The Effect of Feeding Top-Dress Cottonseed Bioactive Peptide and Organic Selenium on Milk Production, Liver Function, Metabolic, and Immunity Responses during the Prepartum of Holstein Dairy Cattle

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ABSTRACT

The objective of this study was to assess the effect of feeding top-dress bioactive peptides from cottonseed (CSBP) and hydroxy seleno methionine (HSM) on the performance, immune system, and health status of Holstein cows during the prepartum period. One hundred and eighty multiparous Holstein dry cows from 21 d before the expected calving date were assigned to one of the four experimental treatments of a randomized complete block design in a 2×2 factorial arrangement including 1) control group (containing inorganic Se recommended in NRC (2001)), 2) control plus 1.2 mg HSM, 3) control plus 300 g CSBP and 4) control plus 1.2 mg HSM and 300 g CSBP. The interaction of HSM and CSBP affected prepartum serum glucose concentration. Prepartum immune indicators and liver enzymes were not affected by the interaction of CSBP by HSM. In the postpartum period, the interaction of HSM by CSBP affected the concentration of serum glucose, cholesterol, total protein (TP), and creatine kinase (CK). Alkaline phosphatase (ALP) tended to be significant by the interaction of HSM and CSBP. The interaction of CSBP with HSM increased milk production. The interaction of CSBP by HSM showed that the Pe_0Se_1 cows had the lowest milk BHB concentration compared to other treatments. These results demonstrate that feeding top-dress CSBP and HSM could improve milk yield. Increasing total antioxidant capacity (TAC) by HSM and peptide separately could be helpful as a tool to pass the inflammatory period peripartum. The liver functionality index wasn't affected by any of the experimental treatments.

KEY WORDS cottonseed protein hydrolysate, cow, hydroxy selenomethionine, transition period.

INTRODUCTION

Throughout the transition period from late gestation to early lactation, dairy cows encounter remarkable alterations in their metabolic and physiological requirements (Goff and Horst, 1997; Drackley, 1999). In addition, changes in immune system functions (Mallard *et al.* 1998), inflammation (Bradford *et al.* 2015), and oxidative stress due to the production of pro-oxidants such as reactive oxygen species (Sordillo and Aitken, 2009) are features of the transition period. Oxidative stress destroys lipids, DNA, proteins, and other macromolecules and compromises defense systems, making animals susceptible to metabolic and infectious diseases (Goff and Horst, 1997; Drackley, 1999). Consequently, much emphasis has been placed on improving cow management around calving, particularly through nutritional and immunomodulators. Bioactive peptides are known for their amino acid

sequences in proteins, which determine their biological function in addition to their nutritional value. The provision of protein nutrition to animals in the form of peptides has the potential to enhance the utilization rate of protein, bolster animal immunity and disease resistance, and improve the quality of animal products. This approach may facilitate the realization of animals' production potential, as suggested by Kumar *et al.* (2022) and Liu *et al.* (2022a). In terms of antioxidant activity, the combination effect of amino acids in a peptide chain is more than the weak impact of a single amino acid unit. Many studies have been conducted on the antioxidant and immunomodulatory properties of plant-derived bioactive peptides (Hou *et al.* 2017). Overstimulation of the immune system destroys the body's natural processes and increases the need for essential amino acids; Therefore, an adequate supply of exogenous protein and amino acids can prevent or limit the consequences of inflammation and immune system activity affecting protein metabolism in the body, muscle, and production capacity of animals (Nathalie *et al.* 2004). To the best of our knowledge, there is no study about the effects of bioactive peptides on metabolic status, immunity, and liver function in transitional cows facing low immune system performance. Cottonseed meal and its protein isolates have garnered significant attention in recent times due to the industrial application of their functional proteins and peptides, as noted by Kumar *et al.* (2021). The peptides are obtained from cottonseed protein via enzymatic hydrolysis (Gao *et al.* 2010) or microbial fermentation (Sun *et al.* 2015). These peptides exhibit various biological activities, such as angiotensin I-converting enzyme (ACE) inhibitory activity (Gao *et al.* 2019), immunomodulatory activity (Liu *et al.* 2018), antimicrobial activity (Filho *et al.* 2020), and antioxidant activity (Wang *et al.* 2021a). Furthermore, a previous investigation conducted by Zhang *et al.* (2011) linked the enhanced antioxidant activity of protein hydrolysates to an elevated concentration of acidic amino acids (such as aspartic acid and glutamic acid) and basic amino acids (including lysine, arginine, and histidine).

Wang *et al.* (2021b) have found that cottonseed protein hydrolysate (CPH) and cottonseed protein fermentation (CPF) exhibit free radical scavenging activities via different mechanisms, with CPH reacting with radicals by hydrogen atom transfer and CPF by electron transfer. The CSBP may contain certain electron donors and thus react with free radicals, resulting in an antioxidant effect and the conversion of the reaction to a more stable product that terminates the free radical chain.

Selenium is one of the essential minerals and antioxidant compounds which used in the synthesis of glutathione peroxidase. The restriction of selenium, whether caused by reduced dry matter intake or lack of dietary selenium or during peak lactation with severe excretion, can result in limited antioxidant capacity (Alhidary *et al.* 2015). An adequate amount of selenium is required for many physiological processes. In livestock, selenium imbalance leads to changes in growth, production, and health (Mehdi and Dufrasne, 2016).

The biological activity of selenium in selenoproteins is due to the presence of selenocysteine (Labunskyy *et al.* 2014; Burk and Hill, 2015). It should be noticed that selenoproteins are different from selenium-containing proteins such as selenomethionine (Se-M). One of the differences is that Se-M is absorbed in the papilla of the gastrointestinal tract via Na-dependent transporters (Wolffram *et al.* 1989). The organic source of selenium is hydroxy selenomethionine (HSM), which is one of the analogs of Se-M, in which the second carbon of the amino group has been replaced by a hydroxyl group. Adding HSM enhances selenium stores in the tissues of growing pigs (Jlali *et al.* 2014). In dairy cows, the administration of HSM promotes blood antioxidant capacity and plasma and milk selenium concentrations (Sun *et al.* 2017).

Selenium exhibits various beneficial properties such as antioxidant, anti-inflammatory, anti-cancer, antiviral, and antibacterial activities, as highlighted by Hosnedlova *et al.* (2017). These effects are attributed to its incorporation into selenoproteins, a group of proteins responsible for its biological function (Sunde, 2021). One crucial selenoprotein, glutathione peroxidase (GSH-Px), plays a pivotal role in the antioxidant pathways by detoxifying lipid peroxides and safeguarding cellular and subcellular membranes against damage caused by reactive oxygen species (ROS) (Bellinger *et al.* 2009). Selenium also plays a non-specific role in the immune response, contributing to chemotactic and phagocytic activities, as well as respiratory burst activities (Hosnedlova *et al.* 2017). In terms of macrophage activity, selenium reduces the cytotoxic effects of ROS, inhibits intracellular pathogen proliferation, and enhances the number and phagocytic potential of macrophages (Dalgaard *et al.* 2018). Furthermore, selenium consumption affects adaptive immunity, including the activation and function of T and B cells (Avery and Hoffmann, 2018; Huang *et al.* 2019). In the context of inflammation, selenium may also regulate the oxidative status of immune cells, thereby influencing inflammatory gene expression (Marciel and Hoffmann, 2019).

Considering the potential impact of introducing bioactive peptides for enhancing immune function (Hou *et al.* 2017), alongside understanding the importance of selenium in improving animal performance and immune system function during the transition period, this research aimed to examine the impact of bioactive peptides administered at a

dosage of 300 gr per head per day, in combination with HSM at a dosage four times higher than the recommended amount in NRC (2001) and NRC (2021), which has not been done previously in dairy cattle, particularly during the transition period, and investigates how this interaction can influence the metabolic status, immune system function, production, and liver function of Holstein cows.

MATERIALS AND METHODS

Cows and Design

The current study was conducted at Taliseh Nemooneh Farm located in Shahriar, Tehran, Iran. The study started on 14 October 2020 and all cows were included in treatment until 4th of November 2020. The experiment takes around 6 months. The basic design of experiment was a randomized complete block design in a factorial arrangement of 2 × 2. To meet the objective of this research, one hundred and eighty multiparous Holstein cows were blocked 21 days before the calving expected date based on parity, Milk production of the previous lactation was assigned to one of the four experimental treatments in a 2 × 2 factorial arrangement including: 1) control group (Pe_0Se_0) (containing inorganic Se recommended in NRC (2001)), 2) control plus 1.2 mg HSM (Pe_0Se_1), 3) control plus 300 g CSBP (Pe_1Se_0), and 4) control plus 1.2 mg HSM and 300 g CSBP (Pe_1Se_1). The treatments were supplemented as top-dressing during morning meal.

The composition of the closeup and fresh cow's diets are shown in Table 1 and the chemical analysis of the diets is shown in Table 2. During the early stages of the dry period, the animals were housed in the same place and then, they were housed in four separate open shed houses for each treatment, which was penned via body condition score (BCS) in each treatment. The pens were similar in size, bunk space, bedding, and water availability. All cows were provided with group-fed close-up diets *ad libitum* twice a day at 0730 and 1730 hours, aiming to achieve 5 to 10% orts.

After demonstrating primary signs of calving, cows were moved to maternity pens. Following that, cows were moved to straw-bedded open-shied pens until 21 days in milk (DIM). The experiment was conducted following the principles and specific guidelines presented in the guide for the Care and Use of Agricultural Animals in Research and Teaching, Fourth Edition, 2020. All cows received the same fresh diet, *ad libitum* three times a day. Cows were milked 3 times a day. The diets were formulated by NRC (2001) software.

The bioactive peptides used in the present study contained plant peptides derived from cottonseed with special structure and special biological functions, they were obtained by multi-enzyme hydrolysis, contained 45% protein and 28% peptides with 2 to 4 amino acids and 96% digestibility. This product encompasses antioxidant and immunomodulatory peptides, heightening macrophage activity and immune factor effectiveness, thereby enhancing both cellular and humoral immunity. This product contains 18 types of amino acids and is rich in histidine, proline, leucine, glutamine, glycine, and phenylalanine. Hydroxy selenomethionine has been used as organic selenium. The used HSM includes 2% selenium equivalent to 20000 ppm selenium per kilogram and provides 100% effective selenium with the chemical formula $CH_3\text{-}Se\text{-}(CH_2)_2\text{-}CH\,(OH)\text{-}COOH$.

Table 1 Feed ingredients (%) of close-up and fresh diets (% of DM)

Ingredients	Close-up	Fresh
Legume forage hay, mature	16.75	21.00
Corn silage, normal	31.36	17.50
Wheat straw	4.90	1.00
Beet sugar pulp, dried	-	6.30
Barley grain, rolled	11.00	8.84
Corn grain, ground, dry	12.32	15.84
Canola meal, mechanical extraction	1.50	3.62
Soybean meal, solvent	2.62	4.80
Meat and bone meal	1.76	2.23
Fish meal	2.20	2.98
Corn gluten meal	1.10	2.00
Cottonseed, whole with lint	4.10	4.50
Soybean seed, whole heated	1.76	2.00
Wheat bran	2.24	1.49
Calcium carbonate	1.28	0.64
Magnesium oxide	0.35	0.24
Magnesium sulfate	0.50	-
Calcium chloride	0.30	-
Ammonium chloride	0.30	-
$NaHCO_3$	-	0.44
Buffer (Kimiabaff)	-	0.34
Salt	-	0.34
Dicalcium phosphate	-	0.10
Glucose precursors (Glyteran)	0.57	0.50
Toxinbinder	0.13	0.10
Yeast	0.44	2.00
Urea	0.39	0.40
Bentonit	1.32	-
Vitamin premix[1]	0.40	0.35
Mineral premix[2]	0.40	0.35

[1] Premix contained per kg: vitamin A: 800000 IU; vitamin D: 150000 IU; vitamin E: 12000 IU; Biotin: 125 mg; Choline: 70000 mg; Niacin: 40000 mg and Monensin: 1850 mg.
[2] Premix contained per kg: Cr: 50000 mg; Cu: 650 mg; I: 90 mg; Mn: 6050 mg; Se: 20 mg and Zn: 2200 mg.
Daily, 1.2 mg of selenium per kg of dry matter was added to the morning feed of Pe_0Se_1 and Pe_1Se_1 treatments.
300 g of bioactive peptide was added daily to the morning feed of Pe_1Se_0 and Pe_1Se_1 treatments.

Table 2 Chemical composition of the diets fed during close-up and fresh periods (% of DM, unless otherwise stated)

Chemical composition	Close-up	Fresh
NE_L, Mcal/kg DM	1.59	1.70
CP	15.50	18.90
MP	9.83	11.46
RDP	10.20	11.70
RUP	5.30	7.20
NDF	31.70	29.00
ADF	20.20	18.90
NFC	4.80	5.60
DCAD	-26	+199

NE_L: net energy for lactation; DM: dry matter; CP: crude protein; MP: metabolizable protein; RDP: rumen degradable protein; RUP: rumen undegradable protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: none fiber carbohydrate and DCAD: dietary cation anion difference.
Based on NRC (2001).

Measurements and analytical methods

Milk yield of individual cows was recorded daily from 1 to 21 DIM, and then monthly until five months postpartum. Milk samples from each cow were collected on days 3, 6, 9, 12, 15, 18, and 21, and then monthly until five months postpartum. Eventually, it was analyzed for fat, protein, lactose, milk urea nitrogen, somatic cell count (SCC), None esterified fatty acids (NEFA), ß-hydroxy butyrate (BHB), free fatty acids (FFA), and fat/protein (Combi Foss 78110; Foss Analytical A/S, Hillerød, Denmark).

Blood samples were taken from the coccygeal vein into evacuated tubes with gel and clot activator (9 mL; Greiner, Germany) for subsequent harvesting of serum. Blood samples collected on days -21, -14, -7, 0, 3, 7, 14, 21, and 28 relatives to the expected calving date. Serum samples were separated following centrifugation at 3000 × g for 15 min and frozen at −20 ˚C for later analysis of glucose (Glucotrend, Roche, Welwyn Garden City, UK; Barham and Trinder, 1972), total protein (TP) (Biuret method, kit no. 1500028; Thomas, 1998), albumin (Bromocresol green method at acidic pH, kit no. 1500001), cholesterol (Cholesterol oxidase-phenol 4-aminoantipyrine peroxidase method, kit no. 1500010), creatinine (Jaffe method; Thomas, 1998), triglycerides (Glycerol-3-phosphate oxidase-phenol 4 amino antipyrine peroxidase method, kit no. 1500032; Cole et al. 1997), bilirubin (dichloroaniline method; Thomas, 1998), aspartate aminotransferase (AST) (International Federation of Clinical Chemistry method, kit no. 1400018; Thomas, 1998) by spectrophotometer (UNICCO, 2100, Zistchemi Co., Tehran, Iran), ALP (Thomas, 1998), alanine transaminase (ALT) (Thomas, 1998), total antioxidant capacity (TAC) (commercial kit Randox; Miller et al. 1993a), malondialdehyde (MDA) using the method Thiobarbiric (Meagher and Fitzgerald, 2000), glutathione peroxidase (GPX) (commercial kit Randox; Paglia and Valentine, 1967), beta hydroxy butyrate (BHB) (commercial calometric kit Randox), creatine kinase (CK) (IFCC method, Stein, 1998) and globulin from the albumin frac-

tion of total protein. To determine liver functionality index (LFI), blood samples were also taken to measure the concentration of cholesterol, albumin, and total bilirubin on days 3 and 28 postpartum.

For all cows, albumin, cholesterol, and total bilirubin levels on days 3 and 28 of lactation were used to calculate LFI according to the formula mentioned in Bertoni and Trevisi (2013).

Statistical analysis

Statistical analyses were performed with SAS software (SAS, 2013). Before data analysis, all data were evaluated for normality and homogeneity of variances. Milk and the concentration of milk composition, serum metabolites, the index of immune status, and serum liver enzymes were analyzed as repeated data in time with the cow effect and in the block in the treatment as subject and the block effect as a random effect using the MIXED procedure of SAS software. Time (lactating days for blood metabolites) was entered into the model as a repeated variable. The covariance structures were tested and the most appropriate covariance structure was selected based on the smallest values for the Akaike information criterion, the corrected Akaike information criterion, and the Bayesian information criterion for each analysis. The same model was used for LFI without the effect of time and the interaction of trial ration by time and with the inclusion of block as a random effect. The data were reported as least square mean (LSM) and the significance level was determined at P < 0.05 and the tendency to significance at the probability level P > 0.05 and smaller and equal to 0.1. If the F-test of fixed effects and their interaction effects were significant, LSMs were analyzed using Tukey's test to compare means. If the interaction effect of experimental ration in time was significant, the differences between ration treatments at each time point were analyzed using the SCLICE option in the LSMEANS command resulting from the MIXED procedure (Schabenberger et al. 2000).

RESULTS AND DISCUSSION

The average amount of dry matter intake (DMI) in Pe_0Se_0, Pe_0Se_1, Pe_1Se_0, and Pe_1Se_1 were 14.97, 15.70, 15.58, and 15.71 kg/d, respectively. The concentration of serum metabolites, status of liver enzymes, and antioxidant status in the peripartum period are presented in Table 3. The concentration of glucose was significantly affected by the interaction of peptides and selenium (P<0.01), but there was no significant effect of the interaction of peptides and selenium on other serum metabolites, although a tendency to significance was also observed in total protein (P>0.05), albumin (P>0.05) and serum triglyceride (P>0.05).

Table 3 The effect of supplementing close-up diet with cotton seed bioactive peptide (CSBP) and hydroxy selenomethionine (HSM) on prepartum serum metabolites, immune status and liver indices of Holstein dairy cows

| Serum metabolites | Pe_0* | | Pe_1* | | SEM | P-value | | | | | | |
	Se_0	Se_1	Se_0	Se_1		Pe	Se	Pe×Se	Time	Pe×Time	Se×Time	Pe×Se×Time
Glucose, mg/dL	61.05[ab]	50.80[c]	56.35[b]	61.80[a]	1.64	0.01	0.07	< 0.01	0.17	0.07	0.71	< 0.01
Insulin, UI/mL	15.33	15.39	15.78	15.10	1.45	0.93	0.76	0.71	0.20	0.69	0.79	0.40
Triglycerides, mL/dL	20.21	23.58	21.98	21.06	1.59	0.77	0.29	0.06	< 0.01	0.58	0.82	0.10
Cholesterol, mg/dL	102.00	102.33	93.68	105.40	7.04	0.60	0.23	0.25	< 0.01	0.24	0.37	0.52
Total protein, g/dL	7.01	7.12	7.76	8.54	0.32	< 0.01	< 0.01	0.08	0.46	< 0.01	< 0.01	< 0.01
Globulin, g/dL	3.80	3.93	4.10	4.11	0.09	0.13	0.30	0.37	0.02	0.41	0.85	0.33
Albumin, g/dL	3.29	3.17	3.66	4.42	0.23	< 0.01	< 0.01	0.06	0.90	< 0.01	< 0.01	< 0.01
Creatinine, mg/dL	0.90	0.96	0.96	0.89	0.05	0.97	0.90	0.16	< 0.01	0.43	0.46	0.27
Bilirubin, mg/dL	0.21	0.20	0.16	0.19	0.02	0.16	0.68	0.26	0.06	0.48	0.19	0.89
TAC, mmol/L	0.23	0.26	0.27	0.33	0.01	< 0.01	< 0.01	0.25	0.03	0.94	0.19	0.12
MDA, nmol/mL	2.04	1.41	1.7	1.6	0.37	0.94	0.30	0.18	0.33	0.67	0.66	0.34
AST, IU/L	65.60	58.17	73.33	64.99	3.57	< 0.01	< 0.01	0.85	0.27	0.24	0.36	0.48
ALT, IU/L	26.82	26.41	24.15	27.32	1.76	0.48	0.27	0.15	0.77	0.27	0.51	0.14
ALP, IU/L	93.90	88.97	87.98	95.48	7.71	0.95	0.81	0.26	0.13	0.55	0.07	< 0.01
CK, IU/L	70.19	139.76	267.91	134.93	61.54	0.06	0.36	0.40	0.80	0.88	< 0.01	0.12

* Pe_0: no bioactive peptide; Pe_1: addition of 300 g of bioactive peptide; Se_0: no organic selenium and Se_1: addition of 1.2 mg/kg dry matter amount of HSM.
Pe_0Se_0: control treatment (without CSBP and HSM); Pe_0Se_1: without CSBP and included HSM; Pe_1Se_0: without HSM and addition of CSBP and Pe_1Se_1: with HSM and CSBS.
TAC: total antioxidant capacity; MDA: malondialdehyde; AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase and CK: creatine kinase.

Table 4 The effect of supplementing close-up diet with cotton seed bioactive peptide (CSBP) and hydroxy selenomethionine (HSM) on milk production and composition in the postpartum period of Holstein dairy cows

| Item | Pe_0* | | Pe_1* | | SEM | P-value | | | | | | |
	Se_0	Se_1	Se_0	Se_1		Pe	Se	Pe×Se	Time	Pe×Time	Se×Time	Pe×Se×Time
Colostrum, L	4.01	3.92	4.47	3.48	0.39	0.97	0.05	0.1				
Milk, L/day	35.03[b]	39.64[a]	37.97[ab]	35.97[b]	1.19	0.66	0.12	0.01	< 0.01	0.14	0.08	0.02
FCM1 3.5%, L/day	40.19	40.58	41.25	41.21	1.75	0.49	0.89	0.86	< 0.01	0.33	0.23	0.44
FPCM, L/day	38.74	38.93	39.61	38.93	1.60	0.68	0.82	0.69	< 0.01	0.35	0.16	0.67
Fat, %	3.20	3.20	3.21	3.57	0.18	0.13	0.15	0.16	< 0.01	0.21	0.03	0.69
Fat, kg/day	1.32	1.36	1.35	1.42	0.07	0.37	0.28	0.75	< 0.01	0.11	0.43	0.14
Protein, %	3.14	3.22	3.07	3.24	0.07	0.60	0.01	0.36	< 0.01	0.06	0.12	< 0.01
Protein, kg/day	1.38	1.37	1.39	1.35	0.05	0.84	0.50	0.64	< 0.01	0.25	0.37	0.62
Fat/protein	1.04	1.02	1.05	1.13	0.07	0.17	0.54	0.24	< 0.01	0.06	0.28	0.87
Lactose, %	4.54	4.49	4.56	4.53	0.04	0.40	0.15	0.70	< 0.01	0.53	< 0.01	0.07
SCC, 10^3/mL	255.43	184.86	252.18	242.69	64.99	0.55	0.38	0.50	0.30	0.51	0.02	0.92
TS, %	11.76	11.70	11.79	11.98	0.15	0.16	0.57	0.28	< 0.01	0.17	0.05	0.20
SNF[5], %	8.61	8.67	8.64	8.71	0.61	0.43	0.13	0.93	0.28	0.07	0.06	0.03
MUN, mg/dL	13.46	13.27	13.16	13.06	0.25	0.16	0.42	0.80	0.92	0.20	0.84	0.49
NEFA, μmol/L	581.51	627.06	591.96	738.61	53.67	0.10	0.01	0.18	< 0.01	0.36	0.07	0.10
BHB, mmol/L	0.084[a]	0.069[c]	0.072[b]	0.092[a]	0.006	0.22	0.60	0.01	< 0.01	0.45	0.30	0.10

* Pe_0: no bioactive peptide; Pe_1: addition of 300 g of bioactive peptide; Se_0: no organic selenium and Se_1: addition of 1.2 mg/kg dry matter amount of HSM.
Pe_0Se_0: control treatment (without CSBP and HSM); Pe_0Se_1: without CSBP and included HSM; Pe_1Se_0: without HSM and addition of CSBP and Pe_1Se_1: with HSM and CSBS.
FCM: fat corrected milk 3.5%; FPCM: fat and protein corrected milk; SCC: somatic cell count; TS: total solids; SNF: solids-not-fat; MUN: milk uninary nitrogen; NEFA: nine esterified fatty acids and BHB: beta hydrixy botirate.

The concentration of serum glucose in Pe_1Se_1 was higher than in Pe_1Se_0 and Pe_0Se_1, but there was no significant difference between Pe_1Se_1 and Pe_0Se_0. The concentration of serum triglycerides tended to be higher in cows assigned to the Pe_0Se_1 group compared to those in the Pe_0Se_0 group (P>0.05).

The total serum protein concentration tended to be higher for cows in Pe_1Se_1 as compared to those from Pe_0Se_0 (P>0.05). Moreover, the concentration of serum Albumin tended to be higher in cows assigned to the Pe_1Se_1 group compared to those in the Pe_0Se_1 (P>0.05).

Cows in Pe_1Se_0 and Pe_1Se_1 groups that were administered CSBP exhibited significantly elevated levels of serum TP compared to Pe_0Se_1 and Pe_0Se_0 groups that were not given CSBP (8.15 *vs*. 7.06 g/dL; P<0.01). This increase is probably the result of the significant increase in serum albumin concentration prepartum by adding CSBP (4.04 *vs*. 3.23 g/dL albumin; *P*>0.01 and 3.97 *vs*. 4.10 g/dL globulin; P>0.05). The interaction effect of time by CSBP showed that cows receiving CSBP on days 14 and 7 before the expected calving date had highly significant serum TP and Albumin than other cows (P<0.01).
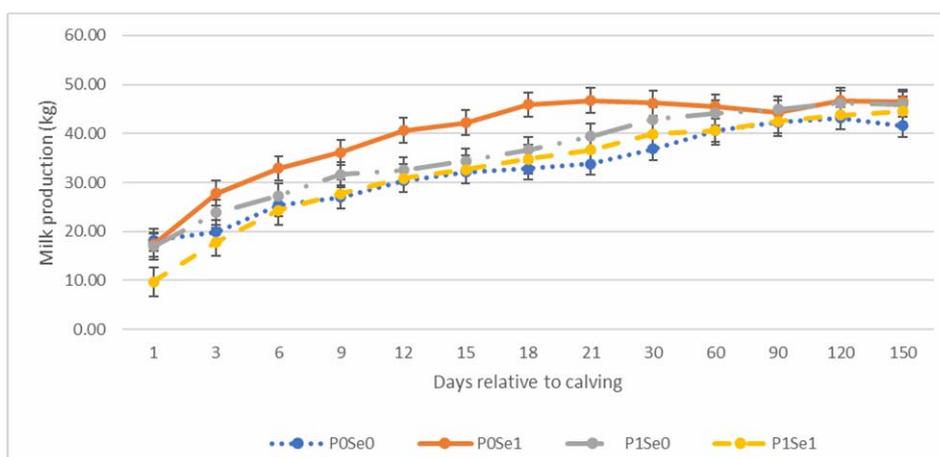
Figure 1 The effect of supplementing close-up diet with cotton seed bioactive peptide (CSBP) and hydroxy selenomethionine (HSM) on milk production in the postpartum period of Holstein dairy cows

Pe_0: no bioactive peptide; Pe_1: addition of 300 g of bioactive peptide; Se_0: no organic selenium and Se_1: addition of 1.2 mg/kg dry matter amount of HSM

Pe_0Se_0: control treatment (without CSBP and HSM); Pe_0Se_1: without CSBP and included HSM; Pe_1Se_0: without HSM and addition of CSBP and Pe_1Se_1: with HSM and CSBS

Table 5 The effect of supplementing close-up diet with cotton seed bioactive peptide (CSBP) and hydroxy selenomethionine (HSM) on postpartum serum metabolites, immune status and liver indices of Holstein dairy cows

Item	Pe_0 *		Pe_1 *		SEM	P-value						
	Se_0	Se_1	Se_0	Se_1		Pe	Se	Pe×Se	Time	Pe×Time	Se×Time	Pe×Se×Time
Glucose, mg/dL	60.36[a]	48.63[b]	56.65[ab]	57.44[ab]	3.22	0.26	0.01	< 0.01	< 0.01	0.1	< 0.01	0.23
Insulin, UI/mL	10.21	7.76	7.08	7.15	1.49	0.08	0.25	0.22	0.02	0.05	0.87	0.35
BHB, mmol/L	0.76	0.61	0.75	0.77	0.12	0.36	0.48	0.33	< 0.01	0.15	0.18	0.06
Triglycerides, mL/dL	10.56	10.79	10.49	10.56	0.55	0.70	0.69	0.83	0.02	0.08	0.017	0.06
Cholesterol, mg/dL	97.62[ab]	98.55[ab]	84.93[b]	111.09[a]	7.16	0.98	< 0.01	0.01	< 0.01	0.07	0.084	0.08
Total protein, g/dL	7.11[bc]	6.97[c]	7.01[bc]	7.70[a]	0.19	0.01	0.042	< 0.01	< 0.01	< 0.01	0.105	0.10
Albumin, g/dL	3.40	3.42	3.50	3.62	0.09	0.01	0.17	0.36	< 0.01	0.32	< 0.01	0.054
Globulin, g/dL	3.86	3.45	4.49	4.17	0.84	0.25	0.52	0.93	0.18	0.43	0.29	0.45
Creatinine, mg/dL	1.08	2.5	1.5	0.62	1.22	0.37	0.77	0.17	0.49	0.36	0.35	0.48
Bilirubin, mg/dL	0.48	0.47	0.55	0.46	0.074	0.53	0.33	0.43	< 0.01	0.66	0.23	0.33
TAC, mmol/L	0.35	1.09	0.42	0.53	0.51	0.47	0.26	0.34	0.34	0.32	0.39	0.35
MDA, nmol/mL	1.55	1.48	1.78	1.60	0.16	0.13	0.62	0.30	0.36	0.49	0.52	0.64
AST, IU/L	82.99	81.06	87.37	80.31	4.27	0.54	0.40	0.13	< 0.01	0.03	< 0.01	<0.01
ALT, IU/L	25.93	25.42	28.65	27.25	2.5	0.19	0.58	0.80	0.79	0.45	0.22	0.64
ALP, IU/L	89.44	75.32	100.31	105.75	8.20	< 0.01	0.42	0.08	< 0.01	0.82	0.12	0.13
CK, IU/L	206.19	182.18	155.11	251.18	41.21	0.75	0.21	0.04	0.03	0.65	0.47	0.15
GPX, IU/gof hemoglobin	57.96	56.17	61.31	47.34	7.79	0.62	0.14	0.27	0.04	0.34	0.07	0.47
LFI	-1.54	-0.22	-1.63	-1.29	1.05	0.51	0.27	0.44	-	-	-	-

* Pe_0: no bioactive peptide; Pe_1: addition of 300 g of bioactive peptide; Se_0: no organic selenium and Se_1: addition of 1.2 mg/kg dry matter amount of HSM.
Pe_0Se_0: control treatment (without CSBP and HSM); Pe_0Se_1: without CSBP and included HSM; Pe_1Se_0: without HSM and addition of CSBP and Pe_1Se_1: with HSM and CSBS.
BHB: beta hydroxy butyrate; TAC: total antioxidant capacity; MDA: malondialdehyde; AST: aspartate aminotransferase; ALT: alanine transaminase; ALP: alkaline phosphatase; CK: creatine kinase; GPX: glutathione peroxidase and LFI: liver functionality index.

Cows that received HSM exhibited significantly elevated levels of TP (7.83 *vs*. 7.38 g/dL; P<0.01) and albumin concentrations (3.80 *vs*. 3.48 g/dL; P<0.01) compared to treatments Pe_0Se_0, and Pe_1Se_0. Also, the interaction effect of HSM by time indicated that HSM was significant and cows in these groups had higher serum TP concentrations on day 14 before calving compared to Pe_1Se_0 and Pe_0Se_0 groups (P<0.01).

None of the treatments had any significant impact on the concentrations of insulin, cholesterol, triglyceride, and creatinine (P>0.05).

The interaction of CSBP by HSM had no significant impact on any of the immune components and liver enzymes (P>0.05). But the TAC increased significantly by adding CSBP (0.30 *vs*. 0.24 mmol/L; P<0.01) and HSM (0.30 *vs*. 0.24 mmol/L; P<0.01). The groups of cows that received

CSBP had a significantly higher prepartum serum concentration of AST compared to Pe_0Se_0 and Pe_0Se_1 groups that did not receive CSBP (69.16 *vs*. 61.90 international units per liter; P<0.01). Cows fed HSM supplements had significantly lower serum AST (61.60 *vs*. 69.47 IU/L; P<0.01). The prepartum serum concentration of CK showed a tendency towards significance in cows belonging to Pe_1Se_0 and Pe_1Se_1 groups that received CSBP (P>0.05).

The effect of supplementing CSBP and HSM (in experimental diets) on colostrum, milk yield, and composition is reported in Table 4 and milk production is shown in Figure 1. Cows in Pe_0Se_1, Pe_1Se_0, and Pe_1Se_1 treatments tended to produce less colostrum in the first milking and the amount of colostrum was higher in the Pe_0Se_0 treatment. The amount of colostrum in the first milking decreased with the addition of HSM (3.7 *vs*. 4.24 kg; P≤0.05). However, there was no significant effect of adding CSBP on the amount of colostrum in the first milking (P>0.05). The interaction of peptide by selenium did not have a significant effect on other compounds of milk (P>0.05) except milk production (P≤0.01) and BHB (P≤0.01). In comparison to Pe_1Se_0, Pe_1Se_1, and Pe_0Se_0, cows in the Pe_0Se_1 group exhibited the highest milk production. The concentration of BHB was significantly influenced by the interaction of HSM and CSBP (P≤0.01), resulting in a higher amount of BHB in Pe_1Se_1 treatment than in Pe_0Se_1, Pe_1Se_0, and Pe_0Se_0 treatments. The addition of CSBP as top dress did not have any significant effect on the milk composition and amount in cows, while significantly affected the nine esterified fatty acids (NEFA) levels. Cows that received HSM as top dress had significantly higher milk protein percentages compared to Pe_1Se_0, and Pe_0Se_0 groups (3.23 *vs*. 3.10%; P≤0.01). Cows that received HSM exhibited significantly elevated NEFA levels in comparison to Pe_1Se_0, and Pe_0Se_0 groups that were not given the treatment (682.83 *vs*. 586.73 µmol/L; P≤0.01).

The effect of supplementing diet with CSBP and HSM on serum metabolites, liver enzymes, and antioxidant status are presented in Table 5. The interaction between CSBP by HSM had no significant effect on the concentration of other serum metabolites (P>0.05), excluding glucose (P<0.01), cholesterol (P≤0.01), and TP concentrations (P<0.01). The CSBP by HSM interaction revealed that cows in Pe_0Se_0 exhibited highly significant elevated glucose concentration in comparison to Pe_0Se_1, however, there were no significant distinctions with other treatments (P>0.05). Cows that were given CSBP exhibited a numerical reduction in serum insulin concentration when compared to Pe_0Se_1 and Pe_0Se_0 groups (P>0.05). Although there was no significant difference in insulin concentrations among the 4 groups, Pe_0Se_1 cows exhibited a lower glucose concentration compared to other groups, suggesting enhanced glucose uptake by the

tissues. Serum cholesterol concentration postpartum was greater in the Pe_1Se_1 group as compared to Pe_1Se_0 (P<0.01). Serum albumin concentration was significantly higher in Pe_1Se_1 compared to Pe_0Se_1 and Pe_0Se_0 (P<0.01). It is important to mention that albumin data supports the finding that cows received the Pe_1Se_1 had significantly higher concentration of serum TP in comparison to Pe_0Se_1, and Pe_0Se_0 (P<0.01).

The impact of CSBP in HSM was not significant in all of the liver indices and immune status during the postpartum period (P>0.05), except for CK (P<0.05). Serum CK concentration after calving was significantly higher in Pe_1Se_1 compared to Pe_1Se_0 and Pe_0Se_1. Also, cows in Pe_0Se_0 showed a higher CK concentration than Pe_1Se_0 and Pe_0Se_1. There was a possible trend toward significance in ALP (P>0.05). Also, the treatments that received CSBP had highly significant serum ALP compared to Pe_0Se_0 and Pe_0Se_1 groups (103.03 *vs*. 82.38 IU/L; P<0.01).

The interaction effect of CSBP by time showed that cows that received CSBP in the prepartum period had significantly higher AST concentration than Pe_0Se_0 and Pe_0Se_1 groups (P<0.05) with increasing days of lactation. However, these differences were within standard ranges (45 to 110 IU/L, Boyd, 1984). Moreover, the interaction effect of HSM by time revealed that the cows that received HSM in the prepartum period had significantly lower AST concentration than Pe_0Se_0 and Pe_1Se_0 groups (P<0.01) with increasing days of lactation.

Few studies evaluated the effects of different sources of Se (sodium selenite and Se-enriched yeast and Se-methionine) on metabolite serum during the transition period (Khalili *et al*. 2019). In contrast to our findings, Khalili *et al*. (2019) did not observe any significant effects on glucose, albumin, and total protein levels. This disparity could be attributed to the variation in the sources and amounts of Se administered in our study. According to a recent *in vitro* study conducted by Zheng *et al*. (2022), the impact of HSM supplementation on the *in vitro* rumen fermentation characteristics and microorganisms of Holstein cows was investigated. The findings revealed that supplementation of 0.6 mg kg−1 DM HSM could enhance cumulative gas production, propionate, and total volatile fatty acids (VFAs) as glucose precursors. These results suggest that HSM has the potential to serve as a regulator for rumen fermentation in Holstein cows. Therefore, increases in serum glucose concentration prepartum in cows assigned to Pe_1Se_1 might be due to increased precursors of glucose. Whereas Seal and Reynolds (1993) pointed out that the major substrates for hepatic gluconeogenesis in ruminants were propionate from ruminal fermentation, lactate from Cori cycle AAs from protein catabolism or net portal drained visceral absorption, and glycerol released during lipolysis in adipose tissue. As

well, Danfær *et al.* (1995) suggested that AAs can account for up to 40% of glucose synthesis in dairy cattle, although this range can vary from 2 to 40%. Therefore, supplementing CSBP in prepartum cows, as a source of available AAs, might provide gluconeogenic amino acid as the substrate of glucose. In the current study, feeding HSM and CSBP before calving promoted TAC, TP, and albumin concentration compared to Pe_0Se_0. Albumin is a negative acute phase protein that decreases with increased inflammation in the body (Bertoni *et al.* 2008), so the results of the present study show that animals that received HSM or CSBP experienced fewer inflammatory conditions with increased albumin. The increase in pre-calving serum TP with the addition of CSBP may be a result of increasing the supply of further AAs. Furthermore, the properties of CSBP may contribute to an improvement in serum albumin concentration and enhance the immune system function.

Liu *et al.* (2022a) observed that broilers fed diets containing CSBP led to enhanced TAC activity in serum and liver, and reduced MDA, and glutathione peroxidase (GSH-Px) activities in serum. In current study, TAC increased in prepartum by supplementing CSBP but MDA changes were not significant in the, but numerically they decreased with supplementing CSBP.

According to Kida (2002), AST is an enzyme catalyzing the reversible transfer of amino groups between aspartate and glutamate, and blood AST concentration can be used as an indicator of liver function in periparturient cows. Although, in line with Beers and Berkow (1998), the present study revealed that supplementing the prepartum diet with CSBP brings about an increase in the serum AST level, However, it remained within the standard range (45 to 110 IU/L, Boyd, 1984). In contrast to our result, Li *et al.* (2019) finding, showed HSM has no effect on AST, and Hachemi *et al.* (2023) showing HSM can elevate AST, Phipps *et al.* (2008) reported a positive linear effect of Selenium yeast on AST activity. While Boyd (1984) reported elevated AST levels, above the normal range, indicating possible damage into the liver and heart tissue. AST is also indirectly involved in Se metabolism, as it converts alpha-ketoglutarate to glutamate during the metabolism of SeCys to selenide (Yasumoto *et al.* 1979).

The previous studies have reported that cows fed HSM exhibited higher levels of SOD, GSH-Px, and TAC, while experiencing a decrease in MDA concentration (Pilarczyk *et al.* 2012; Cao *et al.* 2014; Gong *et al.* 2014; Sun *et al.* 2017; Sun *et al.* 2019). In contrast with these results, Juniper *et al.* (2019) discovered that adding 2-hydroxy-4-methylselenobutanoic acid to the diet in the final 8 weeks of pregnancy had no impact on the levels of GSH-Px and MDA during the prepartum period. These findings align to some extent with the present study, as it also observed an increase in TAC and a numerical decrease in the peroxidation product (serum MDA) after incorporating HSM into the diet.

Previous studies conducted by Juniper *et al.* (2006), Calamari *et al.* (2010), Ceballos-Marques (2012), Machado *et al.* (2013), and Gong *et al.* (2014) verified that the source and concentration of dietary selenium did not have a significant effect on DMI, milk yield, milk fat, protein, lactose, milk urea concentrations, and somatic cell count. In alignment with Ferreira and Petzer (2019) study on the effect of injecting various selenium sources on high-yielding cows, it was observed that the injection of Se-M had no significant effect on milk production and lactose, however, caused a numerical rise in the percentage of total milk protein, which supports our observations. Nevertheless, the present study observed a tendency for HSM to affect milk production (P<0.12). Additionally, their study revealed that SCC levels exhibited a negative correlation after 72 hours following the administration of Se-M. While in our study, HSM did not have a significant effect on SCC. The rise in milk production could potentially be attributed to the reduction of damage caused by free radicals in the mammary gland, as suggested by Miller *et al.* (1993b). This notion is further supported by the data related to the TAC (1.09 *vs.* 0.35, 0.42, and 0.53 mmol/L).

Despite previous studies where increased Se status was reported to be associated with a positive effect on SCC (Weiss, 2005; Ferreira and Petzer, 2019; Reczyńska *et al.* 2019; Wang *et al.* 2021b), we did not observe any significant differences between treatments, but HSM treatments had lower SCC numerically. Miranda *et al.* (2011) and Zhuang *et al.* (2021) found the reduction of somatic cell count (SCC) by HSM could be explained by a decrease in epithelial cells, indicating that HSM reduced tissue damage in the udder. Indeed, HSM has been reported to protect against apoptosis of bovine mammary epithelial cells. Selenium enables to counteract oxidative damage in microorganism's cell membranes by scavenging and neutralizing free radicals (Hidiroglou *et al.* 1968; Surai, 2006).

In studies investigated by Juniper *et al.* (2006), Phipps *et al.* (2008), and Sun *et al.* (2017) reported that Se sources such as Se-yeast and HSM effectively elevate whole blood GPX, TAC, and reduce MDA activity when compared to selenite. In the research of Yong *et al.* (2022), they found that cows that were given HSM exhibited a tendency to enhance GPX and TAC levels while reducing MDA concentration. While other studies (Rutigliano *et al.* 2008; Calamari *et al.* 2010) did not observe any significant differences in DMI, milk yield and composition, or blood biochemical parameters among Se sources.

The impact of peptide supplementation on DMI is often insignificant, which is similar to our findings. Contrary to

Yue *et al.* (2023) and Zhou *et al.* (2022) research, which found hydrolyzed cotton seed meal resulted in a significant increase in milk yield, in the present study, even though CSBP numerically increased the DMI, it did not improve milk production. In contrast, Liu *et al.* (2022b) reported a noticeable increase in DMI and milk production after the administration of small peptide supplement.

It was traditionally believed that one of the *de novo* substrates for producing milk fat is BHB (Antunes-Fernandes *et al.* 2016; Zhao *et al.* 2019; Che *et al.* 2021). In the current study, although Pe_1Se_1 increased BHB, but it had no significant effect on milk fat. This substrate is not only the primary precursor for the synthesis of fatty acids in ruminants but also serves as a significant positive regulator for milk fat synthesis (Urrutia and Harvatine, 2017).

Zheng *et al.* (2022), found by supplementing HSM, the production of microbial protein increased along with the concentration of propionate and total VFAs, which may be due to the improvement of the antioxidant status of rumen microorganisms, thus leading to microbial growth and fermentation of soluble carbohydrates (Hidiroglou *et al.* 1968; Mihalikova *et al.* 2005). The reduction of serum glucose concentration in the Pe_0Se_1 during this study, compared to other groups, might be attributed to its uptake by alternate tissues or its enhanced allocation towards mammary glands and milk synthesis, inducing elevated lactose production in milk in the postpartum period.

Research conducted by Kommisrud *et al.* (2005) examined the impact of supplementing dairy cow diets with selenium and vitamin E before parturition on glucose tolerance. The study found that this supplementation led to improved insulin sensitivity during the first week of lactation. The antioxidant properties of selenium have been observed to have an insulin-like effect in both *in vivo* and *in vitro* studies. This insulin-like effect is associated with safeguarding against abnormalities in glucose metabolism, as highlighted by Kiełczykowska *et al.* (2018). Selenium exerts its antioxidant function through selenoproteins, which play a role in thyroid hormone metabolism, regulation, and cell growth protection. However, preliminary epidemiological studies have indicated that excessive selenium intake may contribute to the development of type 2 diabetes mellitus (Stranges *et al.* 2007; Lippman *et al.* 2009). Further research, such as that conducted by Ferreira *et al.* (2022), has demonstrated that increased levels of selenium and selenoproteins expression can interfere with insulin signaling. In this particular study, the use of HSM resulted in the development of insulin-like characteristics and increased glucose absorption in the Pe_0Se_1 group. However, the level of selenium employed in this investigation disrupted insulin signaling.

A review of animal models concluded that high Se intake, either cooperatively or independently, may contribute to the diabetic potential of Se (Finley *et al.* 2001; Mueller *et al.* 2009; Lei and Vatamaniuk, 2011; Steinbrenner *et al.* 2011; Zhou and Huang, 2013). Supra nutritional doses of Se also decreased insulin sensitivity and induced hyperinsulinemia, insulin resistance, and glucose intolerance due to overexpression of glutathione peroxidase (Rayman and Stranges, 2013; Zhou and Huang, 2013). However, subsequent studies showed that selenite and selenate supplementation in diabetic db/db mice and Wistar rats stimulated β-cell gene expression and increased glucose uptake by peripheral tissues, supporting the insulin-like action of Se (Wang *et al.* 2014; Chen *et al.* 2015; Oztu *et al.* 2015).

In contrast to our findings, Putnam and Varga (1998) discovered that an increase in maternal serum glucose was observed when the prepartum diet contained higher levels of protein than those recommended by the NRC (1989). This suggests that glucogenic amino acids may have a preferential role in maintaining glucose supply during the late pregnancy period. It is crucial to manage nutrition effectively to prevent a decline in maternal serum glucose, as the fetus has a significant demand for glucose. Additionally, Bell (1995) suggests that the presence of non-fiber carbohydrates and proteins in the diet during the early stages of lactation may result in a higher concentration of stable glucose, while Minor *et al.* (1998) found that the concentration of propionate in the rumen notably increases with excessive intake of non-fiber carbohydrates.

The increase in serum protein levels observed in the first 2 weeks after parturition indicates increased mobilization of tissue protein as well as feed protein (Vandehaar *et al.* 1999), as a source of amino acids for mammary metabolism or hepatic gluconeogenesis, which are both very important in the first week after calving. Hall *et al.* (2014) reported that adding selenium to the diet increased serum albumin. Our study revealed that Pe_1Se_1 led to an increase in its value, implying an improvement in liver condition postpartum. Comparable findings have been reported in another study linking increased albumin concentration with better health status (Trevisi *et al.* 2012). Consistent with our results, Bertoni *et al.* (2008) revealed that cows fed by Se-M exhibited elevated levels of serum albumin in comparison to the control group. This observation suggests that cows in the Se-M groups experienced reduced inflammatory conditions, as serum albumin is considered a negative acute phase protein.

Moreover, in line with this experiment, Yue *et al.* (2023) and Baumgard *et al.* (2017) observed elevated levels of TP in the blood, suggesting that the supplementation of enzymatic hydrolyzed cotton seed peptides improved nitrogen efficiency in cows. Similarly, Sabbia *et al.* (2012) conducted a study utilizing yeast-derived microbial protein as a peptide source and found that the treatment groups exhib-

ited superior nitrogen utilization efficiency compared to the control group, which utilized conventional protein sources such as soybean meal.

Contrary to the results of this research, Hall *et al*. (2014) reported adding selenium to the diet decreased serum cholesterol. In contrast with the outcomes reported by Karimzadeh *et al*. (2017), using the peptide sourced from cottonseed produced a steady decrease in serum cholesterol and triglyceride levels. This observation was also noted in a study conducted by Zhong *et al*. (2007) involving both field and laboratory rats. However, our current research indicates that the interaction between CSBP and HSM significantly increases serum cholesterol concentrations in the experimental treatments.

The effects of bioactive peptides on AST, MDA, TAC, CK, ALT, bilirubin, and creatinine in transition dairy cows have not been extensively studied.

In the study conducted by Dolatkhah *et al*. (2020), they investigated the impact of different levels of CSBP on various parameters in the serum of calves. The results showed that glucose, insulin, BHB, albumin, globulin, TP, cholesterol, TG, AST, ALT, and MDA levels remained unaffected. However, they observed that the TAC exhibited a significant increase at 70 days when the calves were fed with 2% and 4% DM of starter.

In the research of Salavati *et al*. (2021), which used 3 levels of bioactive peptides from sesame compared to antibiotics and mannan oligosaccharides, sesame bioactive peptide did not affect triglycerides, cholesterol, and AST, but increased albumin. Interestingly, the current study also found a similar increase in albumin, which was accompanied by an elevation in total protein. However, the findings of Salavati *et al*. (2021) differ from those of Hori *et al*. (2001), and Karimzadeh *et al*. (2017), who observed that the addition of canola and soy peptides resulted in a decrease in serum cholesterol, triglyceride, and AST concentrations. These contrasting findings are also inconsistent with the current study, which found that CSBP had no significant effects on cholesterol, triglyceride, and AST levels. In current study, However, the addition of selenium did not have any impact on ALP levels, but the inclusion of peptides led to an increase in ALP (P<0.01). These findings align with the results reported by Hall *et al*. (2014), who observed that serum GPX concentration remained unaffected by the inclusion of organic selenium in the diet.

While previous studies did not address the impact of the peptide on creatine kinase (CK), the current study revealed that the addition of CSBP resulted in elevated CK levels, indicating the possibility of muscle damage.

However, it has been demonstrated in other studies that certain bioactive peptides derived from cottonseed meal might possess antioxidant characteristics and potentially enhance the overall TAC in animals. Moradian *et al*. (2022) demonstrated that supplementing peptides to mice diet faced by oxidative stress decreased ALT, AST, and ALP as well. They also showed peptides could increase TAC, GPX, and SOD and decrease MDA. Also, Aslam *et al*. (2020) observed that the CSBP effectively inhibited the generation of free radicals, leading to a decrease in the production of antioxidant enzymes. The presence of natural antioxidant substances in biological systems can restrict the production of MDA.

The ability of blood and milk neutrophils to kill bacteria is diminished in dairy cows that suffer from selenium deficiency (Spears and Weiss, 2008). According to Slavik *et al*. (2008), have found that selenium's presence in biochemical pathways can lead to competition with sulfur, especially when it is introduced into amino acids such as cysteine and methionine. This competition has been observed to cause a significant increase in the activity level of red-cell glutathione peroxidase. Bioactive peptides derived from various protein sources have been associated with promoting intestinal health and modulating intestinal ALP activity. ALP in the intestine is involved in nutrient absorption, intestinal barrier function, and immune modulation. Bones and cartilage are classified as forms of connective tissue. Cartilage is secreted surrounding the bone and demonstrates intercellular growth primarily in length and parallel growth primarily in width. ALP activity serves as a phenotype of osteoblasts, and its presence is crucial for the process of mineralization (Bellows *et al*. 1991). In consistent with our results, Kyungae *et al*. (2020), demonstrated that ALP enzyme activity increased by supplementing whey protein hydrolysate.

During the first few months of lactation, dietary calcium intake is generally less than the amount of calcium excreted in milk, feces, and urine (Horst *et al*. 1997), and multiple responses occur due to this imbalance. The first response is an increase in bone resorption, mediated by the secretion of 2 hormones, parathyroid hormone (PTH) and PTH-related Peptide (PTHrP), which allows other organs to utilize the calcium in the bone mineral matrix (Mundy and Guise, 1999). ALP plays an important role in calcium reabsorption through mineralization by calcifying growth plate cartilage (Gaur *et al*. 2005; Gaur *et al*. 2006). It seems that the increase in ALP is a balancing reaction for calcium reabsorption in bones against calcium excretion in milk in early lactation.

Landy *et al*. (2020) and Landy *et al*. (2021) documented that the inclusion of CSBP at three varying levels in the diet can lead to an increase in TAC activity of serum. As Liu *et al*. (2022b) mentioned that the inclusion of CSBP in the diets of broilers resulted in an enhancement of TAC activity in both serum and liver. Additionally, they observed a re-

duction in MDA content, as well as in the activities of T-SOD and GSH-Px in serum. These findings suggest that the appropriate supplementation of CSBP can effectively improve the antioxidant capacity of broilers.

Despite the ongoing financial constraints and the challenge of effectively detecting nutritional deficiencies, metabolic profiling has the potential to serve as a valuable diagnostic tool for managing metabolic disorders. However, its usage remains limited. To address this issue and enhance the detection and management of nutritional issues on dairy farms, the liver function index (LFI) can be utilized. The LFI is a composite index that takes into account changes in plasma biomarker concentrations, including albumin, cholesterol, and bilirubin (Bertoni and Trevisi, 2013). By analyzing these biomarkers, the LFI can provide insights into the inflammatory response and amino acid levels during the transition from pregnancy to lactation. A low LFI suggests a severe inflammatory response and reduced amino acid levels, indicating a challenging transition. Conversely, a high LFI indicates a smooth transition. Cows with a high LFI (LFI>0) during the peripartum period have a lower risk of health problems compared to those with a low LFI. (LFI<0) (Bertoni and Trevisi, 2013; Zhou *et al.* 2016). In the present study, although LFI was observed as negative in all experimental treatments, but it showed a higher level in the treatments that received HSM compared to Pe_0Se_0 and Pe_1Se_0 (-0.75 *vs*. -1.58) and it seems that the liver function in these Groups was better.

CONCLUSION

In response to the peripartum inflammatory state, the incorporation of peptide and selenium could potentially enhance the overall antioxidant capacity during this period, resulting in a positive impact. Despite the lack of statistical significance during the postpartum phase, these modifications exhibited a greater extent compared to the control procedure. Additionally, CSBP × HSM increase prepartum glucose, and enhanced the serum glucose, TP, CK, and cholesterol concentration in postpartum. Also increased milk production. Overall, the inclusion of bioactive peptides in the prepartum diet at the examined concentration intensified inflammation in the postpartum period and reduced the LFI. Moreover, the effect of HSM and CSBP may be influenced by other factors such as the overall health status and DMI of the cows, as well as the management practices implemented on the farm. Drawing definitive conclusions regarding the specific effects of cottonseed bioactive peptides on biomarkers in dairy cows can be challenging. Further investigation is necessary to fully comprehend the effects of bioactive peptides and HSM on dairy cows, particularly during the transition period.

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