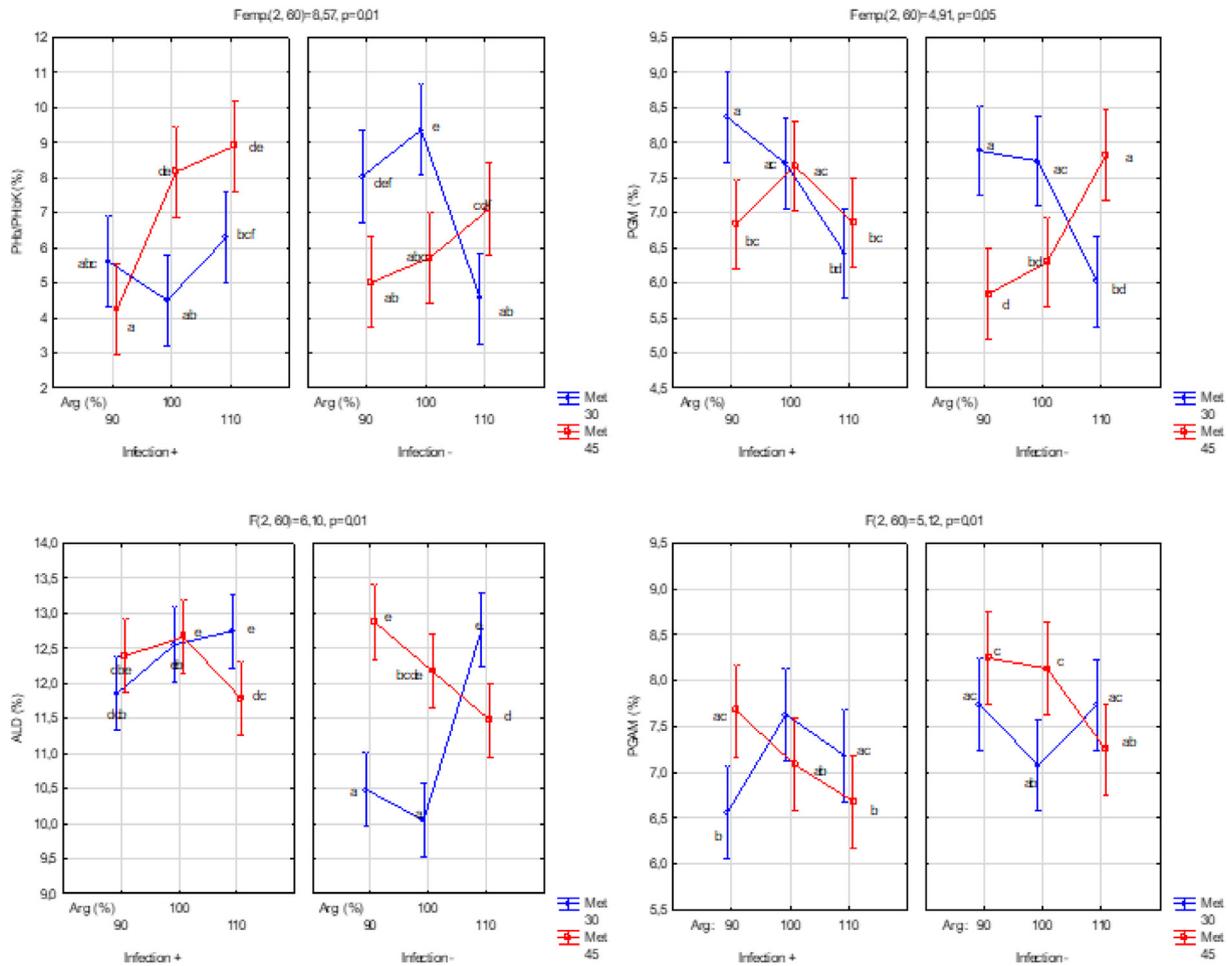
Notably, many studies have presented evidence showing that some glycolytic enzymes play complicated and multifunctional roles with unexpected functional effects, including transcription regulation (HK, LDH, GAPDH, and ENO), apoptosis (HK and GAPDH) and cell motility (GPI) (Kim and Dang, 2005). Among enzymes that perform many simultaneous functions, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is particularly well characterized (Kim and Dang, 2005; Rodacka et al., 2013; Lincet et al., 2015; Konieczna et al., 2015). G3PDH is ~37 kDa and catalyzes the sixth step of glycolysis; that is, it converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in conjunction with the reduction of NAD<sup>+</sup> to NADH (Kim and Dang, 2005). GAPDH is the most frequently identified enzyme in meat exudate (Żelechowska et al., 2012), is known as a

classical glycolytic enzyme, and is encoded by a so-called “housekeeping gene,” which is commonly used (and sometimes misused) as a gene expression control (Kim and Dang, 2005). This protein has been shown to be involved in many cellular processes (in addition to glycolysis), such as DNA repair, transfer RNA export, the regulation of gene expression, membrane fusion and transport, vesicular transport, cytoskeletal dynamics and cell death and/or cell prosurvival functions (Tristan et al., 2011; Ouali et al., 2013). According to Tristan et al. (2011), when cells are exposed to various stressors, GAPDH undergoes dynamic subcellular redistribution, and GAPDH is involved in various diseases, especially neurodegenerative disorders and cancers. In response to cellular stress, GAPDH has been shown to participate in the regulation of cell signaling pathways; for example, it regulates the release of calcium from the endoplasmic reticulum into the cytosol (Rodacka, 2013). In pigs, this enzyme accounts for 15 to 25% of all proteins found in natural drip loss samples, while in sheep and poultry, it accounts for approximately 11 to 15% (Żelechowska et al., 2012; Bowker et al., 2016; Przybylski et al., 2016, 2017). A higher level of GAPDH in chickens with breast myopathy was observed by Kuttappan et al. (2017) and Xing et al. (2020). In the present study, significantly higher levels of this enzyme and LDH were observed when diet Arg levels were 100%, lower levels than 90% or 110% (Table 4). LDH is a tetramer consisting of subunits A and B and catalyzes the conversion of pyruvate to lactate with the simultaneous conversion of NADH to NAD<sup>+</sup>. LDH is a very important enzyme in cellular metabolism, especially in skeletal muscle, where the LDHA form is dominant (Konieczna et al., 2015). LDHA has been identified as a single-stranded DNA (ssDNA)-binding protein and as a DNA-helix-destabilizing protein, and it has been speculated that LDHA is involved in transcription or DNA replication (Kim and Dang, 2005). Xin et al. (2017) suggested that GAPDH and LDH-B activities may play roles in maintaining the color stability of meat. Furthermore, Zheng et al. (2014) showed a significant upregulation of glycolytic enzymes, including LDH-A and GAPDH, in the pectoral muscle of chickens fed the probiotic *Enterococcus faecium*.

As previously stated, EN was found to be the most abundant enzyme (Table 4). EN converts 2-phosphoglycerate (2PG) to phosphoenolpyruvate (PEP) in the 9th glycolytic reaction. The results presented in Table 4 show that infection increased the amount of EN, but an increase in either the Arg or Met level in the feed resulted in an EN level decrease. In addition to its basic role in glycolysis, EN plays many noncatalytic roles, including transcriptional regulation (Kim and Dang, 2005). The cell surface possesses a receptor for human plasminogen, which explains its role in pathogen invasion (Diaz-Ramos et al., 2012). These previous reports of EN activity are consistent with the data presented in Table 4. Zheng et al. (2014) showed downregulation of EN expression in the muscles of chickens fed the probiotic *E. faecium* and suggested that this

downregulation was accompanied by improved meat quality. Many authors have mentioned that EN, GAPDH, and LDH in slaughtered animals are potential biomarkers of meat tenderness (Ouali et al., 2013; Bowker et al., 2014; Marino et al., 2014; Picard et al., 2015). Furthermore, Beauclercq et al. (2017) showed that increased EN levels in chickens were associated with lower ultimate pH.

Aldolase (ALDO) is another abundant glycolytic enzyme with an express pattern similar to that of EN in response to *C. perfringens* infection. ALDO is involved in the aldol cleavage reaction. The substrate for ALDO is fructose-1,6-bisphosphate, which is cleaved to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (Lincet et al., 2015; Przybylski et al., 2016) which is the final reaction in the 1st stage of glycogenolysis. In human tissue, three aldolase isozymes are expressed in a tissue-dependent manner: ALDO A (mainly in muscles), ALDO B (mainly in liver) and ALDO C (in neuronal tissue). ALDO A is the most commonly expressed isozyme in tumor tissue, and it is overexpressed in various cancers, such as squamous cell lung cancer and hepatocellular carcinoma (Lincet et al., 2015). Pirani (2008) showed that ALDO interacts and creates a complex with actin. Dżugaj (2006) demonstrated that fructose-1,6-bisphosphatase forms a complex with muscle ALDO in vivo, and in this complex, fructose-1,6-bisphosphatase is insensitive AMP-induced inhibition. As a result, glycogenesis can proceed from the lactate formed in muscle under anaerobic conditions. This metabolic pathway has recently been acknowledged by researchers (Przybylski et al., 2016). According to Lincet and Icard (2015), in addition to its glycolytic function, aldolase A is involved in several functions independent of its glycolytic role, such as signal transduction, vesicle trafficking, and cell motility. Aldolase A can be phosphorylated by Akt or Erk2 kinases and then be translocate to the nucleus. In this compartment, ALDO A is involved not only in the regulation of transcription of genes implied in cell cycle progression but also in the stabilization of transcripts by linking with AT-rich DNA sequences, which also protects DNA from damage. In the cytosol, ALDO A has been implicated in the endothelial-mesenchymal transition (EMT) as indicated by changes in the expression EMT markers. In this study, the increase in ALDO level due infection and irrespective of the proportion of Arg or Met in the feed, seemed to be associated with the counteracting response of the organism to DNA damage or with the expression of genes related to other defense mechanisms in the organism. Moreover, the results presented in Figure 2 and Table 4 mainly show that the Met level and, to a lesser extent, the Arg level in the feed exerted a significant effect on the amount of ALDO expressed in muscles under normal conditions. The levels of Arg and Met in the feed were also significantly influenced by other very important enzymes, namely, pyruvate kinase (PK) and phosphoglucose isomerase (PGI) (Table 4). PK catalyzes the tenth (final) reaction in glycolysis: the irreversible transfer of phosphate from phosphoenolpyruvate to



**Figure 2.** Triple interaction effects on the sarcoplasmic protein profile of breast muscle exudate in turkeys as determined by analysis of variance. Arg, arginine; Met, methionine; without (–) or with (+) *C. perfringens* infection at 34, 36, or 37 d of age; <sup>a,b,c,d,e</sup> - mean values followed by different superscript letters are significantly different at the  $P \leq 0.05$ .

ADP to produce ATP and pyruvate (Lincet and Icard, 2015). There are 2 different PK genes: PKL/R (pyruvate kinase, in the liver and red blood cells) and PKM2 (pyruvate kinase, in muscles), which expresses 2 isoforms, M1 and M2. PKM1 and PKM2 are produced by alternative splicing of the PKM gene (Lincet and Icard, 2015). The tetramer form of PKM2 can efficiently promote glycolysis and energy production, but the presence of the low-activity PKM2 dimer stops the conversion of phosphoenolpyruvate to pyruvate, which leads to the accumulation of glucose metabolites upstream and the accumulation of a large number of precursor substances required for the synthesis of macromolecules (Xu et al., 2019). Recent studies have shown that PKM2 not only has a glycolytic function but also has a nonglycolytic function. PKM2 can be depolymerized from the tetramer form to dimer form and translocated to the nucleus, mitochondrial membrane, or outside the cell. PKM2 is a protein kinase that phosphorylates various protein substrates at serine/threonine and tyrosine residues. PKM2 interacts with the metabolic substrate, phosphoenolpyruvate, which serves as the phosphate donor for the phosphorylation of a variety of target proteins, including STAT3, histone H3, myosin light chain 2 (MLC2), Bub3, and ERK1/2. Translocated PKM2

dimers are involved in the regulation of gene transcription, metabolic reprogramming, mitosis, apoptosis, and other important cellular events by promoting transcriptional activation, modulating signal transduction, or regulating the phosphorylation of important proteins (Xu et al., 2019). PKM2 can be translocated from the cytoplasm to the nucleus under certain conditions. Nuclear PKM2 is a protein kinase that phosphorylates the transcription factor STAT3, thus boosting IL-6 and IL-1 $\beta$  production. PKM2 has been shown to play a previously unidentified role as a molecular integrator of metabolic dysfunction, oxidative stress and tissue inflammation (Shirai et al., 2016). The results presented in Table 4 show that the increase in the proportion of both Arg and Met in the feed significantly influenced the increase in the levels of PK and PGI, which may have had a significant impact on the abovementioned metabolic processes. Studies by various authors have shown the effect of nutritional factors on the level and activity of PK muscle isozymes. For example, Kawashita et al. (2002) found that decreased PK activity had an effect similar to that of a high-protein diet. In addition, Zheng et al. (2014) showed feeding chickens probiotics exerted a significant effect on the level of PKM. Przybylski et al. (2017) noted a significant

increase in PK/GPI when feed was enriched with fish oil and a decrease when it was added feed additives, increasing antioxidant defenses in the form of carnosic acid and selenium of an inorganic origin. The second enzyme of this band, GPI, also known as PGI and phosphohexose isomerase PHI, is a cytosolic enzyme involved in basal metabolism. PGI plays a central role in both the glycolysis and gluconeogenesis pathways. In the cytoplasm, the dimeric form of PGI catalyzes the reversible isomerization of GPI (also known as aldose) to fructose-6-phosphate (ketose). PGI enzyme may also redirect glucose to the pentose phosphate pathway (PPP) for the production of NADPH and pentoses. GPI is a multifunctional protein (a moonlighting protein) that has acquired other additional functions through evolution (Konieczna et al., 2015; Zong et al., 2015). Beauclercq et al. (2017) showed high GPI gene expression in chickens with a lower ultimate pH.

The effect of adding Arg and Met to the feed on the phosphorylase b/phosphorylase b kinase (PHb/PHbK) level is interesting, especially with regard to the triple interaction effect (Table 4 and Figure 2). A significant effect was found for Arg added to the feed at 100% and 110% compared to that of Arg added at 90% (Table 4). However, an analysis revealed that the triple interaction effect was only clearly observed when the Met dose was 45% (Figure 2); however, it is especially pronounced in infected turkeys. However, at a Met dose of 30%, the Arg addition effect varied and was difficult to interpret. PHb liberates glucose from glycogen to form glucose-1-phosphate. The final product of PHb and hexokinase activity is glucose-6-phosphate (in the case of PHb, some portion of this yield is PGM). Approximately 34.6% of glycogen molecules are directly susceptible to degradation by phosphorylase (Przybylski et al., 2016). Additionally, during glycogenolysis, some portion of the free glucose is liberated by a glycogen-debranching enzyme that hydrolyses  $\alpha$ -1-6 bonds in glucosyl branches (Kylä-Puhju et al., 2005). These results and relationships confirm the higher demand for glucose by the activated immune system in infected animals, as shown by Kvidera et al. (2017) and Sałek et al. (2020). Zhu et al. (2013) stated that many authors have treated glycogen phosphorylase as a crucial index because it indicates the presence of pale, soft, and exudative (PSE) meat in turkeys. Rathgeber et al. (1999) found higher quantities of glycogen phosphorylase in myofibrillar protein extracts obtained from rapidly glycolyzed turkey muscles. The results of a study by Marino et al. (2014) suggested that PHb is a good proteolytic activity marker candidate as well as a meat tenderness indicator. Purintrapiban et al. (2001) and Marino et al. (2014) showed that glycogen phosphorylase is a calpain substrate that is degraded postmortem.

PGM is another glycolytic enzyme that exerted a significant influence on all tested factors and their interactions were noted (Table 4 and Figure 2). PGM is an enzyme in the glycolytic pathway involved in the interconversion of glucose-1-phosphate and glucose-6-phosphate. More PGM is usually found in muscle leakage

undergoing more intense glycolysis (Żelechowska et al., 2012). Ouali et al. (2013) stated that PGM is a potential biomarker of meat tenderness. In the present study, infection was shown to induce a significant increase in the amount of PGM (Table 3). This finding is similar to that of PHb/PHbK and confirms the higher energy demands of inflammatory processes. In contrast, the results shown in Figure 2 indicate that an increase in Arg addition when the Met level was 30% caused a decrease in PGM. On the other hand, with a higher Met content, namely 45%, in healthy turkeys, an increase in PGM was observed with an increase in the addition of Arg from 90% to 110% (Figure 2). Zheng et al. (2014) found a significant effect of feed supplemented with probiotics on the increase in PGM levels in chickens. In addition, Przybylski et al. (2017) found that the addition of fish oil to feed increased the amount of PGM and that the addition of antioxidants (carnosic acid or selenium of inorganic origin) restored the amount of PGM to the initial level.

Additionally, a significant influence of all 3 tested experimental factors was found for another group of enzymes that were found in significant amounts in the effluent, that is, CK and PGAK. CK catalyzes reversible phosphate group transfer from phosphocreatine (CP) to ADP, regenerating ATP, or from ATP to creatine, restoring phosphocreatine stores. CK is a key enzyme in cell bioenergetics, playing an important role in cellular ATP homeostasis. According to Żelechowska et al. (2012), a higher CK level is related to a rapid degradation of CP, glycolysis, a more rapid pH decline, and a high level of drip loss. Przybylski et al. (2017) showed a decrease in CK levels after adding selenium to feed. On the other hand, Ognik et al. (2020) showed that the addition of Arg in feed and infection with *C. perfringens* exerted a significant effect on the blood serum CK levels of 42-day-old turkeys. A similar trend was observed in the results presented in Table 4. The second enzyme present in the same band, that is, PGAK catalyzes the conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate. PGAK is a multifunctional enzyme that is, inter alia, a cofactor of  $\alpha$  DNA polymerase and may participate in the synthesis of delayed DNA strands during replication (Konieczna et al., 2015). As previously mentioned, the effects of feeding fodder supplemented with probiotics and selenium on the level of PGAK have been demonstrated (Przybylski et al., 2017; Zheng et al., 2014).

The level of PGAM, which is critical for the conversion of 3-phosphoglycerate to 2-phosphoglycerate, was influenced by the effect of *C. perfringens* infection, and the triple interaction effect of all main factors was observed. Overall, infection with *C. perfringens* significantly reduced the amount of PGAM in muscles (Table 4 and Figure 2). In addition, Arg in the feed increased the amount of PGAM in muscles when the Met level was 45% (Figure 2). The Arg effect when the Met level was 30% was not sufficiently clear to explain. PGAM activity is commonly upregulated in many cancers, and its inhibition is lethal to cancer cells in culture. PGAM

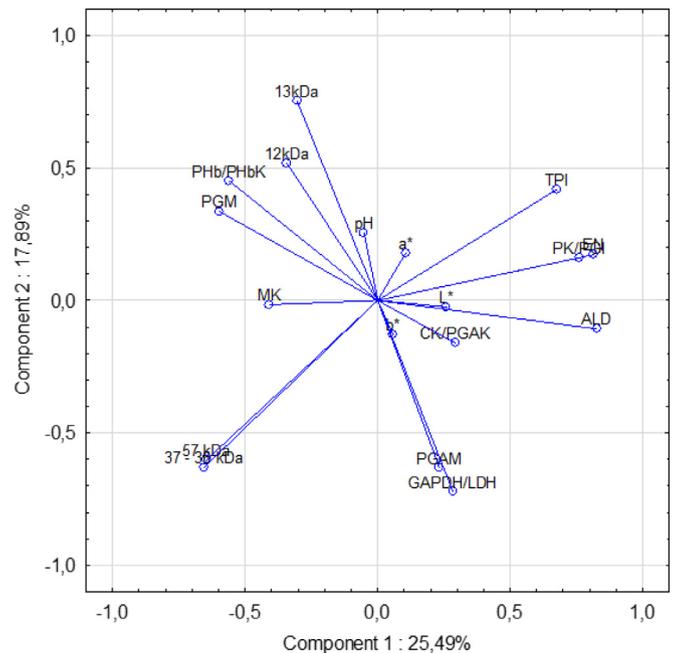
plays a crucial role in coordinating glycolysis and biosynthetic pathways to promote cancer cell proliferation, particularly under hypoxia. PGAM plays a critical regulatory role in glycolysis, as indicated by the lethality of PGAM inhibition induced by epoxide inhibitors in cancer cells (Lincet and Icard, 2015). In other studies, increasing the level of fish oil lowered the level of PGAM, while the addition of probiotics and antioxidants increased its level in muscles (Przybylski et al., 2017; Zheng et al., 2014). Cai et al. (2018) showed overabundant level of PGAM in wooden breast muscle.

The results of the study showed a significant effect of *C. perfringens* infection and the addition of Arg to feed on the level of TPI, with both infection and an increase in Arg resulting in a significant increase in the TPI level in muscles. Notably, TPI accounted for approximately 6% of the protein in this study (Table 4). TPI catalyzes the reversible conversion of phosphodihydroxyacetone to glyceraldehyde-3-phosphate, but only the latter can contribute to glycolysis and thus prevent the accumulation of dihydroxyacetone phosphate. Activation of TPI is critical for ATP production through glycolysis, whereas its inhibition or attenuation drives dihydroxyacetone phosphate into the PPP, which is a key source of reduced NADPH, a cofactor in anabolic pathways (i. e., fatty acid biosynthesis) and in maintaining the redox balance. Thus, TPI regulates the distribution of metabolites between glycolysis and the PPP by coordinating NADPH, H<sup>+</sup> and ATP production with redox metabolism, which depends on reactive oxygen species production. Grüning et al. (2014) demonstrated that an accumulation of phosphoenolpyruvate inhibits TPI activity, which in turn promotes PPP pathway activation, protecting cells against oxidants and, thus, preventing reactive oxygen species accumulation. Przybylski et al. (2017) showed that the addition of fish oil to feed lowered the TPI level, while the addition of antioxidants (carnosic acid or selenium) increased the TPI level in muscles. Teltathum and Mekchay (2010) showed a significant and positive relationship between the TPI level in muscle and the Warner-Bratzler shear force in chicken meat. Additionally, Cai et al. (2018) showed that TPI was overabundant in normal meat compared to wooden breast.

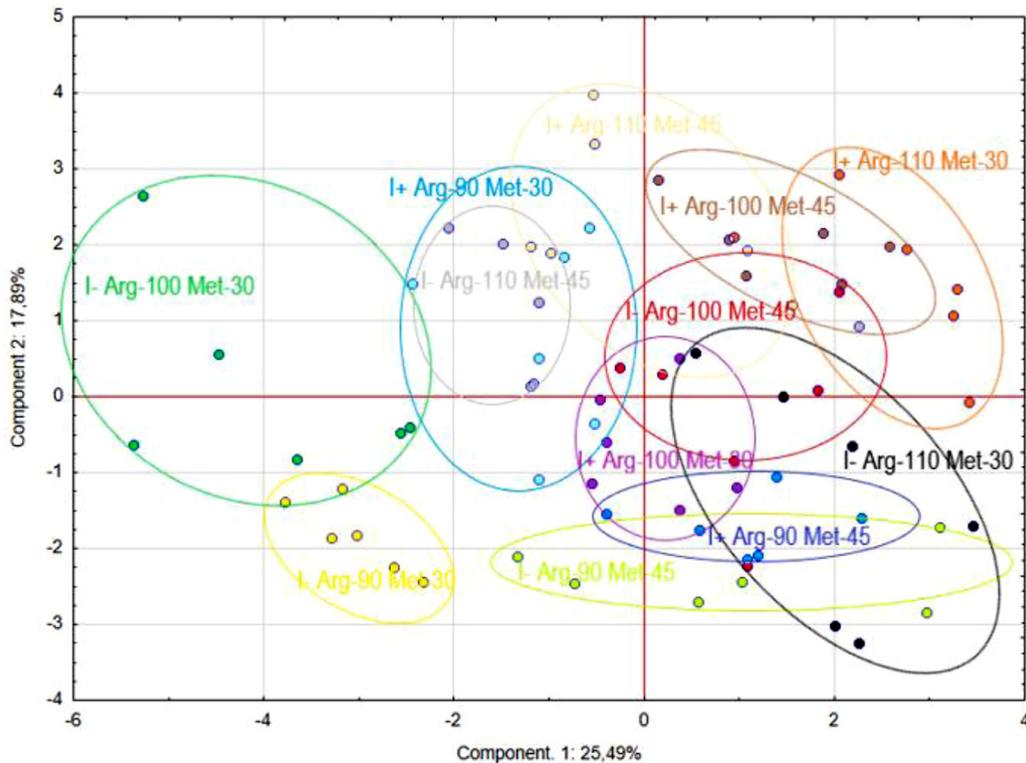
The least-abundant enzyme (2–2.5%) was MK, which was significantly less abundant in the muscles of infected turkeys; however, the addition of Arg significantly increased the amount of MK (Table 4). MK (also known as adenylate kinase or ADK) is a phosphotransferase enzyme that catalyzes the interconversion of adenine nucleotides. ADK is important to cellular energy homeostasis since it can constantly monitor phosphate nucleotide levels inside a cell. The ability of a cell to dynamically measure energetic levels enables easy metabolic process monitoring. ADK can regulate energy expenditure at the cellular level by continually monitoring and altering the adenyl phosphate levels. As energy levels change under different metabolic stresses, ADK can generate AMP, which triggers the activation of signaling cascades. Common factors that have been shown

to influence adenine nucleotide levels and therefore ADK activity include exercise, stress, changes in hormone levels and diet (Dzeja and Terzic, 2009). Therefore, the influence of the studied factors on MK level may also exert a significant impact on the energy metabolism of muscle cells. A significant effect of the examined factors on the number of unidentified proteins with molecular weights of 57 kDa, 37–36 kDa, 13 kDa, and 12 kDa was found (Table 4).

To better understand the total variability in the sarcoplasmic protein profile of all the samples and individual groups, PCA was performed, and the results are shown in Figures 3 and 4: Approximately 43.37% of the total variability was explained by 2 main components (Figure 3). Component 1 was most positively related to PK/PGI, EN, ALD, and TPI, and it was negatively related to PGM and PHb/PHbK. Component 2, on the other hand, was strongly and positively associated with unidentified 12- and 13-kDa proteins and strongly and negatively associated with GAPDH/LDH and PGAM (Figure 3). All tested samples in the same two-dimensional space are presented in Figure 4. Individual groups are identified with different colors to distinguish them. It was clear that the individual groups exhibited unique and specific enzyme profiles. Samples from turkeys infected with *C. perfringens* are in the upper and right sides of the axis in Figure 4. Uninfected groups give feed with Arg at 90 and 100% and Met 30% were notably distinct from the others. The results confirm the significant influence of all examined factors in the muscle juice factors on the differences in the glycolytic enzyme profiles.



**Figure 3.** Results of principal component analysis (PCA) and projection of variables on the PC plane. ALD, aldolase; CK/PGAK, creatine kinase/phosphoglycerate kinase; EN, enolase; GAPDH/LDH, glyceraldehyde-3-phosphate dehydrogenase/lactate dehydrogenase; MK, myokinase; PGM, phosphoglucomutase; PHb/PHbK, phosphor-ylase b/phosphorylase b kinase; PK/PGI, pyruvate ki-nase/phosphoglucose isomerase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; 13 and 12 kDa – unidentified proteins; L\*, a\* – meat color was measured 48 h postmortem on the basis of the CIE L\*a\*b\* system.



**Figure 4.** Results of principal component analysis (PCA) and projection of the cases on the PC plane. Explanation: I+ *C. perfringens* infection; I- no *C. perfringens* infection, three levels of Arg (90%, 100%, and 110%) relative to the content of dietary Met (30% or 45%).

In light of the data from the literature presented in the discussion, these differences may lead to consequences and may be reflected in muscle cell metabolism, especially in terms of inflammatory processes or processes related to DNA replication and stability.

## CONCLUSIONS

The analysis of variance did not show a significant effect on meat quality traits such as pH, L\*, and b\* values; however, an effect of Arg content on the a\* color parameter was found. The results showed that as the Arg level increased, the redness of the meat was more intense. This outcome shows that an increase in the proportion of Arg may change muscle metabolism and lead to an increase in the proportion of muscle pigments (myoglobin). The analysis of variance results showed a significant effect of all studied factors (infection by *C. perfringens* as well as Arg and Met levels) on the sarco-plasmic protein profile. However, the lack of abovementioned effects of the tested factors on pH or color brightness indicates that despite the different enzymatic profiles of individual groups, no significant differences in the extent of postslaughter glycolysis or *postmortem* changes were found in muscle tissue. However, the results of many other studies have shown evidence that some glycolytic enzymes are complex multifunctional proteins with unexpected functional roles, such as transcription regulation (HK, LDH, GAPDH, and ENO), apoptosis (HK and GAPDH) and cell motility (GPI).

Therefore, the results showing an effect of *C. perfringens* infection and feeding with different doses of Arg and Met indicate that these effects may lead to significant consequences in cell metabolism.

## ACKNOWLEDGMENTS

This work was supported by the National Science Centre, Grant No. 2017/27/B/NZ9/01007.

This work was partially supported (applied challenge model) by the Minister of Education and Science under the program entitled “Regional Initiative of Excellence” for the years 2019–2022, Project No. 010/rid/2018/19, amount of funding: PLN 12 000 000.

## DISCLOSURES

The authors declare no conflicts of interest.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2022.102195](https://doi.org/10.1016/j.psj.2022.102195).

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