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Effects of Histidine on the Expression of β-Casein and Phosphorylated Proteins in the JAK2-STAT5/mTOR Signaling Pathway in In Vitro-Cultured Bovine Mammary Epithelial Cells: Postprint

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Abstract

This study aimed to investigate the effects of different concentrations of histidine on the expression of β-casein and phosphorylated proteins related to the tyrosine kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5)/mammalian target of rapamycin (mTOR) signaling pathway in in vitro cultured dairy cow mammary epithelial cells. Primary dairy cow mammary epithelial cells were cultured in vitro and divided into a control group and seven experimental groups using an essential amino acid-free medium; the control group received no histidine supplementation, while the experimental groups were supplemented with 0.15, 0.60, 1.20, 2.40, 4.80, 9.60, and 19.20 mmol/L histidine, respectively. Cell proliferation of primary dairy cow mammary epithelial cells at 12 h was detected using the MTT assay, and Western blotting was employed to detect the expression of β-casein and seven signaling pathway-related phosphorylated proteins. The results showed that: 1) When histidine concentration was 0.15–9.60 mmol/L, the number of dairy cow mammary epithelial cells increased compared with the control group. 2) The expression level of β-casein showed a trend of first increasing and then decreasing with increasing histidine concentration, but all experimental groups were extremely significantly higher than the control group ($P<0.01$). 3) Compared with the control group, histidine supplementation could extremely significantly promote the expression of various signaling pathway-related phosphorylated proteins ($P<0.01$); in the experimental groups, as histidine concentration increased, the expression levels of phosphorylated mammalian target of rapamycin [P-mTOR(Ser2481)] and phosphorylated eukaryotic translation elongation factor 2 [P-eEF2(Thr56)] proteins decreased, while the expression of phosphorylated ribosomal protein

S6 kinase 1 [P-S6K1(Thr389)] protein increased; when histidine concentration was 2.40 mmol/L, the expression levels of phosphorylated tyrosine kinase 2 [P-JAK2(Tyr1007/1008)], phosphorylated eukaryotic initiation factor 4E-binding protein 1 [P-4EBP1(Thr37)], and phosphorylated eukaryotic initiation factor 2α [P-eIF2α(Ser51)] proteins were highest; the expression levels of phosphorylated signal transducer and activator of transcription 5 [P-STAT5(Tyr694)], phosphorylated mTOR regulatory protein [P-raptor(Ser863)], and mTOR complex 1 binding protein (GβL) were highest at a histidine concentration of 9.60 mmol/L. In conclusion, histidine supplementation could regulate β-casein expression by promoting the expression of P-JAK2(Tyr1007/1008) and P-STAT5(Tyr694) proteins in the JAK2-STAT5 signaling pathway. Histidine within the optimal concentration range (0.15–9.60 mmol/L) could also promote β-casein expression through the mTORC1 P-raptor(Ser863) protein acting on the downstream target P-4EBP1(Thr37), ultimately regulating milk protein synthesis.

Full Text

Effects of Histidine on Expressions of β-Casein and Janus Kinase 2-Signal Transducer and Activator of Transcription 5/Mammalian Target of Rapamycin Signaling Pathway-Related Phosphorylated Proteins in In Vitro Cultured Bovine Mammary Epithelial Cells

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Abstract

This study investigated the effects of different concentrations of histidine on the expression of β-casein and phosphorylated proteins related to the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5)/mammalian target of rapamycin (mTOR) signaling pathways in in vitro cultured bovine mammary epithelial cells. Primary bovine mammary epithelial cells were cultured in a medium lacking essential amino acids and divided into a control group and seven treatment groups. The control group received no histidine supplementation, while the treatment groups were supplemented with 0.15, 0.60, 1.20, 2.40, 4.80, 9.60, or 19.20 mmol/L histidine, respectively. Cell proliferation was assessed after 12 hours using the thiazolyl blue (MTT) assay, and protein expression levels of β-casein and seven signaling pathway-related phosphorylated proteins were determined by Western blotting. The results demonstrated that: (1) histidine concentrations ranging from 0.15 to 9.60 mmol/L significantly increased bovine mammary epithelial cell numbers compared to the control; (2) β-casein expression exhibited a dose-dependent increase followed by a decrease, with all treatment groups showing extremely significant elevation compared to the control ($P < 0.01$); (3) histidine supplementation significantly promoted the expression of all signaling pathway-related phosphorylated proteins ($P < 0.01$). Among the treatment groups, the expression levels of phospho-mTOR [P-mTOR(Ser2481)] and phospho-eukaryotic translation elongation factor 2 [P-eEF2(Thr56)] decreased with increasing histidine concentration, whereas phospho-ribosomal protein S6 kinase 1 [P-S6K1(Thr389)] expression increased. The highest expression levels of phospho-JAK2 [P-JAK2(Tyr1007/1008)], phospho-eukaryotic initiation factor 4E-binding protein 1 [P-4EBP1(Thr37)], and phospho-eukaryotic initiation factor 2α [P-eIF2α(Ser51)] were observed at 2.40 mmol/L histidine, while phospho-STAT5 [P-STAT5(Tyr694)], phospho-mTOR regulatory protein [P-raptor(Ser863)], and mTOR complex 1 binding protein (GβL) peaked at 9.60 mmol/L. These findings indicate that histidine promotes β-casein expression by enhancing P-JAK2(Tyr1007/1008) and P-STAT5(Tyr694) expression in the JAK2-STAT5 pathway. Additionally, histidine at optimal concentrations (0.15–9.60 mmol/L) can promote β-casein expression through P-raptor(Ser863) in mTORC1 acting on the downstream target P-4EBP1(Thr37), thereby regulating milk protein synthesis.

Keywords: histidine; cell proliferation; JAK2; mTOR; β-casein

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Milk protein is a crucial nutritional indicator of milk quality, comprising primarily casein and whey protein, with casein accounting for approximately 82% of the total. Both αs1-casein and β-casein constitute 38% of casein content each. β-Casein maintains a constant proportion within casein and exhibits relatively stable structure, making its synthesis and secretion a hallmark of mammary epithelial cell secretory function [1]. Previous studies have confirmed that over 90% of milk proteins in bovine milk are synthesized by mammary epithelial cells using amino acids from blood as precursors [2,3]. Amino acids have become the primary limiting factor in milk protein synthesis [4,5], and besides lysine and methionine, other amino acids are also essential for this process [2,5-7]. Histidine has been identified as a major limiting amino acid in roughage [8] and as a key intracellular factor regulating milk protein synthesis [9]. Furthermore, research indicates that amino acid uptake by mammary tissue from blood does not precisely match the amino acid output in synthesized milk proteins; leucine, isoleucine, valine, arginine, and lysine show greater uptake than output, whereas histidine, threonine, phenylalanine, and methionine exhibit lower uptake than output [10,11]. Therefore, investigating the optimal amino acid concentrations required for milk protein synthesis is essential.

In addition to serving as substrates for protein synthesis, amino acids function as signaling molecules that regulate milk protein synthesis through signaling pathways [12,13]. Yang et al. [14] demonstrated in bovine mammary epithelial cells that methionyl-methionine dipeptide regulates αS1-casein synthesis through the JAK2-STAT5 and mTOR signaling pathways. Gao et al. [15] confirmed that histidine supplementation in bovine mammary epithelial cells promotes casein synthesis-related gene expression via the mTOR signaling pathway. Post-translational chemical modifications represent the final steps in protein biosynthesis for most proteins, with common modifications including glycosylation, methylation, acetylation, and phosphorylation. Phosphorylation is the most extensively studied covalent modification, accounting for one-third of all covalent modifications in mammalian life processes. In eukaryotes, phosphorylation primarily occurs at serine, threonine, and tyrosine residues [16]. Therefore, we hypothesized that histidine may also regulate β-casein synthesis-related phosphorylated protein expression through the JAK2-STAT5 and mTOR signaling pathways.

This study aimed to use in vitro cultured primary bovine mammary epithelial cells as a model to investigate the effects of different histidine concentrations on cell proliferation, β-casein expression, and JAK2-STAT5 and mTOR signaling pathway-related phosphorylated protein expression, providing a scientific basis for understanding the regulatory mechanisms of histidine on β-casein synthesis in milk.

Materials and Methods

1.1.1 Main Instruments The study utilized a Thermo constant-temperature CO_2 incubator, Olympus inverted microscope, Thermo microplate reader, Beijing Junyi Dongfang electrophoresis apparatus, Bio-Rad optical density analyzer, and other equipment.

1.1.2 Main Reagents Reagents included DMEM/F12 medium (Gibco, Cat: 11995-065/11765-054), essential amino acid-free medium (Gibco custom, Cat: ICH11404101), fetal bovine serum (FBS, Gibco, Cat: 10099-141), penicillin-streptomycin (Beyotime, Cat: C0222), trypsin (Beyotime, Cat: C0203), L-histidine (Sigma, Cat: H-5659-25G), thiazolyl blue (MTT, Sigma, Cat: 0793-5G), dimethyl sulfoxide (DMSO, Sigma, Cat: D4540), mTOR (Immunoway, Cat: YT2913), phospho-mTOR [P-mTOR(Ser2481), Immunoway, Cat: YP1134], eukaryotic initiation factor 4E-binding protein 1 (4EBP1, Immunoway, Cat: YT0018), phospho-4EBP1 [P-4EBP1(Thr37), Immunoway, Cat: YP0001], mTOR regulatory protein (raptor, Cell Signaling Technology, Cat: sc-27744), phospho-raptor [P-raptor(Ser863), Santa Cruz, Cat: sc-130214], ribosomal protein S6 kinase 1 (S6K1, Cell Signaling Technology, Cat: nos 9202), phospho-S6K1 [P-S6K1(Thr389), Cell Signaling Technology, Cat: nos 9205], eukaryotic translation elongation factor 2 (eEF2, Cell Signaling Technology, Cat: nos 2332), phospho-eEF2 [P-eEF2(Thr56), Cell Signaling Technology, Cat: nos 2331], eukaryotic initiation factor 2α (eIF2α, Immunoway, Cat: YT1507), phospho-eIF2α [P-eIF2α(Ser51), Immunoway, Cat: YT0093], JAK2 (Santa Cruz, Cat: sc-278), phospho-JAK2 [P-JAK2(Tyr1007/1008), Santa Cruz, Cat: sc-21870], STAT5 (Bioss, Cat: bs-1142R), phospho-STAT5 [P-STAT5(Tyr694), Bioss, Cat: bs-1659R], mTOR complex 1 binding protein (GβL, Cell Signaling Technology, Cat: nos.3274), β-casein (Biorbyt, Cat: orb18512), β-actin (Immunoway, Cat: YT0099), goat anti-rabbit (Sigma, Cat: A9169), and rabbit anti-goat (Millipore, Cat: AP106P) antibodies.

1.2.1 Primary Bovine Mammary Epithelial Cell Culture Primary bovine mammary epithelial cells were cultured using an established in vitro system from our laboratory [17], derived from a 3-year-old primiparous Chinese Holstein cow at 100 days of lactation. Cells were maintained in DMEM/F12 medium supplemented with 10% FBS at 38°C in a 5% CO$_2$ incubator. When cells reached 80-90% confluence in Corning culture dishes (Cat: 430165), they were digested with trypsin solution at 38°C in a 5% CO$_2$ incubator. Upon cytoplasmic retraction and cell rounding, digestion was terminated with DMEM/F12 medium. Cells were collected by repeated pipetting, centrifuged at 900 r/min for 5 minutes at room temperature, resuspended in fresh DMEM/F12 medium containing 10% FBS, and prepared as cell suspensions.

1.2.2 MTT Cell Proliferation Assay Cells were seeded in 96-well plates (Corning) at a density of 5$×10^{4}$ cells/mL (200 L per well) and allowed to adhere for 24 hours in DMEM/F12 medium with 10% FBS. After serum starvation for 6 hours in FBS-free DMEM/F12 medium, cells were treated with essential amino acid-free medium (Gibco custom, Cat: ICH11404101). The experiment consisted of a control group without histidine and seven treatment groups supplemented with 0.15, 0.60, 1.20, 2.40, 4.80, 9.60, or 19.20 mmol/L histidine, respectively. Each treatment had six replicates, and the entire experiment was repeated three times. After 8 hours of treatment, 20 L MTT working solution (5 mg/mL) was added to each well. Following 4 hours of incubation, the supernatant was removed, 150 L DMSO was added, and plates were shaken at 37°C for 10 minutes. Absorbance at 450 nm (OD$_{450}$) was measured using an automated microplate reader to assess cell proliferation. Relative growth rate (RGR) was calculated as: RGR (%) = (OD$_{450}$ of treatment group / OD$_{450}$ of control group) × 100.

1.2.3 Western Blot Analysis 1.2.3.1 Experimental Treatment Primary bovine mammary epithelial cells were seeded in culture dishes (Thermo, 172958) containing DMEM/F12 medium with 10% FBS and allowed to adhere for 24 hours. After overnight serum starvation in FBS-free DMEM/F12 medium, cells were treated for 6 hours with either no histidine (control) or 0.15, 2.40, 9.60, or 19.20 mmol/L histidine. Each treatment had three replicates, and the experiment was repeated three times.

1.2.3.2 Total Protein Extraction

Following treatment, total protein was extracted from primary bovine mammary epithelial cells. RIPA lysis buffer was supplemented with 1 mmol/L phenylmethylsulfonyl fluoride (PMSF) and 1% protease and phosphatase inhibitors, mixed, and kept on ice. Cell lysates were collected using a cell scraper (Costar, Cat: 3008) and centrifuged at 2,000×g for 3 minutes. The supernatant was transferred to centrifuge tubes, aliquoted, and total protein concentration was determined using the BCA method (Beyotime, Cat: P0012). Remaining samples were stored at -80°C.

1.2.3.3 Western Blot Procedure

Samples were mixed with 5× SDS-PAGE loading buffer and heated at 95°C for 10 minutes to denature proteins. Thirty micrograms of protein per sample were loaded for electrophoresis at 80 mV for 30 minutes through stacking gel and 120 mV for 120 minutes through separating gel. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes at 200 mA for 50 minutes at 4°C. Membranes were washed three times with TBST for 5 minutes each, blocked with 3% serum in TBST (300 L chicken serum in 10 mL TBST) on a shaker for 5 hours, then washed again three times with TBST. Membranes were incubated overnight with primary antibodies diluted 1:1,000 in 1× PBS on a shaker, washed three times with TBST, incubated with secondary antibodies diluted 1:3,000 in 1× PBS for 2 hours, and washed three final times with TBST. Protein bands were visualized using an ECL kit (Pierce, 32106), exposed to Kodak film (XBT-1) in a dark box (Guangdong Yuehua, AX-II), scanned with a Bio-Rad GS-800 densitometer, and analyzed using ImageJ2x software.

1.3 Statistical Analysis Data were analyzed using SAS 9.2 software with the ANOVA procedure. Duncan' s multiple range test was used for mean comparisons, with $P < 0.05$ considered significant and $P < 0.01$ considered highly significant. Results are expressed as means ± standard error. Western blot data were analyzed by comparing each treatment group to the control.

Results

2.1 Effects of Histidine on Proliferation of In Vitro Cultured Bovine Mammary Epithelial Cells As shown in [Figure 1: see original paper], compared with the control group, histidine concentrations of 1.20–4.80 mmol/L in essential amino acid-free medium significantly increased bovine mammary epithelial cell proliferation ($P < 0.01$). At 19.20 mmol/L, histidine inhibited cell proliferation, though not significantly different from the control ($P > 0.05$). Other histidine concentrations promoted cell proliferation but showed no significant differences compared to the control ($P > 0.05$).

2.2 Effects of Histidine on β-Casein Expression in In Vitro Cultured Bovine Mammary Epithelial Cells As shown in [Figure 2: see original paper], β-casein expression increased initially and then decreased with rising histidine concentrations from 0.15 to 19.20 mmol/L, with all treatment groups showing significantly higher expression than the control ($P < 0.01$). The highest β-casein expression was observed at 2.40 mmol/L histidine, indicating a dose-dependent effect where optimal concentrations enhance expression while excessive concentrations diminish the promoting effect.

2.3 Effects of Histidine on JAK2-STAT5 Signaling Pathway-Related Phosphorylated Protein Expression As shown in and [Figure 3: see original paper]-a, histidine supplementation significantly increased the expression of P-JAK2(Tyr1007/1008) and P-STAT5(Tyr694) proteins compared to the control ($P < 0.01$). P-JAK2(Tyr1007/1008) expression peaked at 2.40 mmol/L histidine, while P-STAT5(Tyr694) reached maximum expression at 9.60 mmol/L. These results demonstrate that histidine promotes JAK2-STAT5 signaling pathway-related phosphorylated protein expression, though high-dose supplementation reduces this promoting effect.

2.4 Effects of Histidine on mTOR Signaling Pathway-Related Phosphorylated Protein Expression As shown in and [Figure 3: see original paper]-a, compared with the control, histidine supplementation at 0.15-19.20 mmol/L significantly increased the expression of P-mTOR(Ser2481), P-raptor(Ser863), and GβL proteins ($P < 0.01$). P-mTOR(Ser2481) expression was highest at 0.15 mmol/L histidine, while P-raptor(Ser863) and GβL peaked at 9.60 mmol/L, with higher doses showing diminished promoting effects.

As shown in and [Figure 3: see original paper]-b, histidine supplementation significantly promoted the expression of P-S6K1(Thr389), P-4EBP1(Thr37), P-eIF2α(Ser51), and P-eEF2(Thr56) proteins ($P < 0.01$). P-eEF2(Thr56) expression was highest at 0.15 mmol/L histidine, P-4EBP1(Thr37) and P-eIF2α(Ser51) peaked at 2.40 mmol/L, and P-S6K1(Thr389) reached maximum expression at 19.20 mmol/L. These findings confirm that histidine promotes mTOR signaling pathway-related phosphorylated protein expression.

Discussion

3.1 Effects of Histidine on Proliferation of In Vitro Cultured Bovine Mammary Epithelial Cells Amino acids, similar to hormones, vitamins, and growth factors, are important nutrients affecting mammary tissue proliferation, differentiation, and lactation [18]. Xu [19] reported that exogenous arginine addition promotes bovine mammary epithelial cell proliferation. Similarly, Li [20] demonstrated that single supplementation of lysine or methionine enhanced cell proliferation at 24, 48, and 72 hours. Using in vitro cultured primary bovine mammary epithelial cells as a model, our results showed that histidine supplementation promoted mammary epithelial cell proliferation, with proliferation rate initially increasing then decreasing with concentration, consistent with findings by Gao et al. [15]. This may be attributed to toxic effects caused by histidine excess [21].

3.2 Effects of Histidine on Signaling Pathway-Mediated β-Casein Synthesis-Related Phosphorylated Protein Expression Bionaz and Loor [1] investigated the regulatory networks controlling protein synthesis in bovine mammary tissue, examining 44 key genes including JAK2-STAT5 and mTOR signaling pathways, insulin signaling pathway, and amino acid and glucose transporters, revealing that milk protein synthesis initiation is associated with amino acid transport into mammary epithelial cells. Appuhamy et al. [22] and Apelo et al. [23] demonstrated in bovine mammary epithelial cells that essential amino acids regulate milk protein synthesis by modulating the phosphorylation of mTOR, 4EBP1, S6K1, eEF2, and eIF2α through the mTOR signaling pathway. Additionally, Gao et al. [15] found that histidine supplementation at 0.15–9.60 mmol/L in Earle' s balanced salt solution significantly upregulated β-casein gene (CSN2) expression in in vitro cultured bovine mammary epithelial cells compared to the negative control. These studies provide theoretical foundations for further investigation into amino acid regulation of milk protein synthesis in bovine mammary epithelial cells.

Our study employed Western blotting to examine the effects of different histidine concentrations on β-casein expression and JAK2-STAT5 and mTOR signaling pathway-related phosphorylated protein levels in in vitro cultured primary bovine mammary epithelial cells. The results showed that histidine promoted β-casein expression in a dose-dependent manner, with optimal concentrations enhancing expression and high concentrations reducing the promoting effect, consistent with previous reports [24]. The diminished promoting effect at 19.20 mmol/L histidine may be related to decreased cell proliferation at this concentration, as Mercier and Gaye [25] demonstrated that milk protein synthesis rate is largely influenced by mammary epithelial cell number. This suggests that histidine regulates milk protein synthesis and secretion by increasing bovine mammary epithelial cell number. In production settings, Lee et al. [26] confirmed that rumen-protected histidine supplementation in dairy cows fed metabolizable protein-deficient diets increased feed intake and milk protein yield. Similarly, Kim et al. [8] demonstrated through histidine infusion experiments that histidine significantly improved milk yield and milk protein production, consistent with our findings.

STAT5 was first identified in studies of prolactin-stimulated mammary epithelial cells and can be activated by various cytokines including prolactin, growth hormone, and erythropoietin [27,28], thereby maintaining normal cellular functions and regulating proliferation and differentiation [29]. Shi [16] demonstrated that hormone supplementation in bovine mammary epithelial cells affects β-casein synthesis through the JAK2-STAT5 signaling pathway. In this pathway, phosphorylation of tyrosine residues at the C-terminus of STAT5 (STAT5a Tyr694 and STAT5b Tyr699) plays a crucial role in STAT5 nuclear translocation and DNA binding capacity [30]. Activated JAK2 kinase phosphorylates STAT5, which then acts on corresponding DNA regions in the nucleus to regulate transcription and affect protein synthesis. Our results showed that histidine supplementation enhanced P-JAK2(Tyr1007/1008) and P-STAT5(Tyr694) expression, with P-STAT5(Tyr694) peaking at 9.60 mmol/L histidine, which did not completely coincide with β-casein expression, possibly due to involvement of other hormonal factors in pathway regulation.

mTOR, raptor, and GβL are components of the mammalian target of rapamycin complex 1 (mTORC1). As a key regulator of milk protein synthesis, mTOR has been shown to have important regulatory sites including P-mTOR(Ser2448) and P-mTOR(Ser2481) [24,31]. Our study confirmed that histidine supplementation significantly increased P-mTOR(Ser2481) expression compared to the control. Raptor binds mTOR to downstream signaling proteins S6K1 and 4EBP1, thereby regulating intracellular protein expression and ultimately altering cell growth and proliferation status [32,33]. GβL positively regulates mTOR activation as a binding protein that, together with mTOR and raptor, modulates mTOR activity. Previous studies demonstrated positive correlation between GβL and P-mTOR(Ser2448) [34], and our research confirmed a similar positive correlation between GβL and P-mTOR(Ser2481), with both P-raptor(Ser863) and GβL expression reaching highest levels at 9.60 mmol/L histidine.

S6K1, 4EBP1, eEF2, and eIF2α are important downstream target proteins of the mTOR signaling pathway, with their phosphorylation status closely related to milk protein synthesis. S6K1 is ubiquitously expressed in mammalian cells and can be activated by various extracellular signals, with mTOR-phosphorylated S6K1 activity increasing nearly 100-fold. Previous studies revealed that mTOR regulates multiple phosphorylation sites in S6K, with P-S6K1(Thr389) being directly phosphorylated by mTOR and indirectly regulated through phosphatase activity modulation [35]. Our results confirmed that histidine supplementation significantly promoted P-S6K1(Thr389) expression, which increased with histidine concentration. The incomplete correlation between β-casein expression and P-S6K1(Thr389) may be because high histidine concentrations promote expression of other caseins such as αS-casein and -casein. Additionally, mTOR regulates protein translation by inducing 4EBP1 phosphorylation [22]. Gao et al. [15] reported that histidine promotes casein expression through mTORC1' s P-raptor(Ser792) acting on downstream target P-S6K1(Thr389). Our study further demonstrates that optimal histidine concentrations also promote casein expression through mTORC1's P-raptor(Ser863) acting on downstream target P-4EBP1(Thr37).

During protein synthesis, eEF2 induces translocation of peptidyl-tRNA from the ribosomal A site to P site to facilitate peptide chain elongation. Phosphorylation of eEF2 at threonine 56 reduces its affinity for ribosomes, thereby terminating peptide chain extension [36]. Our results showed that P-eEF2(Thr56) expression decreased with increasing histidine concentration but remained significantly higher than the control. Additionally, eukaryotic cells regulate gene expression at the translational level by activating protein kinases that phosphorylate eIF2α, an important regulatory mechanism. Phosphorylated eIF2α not only inhibits translation of most mRNAs but also specifically activates translation of certain mRNAs to synthesize specific proteins that regulate target gene expression [37]. Our results demonstrated that histidine supplementation initially increased then decreased P-eIF2α(Ser51) expression, with maximum expression at 2.40 mmol/L.

Conclusion

In conclusion, using in vitro cultured bovine mammary epithelial cells as a model, histidine supplementation promotes β-casein synthesis and regulates milk protein production by enhancing expression of JAK2-STAT5 and mTOR signaling pathway-related phosphorylated proteins. Histidine at concentrations of 0.15-9.60 mmol/L in essential amino acid-free medium promotes proliferation of in vitro cultured primary bovine mammary epithelial cells, while 19.20 mmol/L inhibits proliferation. The optimal histidine concentration for maximal β-casein expression is 2.40 mmol/L.

Histidine promotes β-casein expression by enhancing P-JAK2(Tyr1007/1008) and P-STAT5(Tyr694) expression in the JAK2-STAT5 signaling pathway. Additionally, histidine at optimal concentrations (0.15-9.60 mmol/L) can promote β-casein expression through mTORC1' s P-raptor(Ser863) acting on the downstream target P-4EBP1(Thr37), thereby regulating milk protein synthesis.

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