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Practical considerations in the selection of creatine forms for calf feeding

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Abstract. The purpose of the study was to evaluate the feasibility of adding different forms of creatine to milk and whole milk replacer. The following creatine sources were examined: creatine hydrochloride, creatine monohydrate, creatine phosphate, and creatine malate. Creatine preparations, at a dosage equivalent to 15 g per 6 L of milk, were assessed for water solubility and subsequently added to milk and milk replacer. Creatine hydrochloride demonstrated excellent water solubility and readily passed through the filter after mixing with milk. Amount of dry matter retained on the filters decreased by 0.2 g/L compared with milk, indicating improved filtration properties. After one hour of standing, the residue in the lower fraction remained 0.1 g/L lower than in the corresponding milk fraction. Creatine monohydrate remained insoluble in water but did not induce milk coagulation. Residue retained on the filters increased by 0.1 g/L compared with milk, while after one hour of standing the residue in the lower fraction increased by 0.5 g/L relative to the control. Creatine phosphate dissolved well in water; however, mixing with milk increased the residue on the filters by 0.1 g/L immediately after mixing, and after one hour of storage the mixture separated, forming protein coagulates. The residue retained on the filters in the lower fraction exceeded the control by 3.5 g/L. Creatine malate showed slower dissolution in water due to the presence of large agglomerates. In prepared mixtures with milk, the residue retained on the filters increased by 0.6 g/L compared with the control, and after one hour of standing the residue in the lower fraction exceeded the control by 5.3 g/L. Protein coagulates formed

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in the upper fraction of the mixture after one hour of standing, making it unsuitable for filtration. In mixtures with whole milk replacer, all forms of creatine slightly accelerated the sedimentation of suspension components; however, this effect was not critical. For calf milk mixtures, the inclusion of creatine hydrochloride was recommended due to its high solubility and lack of rapid milk coagulation

Keywords: milk; milk replacer; cattle; solubility; milk protein coagulation; esophageal groove reflex

Introduction

Creatine was a nitrogen-containing compound involved in energy storage in muscle tissue. Various forms of creatine were widely used in sports nutrition to enhance muscle energy supply and promote muscle growth. B. Wax *et al.* (2021) reported that creatine-containing drugs have been shown to increase intracellular creatine concentration, thereby enhancing adenosine triphosphate resynthesis, improving work performance, increasing maximal strength, and promoting lean muscle mass accretion. Creatine supplementation also accelerated the recovery of muscle strength following intensive exercise. Approximately half of the daily creatine requirement was synthesised endogenously from amino acids, particularly arginine, glycine, and methionine, while the remainder was obtained from dietary sources. According to S.M. Ostojic (2021), low creatine intake was associated with slower growth, reduced muscle mass, higher body fat content, and other functional impairments. In cattle production, the ability of various creatine forms to increase muscle mass may be beneficial as a strategy to enhance meat productivity and carcass quality. The utilisation of nitrogen-containing compounds in cattle was closely associated with rumen digestion. The rumen hosted a complex microbiome, including associations of proteolytic, peptidolytic, and ureolytic bacteria, as reported by P. Tan *et al.* (2021), which degraded available protein to ammonia, followed by its incorporation into microbial protein synthesis. Creatine, like other free amino acids, was utilised by rumen microorganisms; therefore, its direct inclusion in feed as a supplement was ineffective. One of the potential approaches to creatine supplementation was its inclusion in ruminant diets in a bypass form. Another option was the addition of creatine to milk replacers during calf feeding. Feeding creatine mixed with milk may be technologically feasible due to the oesophageal groove reflex, which was active in calves during milk consumption. M. Marszałek *et al.* (2022) reported that the high concentration of cholinergic and nitrergic neurons in the oesophageal groove of calves, which facilitated its closure reflex, liquid feeds and feed additives mixed with milk bypass the rumen and were directly delivered to the abomasum.

Studies by J. de Souza Pinheiro *et al.* (2024) in goat kids demonstrated that regulating milk replacer intake can influence not only animal performance and body development but also metabolism and cellular hypertrophy. Based on this, exploring strategies to influence muscle cell hypertrophy in calves through creatine supplementation in milk feeds was of particular interest. There were various forms of creatine that can theoretically be used in animal feeding. Guanidinoacetic acid, a naturally synthesised precursor of creatine derived from amino acids, was commonly used as a feed additive. A study by K.J. Hazlewood *et al.* (2024) demonstrated that supplementation of milk replacer with guanidinoacetic acid in Holstein calves for 7 weeks, starting from one week of age, affected growth performance. The authors reported that supplementation at 1 or 2 g/day increased plasma creatine concentration, improved average daily gain and body weight at 8 weeks of age, and enhanced starter feed intake, without adverse effects on animal health. However, the EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in

Animal Feed) *et al.* (2022) concluded that the use of guanidinoacetic acid as a feed additive was acceptable only under specific conditions, including low inclusion levels in feed or water, due to safety considerations and potential adverse effects observed in animal studies. Therefore, it was advisable to evaluate other sources of creatine in animal nutrition, particularly those widely used in human sports nutrition that have demonstrated efficacy and safety. According to C. Fazio *et al.* (2021), creatine monophosphate was considered one of the most bioavailable forms with proven efficacy; however, other forms such as creatine magnesium chelate, creatine citrate, creatine malate, creatine ethyl ester, creatine nitrate, and creatine pyruvate were also widely used. According to R.B. Kreider *et al.* (2022), creatine monohydrate remained the primary source of creatine in sports nutrition, with advantages in bioavailability, efficacy, and safety. The potential use of creatine as a feed additive in animal nutrition remained insufficiently studied. J.R. Stout *et al.* (2025) reported that one of the first attempts to evaluate the effectiveness of creatine monohydrate supplementation in a racehorse mare encountered difficulties due to its low solubility in water, which hindered the preparation of a solution for oral administration. The use of creatine monohydrate as an additive to milk may also be problematic due to low solubility and sediment formation, which could reduce intake by calves. Other creatine forms with higher solubility and better dispersion in milk mixtures may induce coagulation of milk proteins, which must be taken into account. The aim of this study was to evaluate, under laboratory conditions, the technological feasibility of incorporating the most commercially available forms of creatine into milk feeds for calf feeding.

Materials and Methods

The study was conducted in 2025 at the Laboratory of Milk and Dairy Product Quality, Department of Milk and Meat Production Technologies, National University of Life and Environmental Sciences of Ukraine. The study evaluated the solubility of different creatine forms in water and their suitability for incorporation into milk and milk replacers. Four of the most commercially available creatine compounds were used for analysis: creatine hydrochloride, creatine monohydrate, creatine phosphate, and creatine malate. Creatine hydrochloride was a sports nutrition product manufactured by LLC "Sport Nutrition Import", containing added colorant and flavouring. Creatine monohydrate was produced by PE "Powerful Progress Ukraine" and contained flavouring. Creatine phosphate and creatine malate were manufactured in People's Republic of China and supplied by LLC "Himlaborreaktyv". To assess the solubility of creatine preparations in water, three experimental flasks (250 cm^3) were prepared for each creatine compound, each containing 200 cm^3 of water at 38-40°C. Flasks containing water without additives served as controls. A 0.5 g sample of each creatine preparation was added to the experimental flasks and thoroughly mixed. The sample weight was calculated based on an estimated daily creatine intake of 15 g, intended to be dissolved in 6 L of liquid feed, corresponding to the average daily milk allowance per calf. Each samples were visually evaluated for sediment formation, and the presence of undissolved material was determined by filtration followed by weighing of the filters after drying. Filters made of black spunbond (10 × 10 cm) were used for filtration. Filters were weighed before filtration, and the presence of residue was visually assessed after filtration. To determine potential losses of the preparation during filtration that were not visually detectable, the filters were dried in a drying oven for 40 minutes at 105 ± 2°C and reweighed after cooling. Weighing was performed using RADWAG THB-600 (Poland) laboratory scales (d = 0.01 g).

To assess the feasibility of incorporating creatine preparations into milk, samples were prepared analogously to the water-based experiment. The mixtures were thoroughly stirred and visually compared with the control samples. Immediately after mixing, 50 cm^3 samples were taken from each flask. Composite samples (150 cm^3) were prepared from the three experimental replicates and the control and filtered through pre-weighed black spunbond filters. The filters were cooled to room temperature, and reweighed. The remaining milk-creatine mixtures were held for one hour, after which the consistency of the upper and lower layers was assessed. From the upper third of each experimental and control flask, 50 cm^3 samples were collected and combined into composite samples (150 cm^3), which were filtered through pre-weighed filters. Filtration results were evaluated visually, and the filters were dried, cooled, and re-weighed. The lower layer of milk and milk-creatine mixtures (100 cm^3 per flask) was filtered in the same manner, and the differences in the mass of dried filters were determined. To evaluate the possibility of adding creatine to milk replacer, a working solution was prepared containing 130 g of milk replacer powder per 1 L of water. The study used milk replacer "Telyatko Ultra Active" (Shencon Corporation, n.d.) intended for calves from 15 days of age, containing up to 70% dairy components. Creatine compounds were added at a rate of 15 g per 6 L of solution. For this purpose, 1.25 g of creatine preparation was added to 65 g of milk replacer powder and dissolved in 0.5 L of water at 50-55°C. The liquid mixtures were visually evaluated immediately after dissolution and after 15 minutes. Subsequently, the mixtures were remixed, and changes were visually assessed after 5, 10, and 15 minutes. To determine potential losses of creatine and milk components during sediment filtration, arithmetic means, standard errors, and significance of differences (p-values) were calculated.

Results and Discussion

Creatine compounds were promising for inclusion in animal diets as a means of stimulating productivity; however, in cattle production their application was limited due to the physiological characteristics of ruminant digestion. There were two most practical approaches for incorporating creatine into cattle diets. The first involved supplementation of adult animals and growing stock in a rumen-protected form that ensured bypass of the rumen. The second approach involved administration to calves via liquid feeding with milk-based feeds, allowing the utilisation of the oesophageal groove reflex. The inclusion of novel feed components for calf nutrition required preliminary evaluation of their compatibility with milk feeds prior to conducting efficacy studies. In particular, when using different forms of creatine as additives to milk feeds, losses of the preparation due to sedimentation may occur, as well as possible alterations in the structure of milk mixtures resulting from interactions with the additives. The investigated feed-grade creatine sources were therefore evaluated for solubility in water and their interaction with milk and milk replacer. Creatine compounds exhibited different solubility characteristics in water (Fig. 1). Creatine hydrochloride dissolved rapidly, resulting in a clear solution with a pink coloration (due to added colorants). No sediment was observed, only a small number of air bubbles were present on the bottom and walls of the flask.

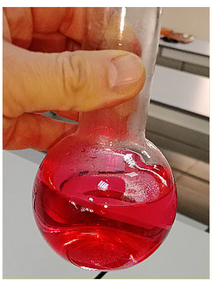
a  b  c  d 

Figure 1. *Dissolution of creatine compounds in water at 38-40°C*
Note: a – creatine hydrochloride; b – creatine monohydrate; c – creatine phosphate; d – creatine malate
Source: developed by the authors

After dissolution of creatine monohydrate, a clear solution with a slightly yellowish tint (due to the colorant) was obtained. However, sediment remained at the bottom of the flask and did not dissolve even after prolonged and thorough mixing. The undissolved residue appeared as a homogeneous, well-wetted powder. The creatine phosphate solution was a clear, homogeneous liquid with no visible difference in colour compared to the control (water). The compound dissolved rapidly, with only a small number of fine air bubbles remaining at the bottom of the flask. Dissolution of creatine malate in warm water resulted in a clear solution with a slightly yellowish colour. In two out of three flasks, no sediment was observed; in one flask, a small number of crystals of undissolved compound were present, which completely dissolved over time. The control sample and experimental aqueous solutions of creatine preparations were filtered through spunbond filters. Visually, the filters remained clean, and no residue was observed on their surface. Potential retention of creatine compounds on the filter surface or within its structure, not detectable visually, was assessed by weighing before and after drying (Table 1).

Table 1. *Determination of creatine preparation residues on filters (after filtration of aqueous solutions)*

Sample	Mass before filtration (n = 3), g	Mass after filtration (n = 3), g	Difference before and after filtration, g	p-value
Control	0.57 ± 0.015	0.57 ± 0.018	0.01	0.091752
Creatine hydrochloride	0.62 ± 0.011	0.62 ± 0.019	0.00	0.370901
Creatine monohydrate	0.56 ± 0.007	0.56 ± 0.008	0.00	0.211325
Creatine phosphate	0.66 ± 0.023	0.66 ± 0.029	0.00	0.500000
Creatine malate	0.65 ± 0.028	0.66 ± 0.025	0.01	0.091752

Source: developed by the authors

The weights of the dried filters before and after filtration did not differ significantly. The observed differences were within the limits of statistical error, indicating that the preparations were well dissolved in water. In the case of creatine monohydrate, which appeared visually undissolved, its fine particle structure allowed it to pass through the filter pores without noticeable losses. In terms of water solubility, creatine hydrochloride appeared to be the most suitable for practical application. Creatine phosphate and creatine malate required slightly longer dissolution time, whereas creatine monohydrate required specific handling conditions to minimise sedimentation of undissolved particles. Creatine preparations were also mixed with milk. During mixing, no visible changes in the milk were observed, except for coloration caused by preparations containing added colorants (Fig. 2).

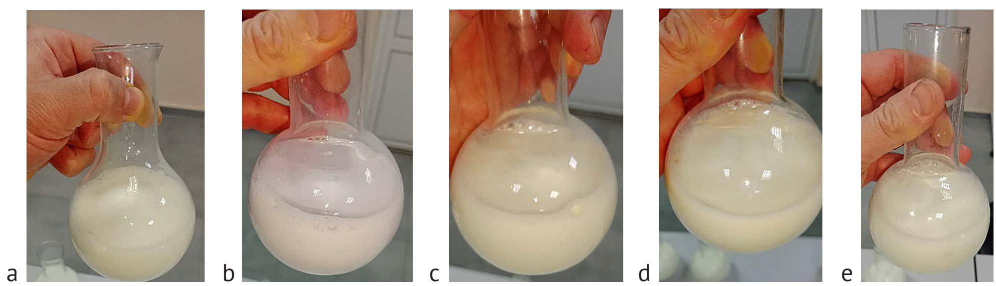


Figure 2. *Dissolution of creatine compounds in milk at 38-40°C*
Note: a – milk (control); b – creatine hydrochloride; c – creatine monohydrate; d – creatine phosphate; e – creatine malate
Source: developed by the authors

Since it was difficult to visually assess solubility in milk, this was evaluated by filtration. Immediately after mixing creatine preparations with milk, composite samples from the experimental flasks were prepared and filtered (Fig. 3). The filters remained relatively clean, and the mixtures passed through them readily, indicating the absence of visible coagulated particles or large insoluble residues. This suggested that, immediately after mixing, the creatine preparations were sufficiently dispersed in milk and did not noticeably interfere with its filtration properties.

a b c

Figure 3. *Filters after filtration of milk mixtures with creatine preparations*

Note: a – immediately after mixing (from left to right, starting from the top row: creatine hydrochloride; creatine monohydrate; creatine phosphate; creatine malate); b – after an hour, upper layer (from left to right, starting from the top row: creatine hydrochloride; creatine monohydrate; creatine phosphate; creatine malate); c – after an hour, lower layer (from left to right, starting from the top row: milk; creatine hydrochloride; creatine monohydrate; creatine phosphate; creatine malate)

Source: developed by the authors

The filters were dried and weighed. It was established that filtration of milk and milk-creatine mixtures resulted in an increase in filter mass (Table 2). Based on the difference in filter weight, the loss of dry matter from milk or milk mixtures was calculated per 1 L of filtered liquid. The mass of the filters increased in all samples, indicating deposition of milk components or milk-creatine mixture constituents on their surface. Immediately after mixing, dry matter losses during filtration in mixtures containing creatine monohydrate and creatine phosphate were similar and slightly higher (by 0.1 g/L) than in the control. The inclusion of creatine hydrochloride improved filtration properties and reduced dry matter losses on the filter by approximately 50%. The highest dry matter loss (1.0 g/L) was observed in the mixture containing creatine malate, with filter deposition exceeding the control by 0.6 g/L (2.4 times higher).

Table 2. *Determination of residues on filters (prepared milk mixtures with creatine preparations)*

Sample	Volume, cm^3	Filter mass, g	Filter mass after filtration, g	Dry matter loss per 1 L of milk mixture, g
Milk (control)	150	0.65	0.71	0.4
Creatine hydrochloride	150	0.55	0.58	0.2
Creatine monohydrate	150	0.62	0.69	0.5
Creatine phosphate	150	0.60	0.67	0.5
Creatine malate	150	0.55	0.70	1.0

Source: developed by the authors

Milk feeds and mixtures for calves were not always administered immediately, depending on feeding management practices and herd size. During storage, even short-term (e.g., during transport within the farm and prior to feeding), phase separation and chemical interactions may occur, altering the structure of the mixtures. To simulate the period between preparation of milk mixtures with creatine sources and their actual administration, control samples and experimental mixtures were held for one hour. Since storage of milk led to separation of the fat fraction, the upper third and the lower settled fraction were filtered separately. It was found that the upper layer, characterised by higher fat content, in milk and mixtures containing creatine hydrochloride, monohydrate, and phosphate passed through the filter, leaving only minor coagulated particles on the surface (Fig. 3). In contrast, the mixture containing creatine malate exhibited increased viscosity and did not pass through the filter (Fig. 4a). After filtering, a substantial amount of coagulated material with a syrup-like consistency remained on the filter.

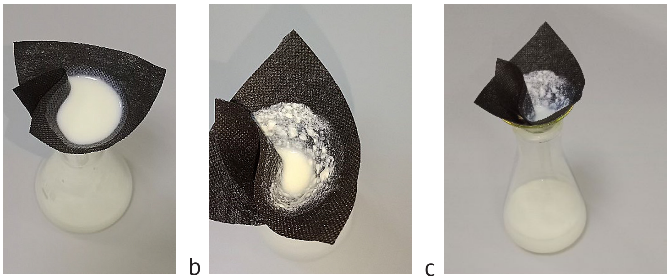

a b c

Figure 4. *Filtration of milk-creatine mixtures after one hour of incubation*
Note: a – mixture with creatine malate, upper layer; b – mixture with creatine malate, lower layer; c – mixture with creatine phosphate, lower layer
Source: developed by the authors

Drying of the filters after filtration of the upper fraction (Table 3) showed that, for milk, the proportion of filtered dry matter remained unchanged compared with the fresh sample (Table 2). In mixtures containing creatine hydrochloride, this parameter increased by 0.3 g/L relative to the corresponding values in Table 2, whereas in mixtures with creatine monohydrate and creatine phosphate it decreased by 0.2 g/L and 0.1 g/L, respectively. Overall, the changes after one hour were minor. The inability to filter the upper layer of the mixture containing creatine malate was likely associated with the acidic properties of oxalic acid present in the compound, leading to casein coagulation and curd formation. The lower layer of milk mixtures, containing less fat, exhibited different filtration behaviour compared to the upper layer. Mixtures containing creatine phosphate and creatine malate filtered slowly and produced a large amount of curd residues (Fig. 4b, 4c). As a result, the volume of filtered mixture had to be reduced. The filters after these mixtures contained substantial amounts of curd deposits, indicating interaction between the creatine preparations and milk components.

Table 3. *Determination of residues on filters (milk mixtures with creatine drugs, one hour after mixing, upper layer)*

Sample	Volume, cm^3	Filter mass, g	Filter mass after filtration, g	Dry matter loss per 1 L of milk mixture, g
Milk (control)	150	0.58	0.64	0.4
Creatine hydrochloride	150	0.71	0.78	0.5
Creatine monohydrate	150	0.52	0.57	0.3
Creatine phosphate	150	0.61	0.67	0.4
Creatine malate	150	0.63	**not filtered**	

Source: developed by the authors

Drying of filters after filtration of the lower fraction (Table 4) showed that dry matter losses in the control (milk) and in mixtures containing creatine hydrochloride decreased compared to the upper fraction and to filtration immediately after mixing. This suggested that the previously retained material on the filters was mainly associated with the fat fraction. The addition of creatine hydrochloride to milk, at the concentrations specified in the methodology, did not induce coagulation; on the contrary, it reduced milk viscosity. Consequently, dry matter losses during filtration – both immediately after mixing and in the lower fraction after one hour of settling – were lower than in the control. Due to reduced viscosity, fat globules rose more rapidly; therefore, after filtration of the upper layer, an increase in retained dry matter on the filter was observed due to partial absorption of fat by the spunbond material. The addition of creatine phosphate and creatine malate acidified the milk and led to partial casein coagulation, increasing the viscosity of the system. As a result, after one hour of incubation, a substantial amount of curd formed in the lower fraction, and total dry matter losses during filtration increased to 3.7 g/L (creatine phosphate) and 5.5 g/L (creatine malate), which was 7.4 and 5.5 times higher than immediately after mixing, and 18.5 and 27.5 times higher than in the control, respectively. In absolute terms, the amount of dry matter retained on the filters exceeded the control by 3.5 g/L in the mixture containing creatine phosphate and by 5.3 g/L in the mixture containing creatine malate. Creatine monohydrate should be considered separately. Its inclusion led to a slight increase in dry matter losses during filtration compared to milk and caused a minor increase in viscosity, reflected in reduced losses in the upper fraction and increased losses in the lower fraction. Overall, these changes were not critical, and the increase in viscosity may help maintain mixture homogeneity for a longer period and prevent rapid sedimentation of undissolved particles.

Table 4. *Determination of residues on filters (milk mixtures with creatine drugs, one hour after mixing, lower layer)*

Sample	Volume, cm^3	Filter mass, g	Filter mass after filtration, g	Dry matter loss per 1 L of milk mixture, g
Milk (control)	300	0.57	0.64	0.2
Creatine hydrochloride	300	0.58	0.62	0.1
Creatine monohydrate	300	0.63	0.83	0.7
Creatine phosphate	100	0.68	1.05	3.7
Creatine malate	100	0.56	1.11	5.5

Source: developed by the authors

Creatine can be added not only to whole milk but also to milk replacer. This approach was technologically more convenient, as it allowed preparation of a balanced mixture in advance. Such a mixing strategy was particularly relevant in specialised beef production systems, where calves were raised without access to a lactating herd and were fed exclusively with milk replacer. Creatine preparations were added to artificial milk mixtures, and their dissolution and phase separation during short-term storage were evaluated. According to the manufacturer's instructions for the milk replacer used in the study (Shencon Corporation, n.d.), the recommended time for feeding reconstituted milk replacer was 5 to 15 minutes, due to sedimentation of components and loss of homogeneity. Freshly reconstituted milk replacer was a turbid suspension of light beige colour. After 15 minutes, sedimentation and phase separation occurred, with a visible boundary between the whey fraction and the sediment, the latter occupying approximately the lower quarter of the flask. Mixtures containing creatine preparations also exhibited phase separation after 15 minutes (Fig. 5).

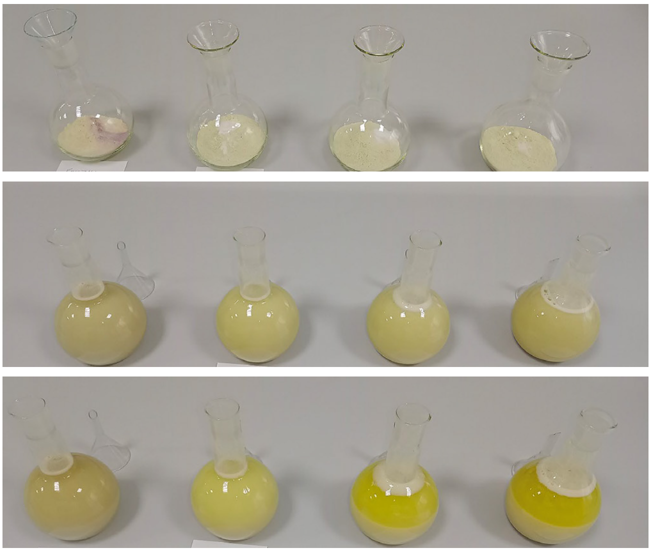

a
b
c

Figure 5. *Addition of creatine compounds to milk replacer*

Note: flasks from left to right: milk replacer + creatine hydrochloride; milk replacer + creatine monohydrate; milk replacer + creatine phosphate; milk replacer + creatine malate; a – dry mixture; b – mixture after dissolution; c – mixture 15 minutes after dissolution

Source: developed by the authors

The addition of creatine affected the coagulation behaviour of the milk replacer. After 15 minutes of settling, separation into whey and sediment phases was observed. In the flask containing creatine hydrochloride, the overall pattern of phase separation was similar to that of the control sample. In the presence of creatine monohydrate, sedimentation of components occurred, but a distinct phase boundary was not clearly visible, indicating improved retention of mixture homogeneity. In contrast, samples containing creatine phosphate and creatine malate exhibited a clearly defined phase boundary, and the whey fraction appeared more yellow and significantly more transparent than in the control and the other experimental samples. This indicated reduced viscosity and pronounced phase separation, which was undesirable for feeding calves with standardised milk replacers. To evaluate the dynamics of phase separation, the mixtures were remixed and sedimentation was monitored at 5-minute intervals. In the control sample (milk replacer without additives), slight clarification of the upper third was observed after 5 minutes. After 10 minutes, clarification extended to a larger portion of the upper layer, accompanied by the formation of a visible sediment occupying approximately the lower quarter of the flask. After 15 minutes, a distinct boundary between whey and sediment was evident. The pattern of phase separation was similar to that observed immediately after preparation, indicating that the functional properties of the milk replacer were preserved. Therefore, additional mixing can restore homogeneity prior to feeding. In mixtures containing creatine preparations, the rate of sedimentation after remixing increased compared to the control (Fig. 6). After 5 minutes, all samples showed clarification of the upper layer and formation of visible sediment. After 10 minutes, a distinct phase boundary had already formed, and after 15 minutes, the transparency of the whey fraction further increased. This indicated that storage beyond the recommended time was associated with loss of homogeneity, which should be considered in feeding management. The most pronounced signs of accelerated component deposition, consistent with the initial mixing, were observed in samples containing creatine phosphate and creatine malate. These effects were likely due to interactions between these compounds and mixtures components, similar to those observed, when added to whole milk.

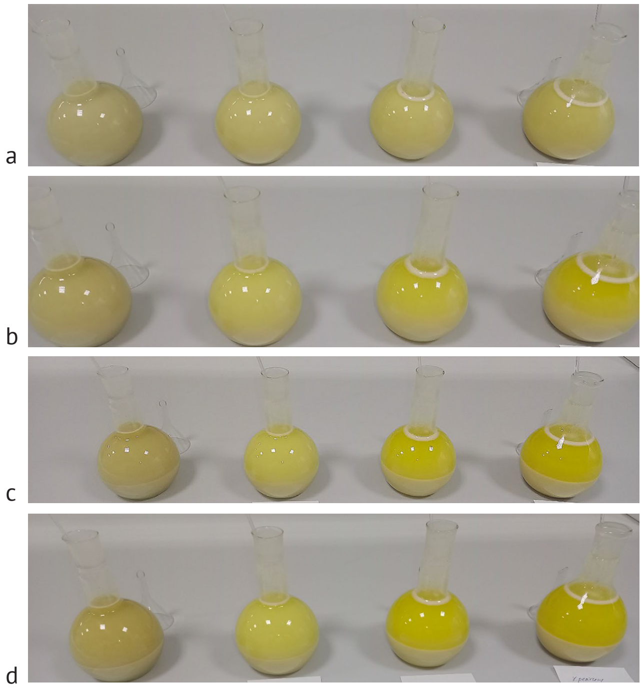


Figure 6. *Changes in the consistency of milk replacer-creatine mixtures over 15 minutes*
Note: flasks from left to right: milk replacer + creatine hydrochloride; milk replacer + creatine monohydrate; milk replacer + creatine phosphate; milk replacer + creatine malate; a – mixture after re-mixing; b – mixture 5 minutes after re-mixing; c – mixture 10 minutes after re-mixing; d – mixture 15 minutes after re-mixing
Source: developed by the authors

The search for strategies to enhance muscle development in calves raised for beef production remains relevant even during the milk-feeding period. In studies by R.E. Carter *et al.* (2025), it was demonstrated that feeding calves milk replacer with increased crude protein and crude fat content prior to weaning promotes higher body weight and greater muscle fibre cross-sectional area. In the present study, various creatine compounds were considered as potential factors influencing muscle growth. Creatine was synthesised endogenously in animals; however, under conditions of increased physiological demand and rapid muscle growth, additional dietary intake was desirable. As creatine was classified as a carninutrient, it was available primarily from animal-derived products, including milk. M.E. Brosnan & J.T. Brosnan (2016) reported that creatine concentrations in blood plasma and muscle tissue declined, when animals were fed plant-based diets, which often occurred, when whole milk feeding was restricted to reduce rearing costs. Creatine sources intended for inclusion in milk-based feeds must possess appropriate technological properties. The present study confirmed that different creatine preparations differed in their solubility in water. Creatine hydrochloride exhibited the highest solubility and can be effectively used in liquid feeding systems. Creatine phosphate also demonstrated high solubility and ease of incorporation into solutions. In contrast, creatine malate required longer dissolution time due to its heterogeneous powder structure, including the presence of larger crystals and aggregates, which should be considered, when incorporating it into liquid feeds. In the case of creatine monohydrate, its low solubility may result in sedimentation and reduced effectiveness, when administered in liquid feeds, as reported by J. Antonio *et al.* (2021). However, in this study, no significant residue was detected on filters, indicating sufficient dispersion within the solution. Therefore, minimising prolonged settling can reduce potential losses due to sedimentation. According to G. Escalante *et al.* (2022), the solubility of creatine monohydrate can be improved in solutions with lower pH; however, this was not feasible in milk due to its buffering capacity and the risk of protein coagulation under acidic conditions. Mixing with carbohydrate or protein powders was recommended to improve suspension stability and reduce sedimentation, which was applicable, when incorporating creatine into milk or milk replacer.

Milk feeds for calves were commonly supplemented with various additives aimed at optimising nutrition, balancing diets, and improving productivity and immune status. J.N. Wilms *et al.* (2024) demonstrated that the fat composition of milk replacer significantly influenced metabolism in dairy calves, indicating that lipid sources can be strategically used to modulate metabolic responses. P. Górka *et al.* (2021) reported that supplementation of milk replacer with probiotics and nucleotides positively affected growth performance and altered the composition of microbiota, suggesting improved gut health and digestive efficiency in calves. M.G. Coelho *et al.* (2023) investigated the inclusion of essential oil blends, including peppermint and eucalyptus, as well as menthol crystals, and found that such additives can enhance calf performance and health status, likely due to their antimicrobial and immunomodulatory properties. Additives may also be introduced directly into whole milk. For instance, S. Guven & T. Cimrin (2023) evaluated the effects of commercial supplements containing organic acids, plant extracts, and prebiotics. Sodium butyrate has been widely studied as a growth-promoting additive and as a factor improving calves' adaptation to weaning. Its supplementation has been shown to enhance growth performance and modulate gut microbiota in preweaning calves, as demonstrated by D. Wu *et al.* (2023). These effects were largely attributed to its role in supporting intestinal function through the nourishment of epithelial cells, as reported by A.B. Amin *et al.* (2022). In addition, sodium butyrate has been proposed as a potential alternative to antibiotic growth promoters due to its positive effects on gut health and its safety profile, which was highlighted in studies by W. Liu *et al.* (2021). Most studies have focused on the effects of additives on calf performance, gut microbiota composition, and disease incidence, while the technological properties of the mixtures have received less attention. In this study, certain creatine forms added to milk altered its physicochemical properties and induced coagulation. This can be explained by the formation of creatine salts through the addition of acidic moieties to the creatine molecule, which lowers the pH of aqueous solutions. While this enhanced creatine solubility, it negatively affected milk stability. Under these conditions, creatine monohydrate appeared to be the most suitable option for inclusion in milk, as it was not exert a pronounced acidifying effect. In present study, creatine hydrochloride did not cause significant coagulation even after one hour of incubation in milk, although Z.-M. Murillo *et al.* (2022) reported its ability to reduce pH.

The inclusion of feed additives in milk and milk replacers may affect not only the structure of the mixture but also its palatability. M. Terré *et al.* (2022) noted that the addition of sensory additives with a bitter taste – characteristic of creatine phosphate and creatine malate – can lead to feed aversion in calves and reduced intake. According to G. Escalante *et al.* (2022), who analysed the chemical properties and bioavailability of various creatine forms, creatine monohydrate remained the only form with strong evidence supporting its high bioavailability, efficacy, and safety. Milk replacers played an important role in calf feeding. Although they do not always reduce the cost of rearing, as reported by K.T. Sharpe & B.J. Heins (2021), they can compensate for milk shortages and were well suited for automated feeding systems. Their composition included dairy components but may be supplemented with more affordable and accessible ingredients, as well as deficient nutrients. However, due to the inclusion of plant-based components aimed at reducing production costs, the content of carninutrients, including creatine, was reduced. In present study, the inclusion of creatine preparations in milk replacer resulted in a slight acceleration of phase separation, particularly with creatine phosphate and creatine malate. However, the overall separation pattern was similar to that observed in the control sample, indicating the feasibility of incorporating creatine into milk replacers.

Conclusions

Among the key parameters influencing the quality of milk mixtures it was determined the solubility of creatine preparations and their potential interactions with milk components, which may alter structural and physicochemical properties. Creatine compounds differed in their degree of solubility in water. Creatine hydrochloride dissolved most rapidly, creatine phosphate demonstrated relatively good solubility. Creatine malate tended to form aggregates and required longer dissolution time. Creatine monohydrate exhibited low solubility and tended to sediment; therefore, its inclusion in calf milk mixtures must take this characteristic into account. The study demonstrated that mixing creatine preparations with milk may lead to changes in its properties and consistency. In prepared mixtures containing creatine hydrochloride, filtration properties improved compared with milk, and the deposition of dry matter on the filters decreased by 0.2 g/L. In mixtures containing creatine monohydrate, creatine phosphate, and creatine malate, the amount of residue retained on the filters increased by 0.1, 0.1, and 0.6 g/L, respectively. After one hour of standing, the filtration properties of the mixtures changed, most notably in the lower fraction. Compared with the lower fraction of milk, the amount of residue retained on the filters decreased by 0.1 g/L in mixtures with creatine hydrochloride, whereas it increased by 0.5, 3.5, and 5.3 g/L in mixtures containing creatine monohydrate, creatine phosphate, and creatine malate, respectively. Creatine monohydrate impaired filtration properties but, during storage, partially reduced the rate of phase separation. Creatine hydrochloride decreased milk viscosity, resulting in altered filtration characteristics of the upper and lower fractions during storage; however, dry matter retention on filters did not exceed that of the control. Creatine malate and creatine phosphate induced coagulation when mixed with milk. So, creatine hydrochloride proved to be the most technologically suitable additive for milk mixtures, due to its high solubility and the absence of significant coagulation effects at a dosage of 15 g per 6 L within one hour, as well as its ability to reduce the viscosity of milk replacer. The use of creatine monohydrate was feasible, provided that its low solubility was taken into account and the suspension was maintained in a homogeneous state prior to feeding. Creatine malate and creatine phosphate altered the structure of milk mixtures; therefore, their inclusion as feed additives may have adverse technological effects. Further research should investigate the effects of creatine preparations on calf growth when administered with milk or milk replacer.

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Conflict of Interest

None.

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Практичні аспекти вибору форм креатину для згодовування телятам

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Анотація. Метою дослідження було проаналізувати можливість додавання різних форм креатину в молоко і замінник незбираного молока. Було проаналізовано креатин гідрохлорид, креатин моногідрат, креатин фосфат і креатин малат. Препарати креатину, у дозуванні еквівалентному 15 г на 6 л молока було перевірено на розчинність у воді при додаванні до молока і замінника незбираного молока. Креатин гідрохлорид характеризувався відмінною розчинністю у воді, після додавання до молока суміш добре проходила через фільтр. Кількість сухої речовини, що затримується на фільтрах, зменшилася на 0,2 г/л, порівняно з молоком, що свідчить про покращені фільтраційні властивості. Після однієї години відстоювання залишок у нижній фракції залишався на 0,1 г/л меншим, ніж у відповідній фракції молока. Креатин моногдірат у воді залишався нерозчиненим, після розведення в молоці утворення згустків не викликав. Було визначено, що залишок у сумішах, що затримується на фільтрах, збільшився на 0,1 г/л порівняно з молоком, тоді як після однієї години відстоювання залишок у нижній фракції збільшився на 0,5 г/л відносно контролю. Креатин фосфат добре розчинявся в воді, однак змішування з молоком збільшувало залишок на фільтрах на 0,1 г/л одразу після змішування, а через годину зберігання суміш розділялася, утворюючи білкові коагуляти. Залишок, що затримувався на фільтрах у нижній фракції, перевищував контрольний на 3,5 г/л. Креатин малат мав подовжений час розчинення у воді через наявність великих агломератів. У приготованих сумішах з молоком залишок, що затримувався на фільтрах, збільшувався на 0,6 г/л порівняно з контролем, а через годину відстоювання залишок у нижній фракції перевищував контрольний на 5,3 г/л. Білкові коагуляти у верхній фракції суміші після години відстоювання зробили її непридатною для фільтрації. В суміші із замінником незбираного молока всі форми креатину прискорювали швидкість осідання компонентів суспензії, але цей вплив не був критичним. Було визначено, що в молочні суміші для телят доцільно включати креатин гідрохлорид, який має високу розчинність і не призводить до швидкого згортання молока

Ключові слова: молоко; замінник незбираного молока; велика рогата худоба; розчинність; коагуляція молочного білка; рефлекс стравохідного жолоба