Effects of dietary chromium supplementation on blood biochemical parameters in dairy cows: A multilevel meta-analytical approach

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ABSTRACT

Chromium (Cr) has been reported to modulate blood biochemistry in dairy cows. However, there is a discrepancy in the literature regarding the effects of dietary Cr supplementation on various blood parameters. This meta-analysis aimed to evaluate the effects of Cr supplementation in dairy cows on blood glucose, insulin, glucagon, nonesterified fatty acid (NEFA), cortisol, and serum total protein (STP) concentrations. Following relevant literature data extraction, a 3-level meta-analytical random effect model was fitted to the data expressed as standardized mean difference (SMD) of outcome measures of control vs Cr-supplemented cows (i.e., difference in mean between control and treatment group / pooled standard deviation). The SMD can be categorized as having a small effect = 0.20, a moderate effect = 0.50, and a large effect = 0.80. The meta-regression identified the potential sources of heterogeneity, including the body weight (BW) of cows, experimental duration/duration of Cr supplementation, blood sampling time (3 weeks before parturition till 4 weeks after parturition categorized as the transition period, else as the non-transition period), and form of Cr complexes. Blood glucose did not differ significantly between control and Cr-supplemented cows with an estimated SMD of $\mu^* = 0.0071$ [95% confidence interval (CI): −0.212 to 0.226]. The effect of Cr supplementation on blood insulin was also non-significant with an SMD of $\mu = 0.0007$ (95% CI: −0.191 to 0.193). Cows receiving Cr supplements had significantly higher levels of glucagon than controls (95% CI: 0.116 to 0.489), with an estimated SMD = 0.303. Combined transition and non-transition data suggest Cr supplementation did not impact the concentration of NEFA. However, in transition cows, Cr supplementation significantly decreased blood NEFA levels as compared with controls (95% CI: −0.522 to −0.0039), with estimated SMD = −0.263. The estimated SMD was $\mu^* = −0.1983$ (95% CI: −0.734 to 0.337) for cortisol and −0.0923 (95% CI: −0.316 to 0.131) for total protein. In summary, Cr supplementation in the transition cows decreased NEFA concentration. Blood glucose, insulin, cortisol, and STP concentrations were unaffected. However, Cr supplementation increased glucagon concentration.

Key words: Blood biochemical parameters, chromium, dairy cows, insulin, meta-analysis, NEFA, transition cow

INTRODUCTION

The dairy industry has experienced a global expansion in recent years, and ruminant nutritionists have been investigating strategies to optimize the production performance and health of dairy cows. Achieving an ideal balance of nutrients, including microminerals, is crucial to maximizing the potential of high-performance dairy cows. Chromium is an essential microminerals that plays a crucial role in ruminant animal metabolism (Lashkari et al., 2018). Numerous studies have demonstrated that Cr supplementation can enhance dry matter intake, growth performance, milk production, and milk composition in ruminants (Lashkari et al., 2018). The Cr supplementation improves DMI and milk production, while milk protein, milk fat, milk lactose, and solids-not-fat are not influenced, which was published in a meta-analytical study (Malik et al., 2023).

Chromium is believed to act by inducing the up-regulation and downregulation of blood metabolites (García-Roche et al., 2019). It has been postulated that Cr is a component of the glucose tolerance factor. The primary role of the glucose tolerance factor is to potentiate glucose metabolism via oligopeptide chromodulin and consequently potentiate the auto-amplification of glucose metabolism.
system for insulin signaling, which reinforces the effects of insulin and increases glucose tolerance. The stimulatory effect of chromodulin appears to occur without affecting insulin concentration, indicating that this oligopeptide has an intrinsic role in insulin sensitivity (Khan et al., 2014). The responses to Cr supplementation regarding glucose and blood insulin concentration, however, have been inconsistent among studies (Hayirli et al., 2001, Kafari and Targhibi, 2012, Wu et al., 2021).

Furthermore, Cr supplementation has been found to have beneficial effects on lipid metabolism in animals (Vincent, 2010). In cows, Cr supplementation can reduce blood nonesterified fatty acid (NEFA) concentration (Hayirli et al., 2001) and cortisol levels (Kafari and Targhibi, 2012). Reduced blood NEFA concentrations have been associated with a decreased risk of periparturient metabolic disorders (Drackley, 1999) and reduced cortisol and NEFA concentrations could be associated with stress alleviation (Soltan, 2010). Nonetheless, the reported effects of Cr supplementation on blood metabolites and hormones have been inconsistent.

To deepen our understanding of Cr supplementation and its impact on blood metabolites and hormones, a multilevel meta-analysis was conducted to evaluate the effects of Cr supplementation in dairy cows on blood glucose, insulin, glucagon, cortisol, NEFA, and serum total protein (STP). This study aims to provide insights into the potential role of Cr supplementation in ruminant animal nutrition and identify areas for further research.

**MATERIALS AND METHODS**

**Search Strategy**

A systematic and comprehensive literature search was conducted using the following databases: PUBMED (https://www.ncbi.nlm.nih.gov/pubmed/), Agricola (https://agricola.nal.usda.gov/), CABI (https://www.cabi.org/publishing-products/animal-science-database/), and Google Scholar (https://scholar.google.com/). The following keywords were used to search publications in each search engine: “chromium and cattle,” “chromium and cow,” “chromium and cows,” “chromium and dairy cattle,” “chromium and dairy cow,” “chromium and dairy cattle,” “chromium supplementation,” “chromium supplementation and cattle,” “chromium supplementation and cow,” “chromium supplementation and cows,” “chromium supplementation and dairy cow,” “chromium supplementation and dairy cows,” and “chromium supplementation and dairy cattle.” Google Scholar was searched for each keyword for up to 10 pages. Additionally, we searched Journal of Dairy Science (https://www.journalofdairyscience.org/action/doSearch?text1 = chromium&field1 = AbstractTitleKeyword-FilterField) with only a single keyword “chromium” by restricting the search to the article title, abstract, and keywords only.

**Inclusion Criteria**

The following criteria were set for publications included in the meta-analysis: 1) journal articles were published in the English language, 2) the concentrations of glucose, insulin, glucagon, NEFA, cortisol, and STP were reported, and 3) the experimental cows were fed a diet without (control) and with Cr-supplementation. The means, standard deviation (SD), or standard error (SE) for outcome variables from cows fed a control or Cr-supplemented diet was extracted only if the response of interest (glucose and insulin, glucagon, NEFA, cortisol, and STP) were evaluated during Cr supplementation. The data for the outcome variables were not included if the supplementation of Cr occurred for less than a week. Additionally, the studies were excluded for data extraction if the outcome variables were recorded after Cr supplementation was stopped. The data of body weight, experimental duration/duration of Cr supplementation (the experimental duration was converted to weeks), blood sampling time, and Cr complexes (methionine, amino acids, yeast, picolinate, or propionate) were also extracted from the publications. The experimental design data indicates that all the studies included in the meta-analysis employ either a completely randomized design or a randomized complete block design, with no Latin-square or crossover designs. As a factor in meta-regression analysis, blood samples collected 3 weeks before parturition till 4 weeks after parturition were categorized as the transition (TRAN) period and other sampling times were categorized as the non-transition (N-TRAN) period. The effect size extracted from Gultepe et al. (2018), based on blood samples collected during the dry period (4 weeks before parturition), and the study conducted by Khalili et al. (2011), which included blood sample collections at 4 and 5 weeks before parturition, were both utilized as N-TRAN in the analysis. The publications that did not report the variance (SE or SD), mean, or data given in figures without mentioning the mean, were excluded from the database. The data extraction was carried out manually by the first author and cross verified by the corresponding authors.

The Preferred Reporting Items of Systematic reviews and Meta-Analyses (PRISMA) flowchart (Figure 1) provides information on the studies included and excluded from the meta-analysis. This systematic approach ensured that the publications analyzed met...
specific criteria to increase the reliability and validity of the meta-analysis. By considering multiple variables, the meta-analysis provides a comprehensive overview of the impact of Cr-supplementation on various metabolic responses in cows, which can inform future research and dietary recommendations in animal science.

**Statistical Analysis**

A 3-level meta-analytical random effect model was fitted to the extracted data because multiple effect sizes were extracted from the same studies (i.e., the same control vs multiple treatment groups within a publication). The application of the 3-level random effects model is the most appropriate model for this type of data when there are multiple effect sizes from the same study. The multilevel model takes into consideration the studies’ hierarchical structure as well as any additional levels of variance in the data. The effect sizes from the same study were nested as higher-level in the model and this approach can be particularly useful when there is significant heterogeneity between studies. By accounting for the different levels of variation within and between studies, multilevel meta-analysis can provide more accurate estimates of treatment effects and help to identify sources of heterogeneity (Cheung, 2014, Assink and Wibbelink, 2016). This model allows for quantifying the difference between studies and levels, assessing the overall effect size, and allowing for the evaluation of moderators or confounders that could explain the observed heterogeneity between studies (Cheung, 2014). The analysis was conducted using the standardized mean difference (SMD) as the outcome measure. The SMD is a statistical method used in meta-analysis to compare and combine results from different studies that use different scales of measurement (Cohen, 2013). The SMD was calculated as
SMD = (treatment mean - control mean) / the pooled standard deviation

An SMD >0 indicates the treatment group had a higher mean than the control group. An SMD <0 indicates the control group had a higher mean than the treatment group (Andrade, 2020). The SMD is interpreted as, small effect = 0.20, moderate effect = 0.50, and large effect = 0.80 (Cohen, 2013). The significance was declared at P ≤ 0.05, while the values between 0.05 and 0.10 were considered as a trend toward significance. The multilevel random effect meta-analysis model was carried out to evaluate the heterogeneity at 3 levels. The variance distribution was: level 1 = sampling variance; level 2 = effect sizes extracted from the same publication; and level 3 = variance between publications (Cheung, 2014). The multilevel meta-analysis was performed using the R package metafor (version 3.4.0) (Viechtbauer, 2010) and variance distribution was evaluated using the R package dmetar (version 0.0.9000) (Harrer et al., 2019).

The amount of heterogeneity (τ²) was estimated using the restricted maximum-likelihood estimator (Viechtbauer, 2005). In addition to the estimate of τ², the Q-test for heterogeneity (Cochran, 1954) and the I² statistic (Higgins and Thompson, 2002) were reported. The I² value was defined as

\[ I^2 = \left( \frac{Q - df/Q}{Q} \right) \times 100, \]

where Q is the χ² statistic and its degree of freedom. Values of I² at 0 to 40% were considered possibly not important, 30 to 60% were considered moderate, 50 to 90% were considered substantial, and 75 to 100% were considered considerably heterogeneous (Higgins et al., 2019). Additionally, meta-regression was carried out to identify the potential source of heterogeneity. For meta-regression, the moderators were the body weight (BW) of cows, experimental duration/duration of Cr supplementation, blood sampling time (TRAN and N-TRAN), and form of Cr complexes. All the moderators were included in the meta-regression model individually and the analysis was carried out using R version 4.1.3 (R Core Team, 2020) and the metafor package (version 3.4.0) (Viechtbauer, 2010).

**RESULTS**

**Blood Glucose**

A total of 24 studies with 72 comparisons of blood glucose from 913 control and 929 Cr-supplemented cows were included in the database for analysis (Supplementary Table 1; 10.6084/m9.figshare.23804607). The observed SMD in glucose concentration between control and Cr-supplemented cows ranged from −2.2264 to 2.264, with the majority of the estimates being positive (54%). A multilevel random-effects model was employed to estimate the average SMD, which was found to be μ̂ = 0.0071 [95% confidence interval (CI): −0.212 to 0.226] (Figure 2). The average SMD for glucose concentration did not significantly differ between control and Cr-supplemented cows (P = 0.948). The multilevel variance was also assessed and showed that the sampling variance (level 1) of the effect size was 27.27%, the variance between effects sizes extracted from the same study (level 2) was 59.94%, and the variance among studies (level 3) was 12.94% (Supplementary Figure 1; 10.6084/m9.figshare.23804607).

The true SMD outcomes appear to be heterogeneous based on the Q-test (I² = 72.74%, and Q statistic: χ² = 227.09) and none of the studies had a value larger than ± 3.391 based on examination of the studentized residuals indicating that there were no outliers. The symmetrical distribution of all studies around the calculated SMD indicated no risk of bias, and the asymmetrical distribution around SMD was an indication of the potential risk of bias. The presence of bias was identified by Egger’s test (Egger et al., 1997), and P < 0.05 indicated the presence of bias in the funnel plot. The analysis was carried out using R (version 4.1.3) (R Core Team, 2020) the metafor package (version 3.4.0) (Viechtbauer, 2010). The contribution of heterogeneity in meta-analysis was also presented in the Baujat diagnostic plot which shows the contribution of each study to overall heterogeneity as measured by the Q test on the x-axis and its influence on the y-axis. The influence analysis by using Cook’s distance was conducted for each parameter based on the leave-one-out method for studies incorporated in the meta-analysis. In this approach, the meta-analysis outcomes were recomputed sequentially after omitting one study at a time, enabling the identification of studies that substantively impact the aggregated effect size estimate of the meta-analysis.

**Publication Bias**

A contour enhanced funnel plot of SMD against standard error was used to test for publication bias in the studies included in the meta-analysis. The standard error of the observed outcomes as a predictor was used to check for funnel plot asymmetry. The symmetrical distribution of studies around the calculated SMD indicated no risk of bias, and the asymmetrical distribution around SMD was an indication of the potential risk of
Figure 2. Forest plot of the multilevel random-effects meta-analysis for glucose. The effect size was calculated as the standardized mean difference (SMD). The dotted vertical line represents the average effect size for glucose in cows supplemented with chromium. The negative value under the SMD heading indicates a decrease in blood glucose concentration and vice versa. Ne = number of cows in the experimental group, Me = mean in the experimental group, Se = standard deviation for the experimental group, Nc = number of cows in the control group, Mc = mean in the control group, Sc = standard deviation for the control group.
could be considered to have a large influence based on the Cook’s distances test, and based on the Baujat diagnostic plot (Supplementary Figure 3; 10.6084/m9.figshare.23804607) and the influence analysis (Supplementary Figure 4; 10.6084/m9.figshare.23804607).

The results of the meta-regression analysis indicated that the selected moderators, such as body weight, rate of Cr supplementation, duration of the experiment, phase of blood sampling, and type of Cr complexes used, had no significant impact on blood glucose levels in dairy cows that received Cr supplementation (Table 1). The findings were derived from a comprehensive evaluation of various factors, including differences in body weight ($P = 0.156$, 95% CI: $-0.0001$ to $0.0006$, $Q$ statistic = 149.98, $I^2 = 72.30$), the rate of Cr supplementation ($P = 0.854$, 95% CI: $-0.0245$ to $0.029$, $Q$ statistic = 227.01, $I^2 = 72.66$), the duration of the experiment ($P = 0.764$, 95% CI: $-0.020$ to $0.015$, $Q$ statistic = 229.39, $I^2 = 72.61$), the phase of blood sampling (i.e., for transition or non-transition cows) ($P = 0.496$, Q statistic = 219.91, $I^2 = 72.4$), and the type of Cr complexes used ($P = 0.462$, Q statistic = 206.52, $I^2 = 72.33$).

### Insulin

A total of 15 studies with 48 comparisons were reviewed to determine the effects of Cr supplementation on insulin concentration in cows. The analysis included 598 control cows and 612 Cr-supplemented cows (Supplementary Table 2; 10.6084/m9.figshare.23804607). The observed SMD in insulin concentration between control and Cr-supplemented cows ranged from $-1.186$ to $1.300$, with half of the estimates being negative (50%) (Figure 3). The estimated average SMD for insulin concentration based on the random-effects model was estimated to be $\mu = 0.0007$ (95% CI: $-0.191$ to $0.193$) and the difference for insulin concentration between control and Cr-supplemented cow was not found to be significant ($P = 0.994$). The multilevel variance (Supplementary Figure 5; 10.6084/m9.figshare.23804607) shows that the sampling variance (level 1) of the effect size was 68.81%, the variance between effects sizes extracted from the same study (level 2) was 3.32%, and the variances associated between studies was 27.87% (level 3).

The heterogeneity analysis for the SMD of insulin revealed non-important results ($I^2 = 31.26\%$, and $Q$ statistic: $\chi^2 = 69.48$). The symmetrical distribution of all studies around pooled effect size (SMD) in the funnel plot (Supplementary Figure 6; 10.6084/m9.figshare.23804607) indicates that there was no publication bias for studies included in the meta-analysis for blood insulin concentration which was supported by the non-significance ($P = 0.393$) of Egger’s test for publication bias. However, the study by Kafilzadeh and Targhibi (2012) may have exerted undue influence, as evidenced by the Baujat diagnostic plot (Supplementary Figure 7; 10.6084/m9.figshare.23804607).

The results of the meta-regression model analysis showed that insulin concentration in cows was not significantly affected by body weight ($P = 0.956$, 95% CI: $-0.0004$ to $0.0004$, Q statistic = 62.43, $I^2 = 42.55$), dose of Cr supplementation ($P = 0.983$, 95% CI: $-0.0208$ to $0.0203$, Q statistic = 69.43, $I^2 = 30.95$), duration of Cr supplementation/experiment duration ($P = 0.701$, 95% CI: $-0.0180$ to $0.0122$, Q statistic = 69.60, $I^2 = 30.98$), form of Cr complexes used ($P = 0.371$, Q statistic = 60.55, $I^2 = 30.61$), and timing of blood sampling (for

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**Table 1. A summary of statistical model and moderators for the blood glucose meta-analysis**

<table>
<thead>
<tr>
<th>Model</th>
<th>Estimate</th>
<th>Standard error</th>
<th>z-value</th>
<th>$P$-value</th>
<th>CI-LB</th>
<th>CI-UB</th>
<th>tau$^2$</th>
<th>N$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilevel random effect</td>
<td>0.0071</td>
<td>0.110</td>
<td>0.064</td>
<td>0.948</td>
<td>0.212</td>
<td>0.226</td>
<td>0.447</td>
<td>72</td>
</tr>
<tr>
<td>Moderator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW $^1$</td>
<td>0.0003</td>
<td>0.000</td>
<td>1.440</td>
<td>0.1566</td>
<td>0.0001</td>
<td>0.0006</td>
<td>0.419</td>
<td>47</td>
</tr>
<tr>
<td>Dose</td>
<td>0.0025</td>
<td>0.013</td>
<td>0.184</td>
<td>0.854</td>
<td>0.0245</td>
<td>0.0295</td>
<td>0.445</td>
<td>72</td>
</tr>
<tr>
<td>Experimental duration</td>
<td>-0.0227</td>
<td>0.009</td>
<td>0.300</td>
<td>0.764</td>
<td>0.0208</td>
<td>0.0154</td>
<td>0.446</td>
<td>72</td>
</tr>
<tr>
<td>Blood sampling time $^1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAN</td>
<td>0.108</td>
<td>0.136</td>
<td>0.794</td>
<td>0.429</td>
<td>0.164</td>
<td>0.381</td>
<td>0.439</td>
<td>70</td>
</tr>
<tr>
<td>N-TRAN</td>
<td>-0.139</td>
<td>0.165</td>
<td>0.841</td>
<td>0.402</td>
<td>0.469</td>
<td>0.190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr-complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.062</td>
<td>0.154</td>
<td>0.401</td>
<td>0.689</td>
<td>0.246</td>
<td>0.370</td>
<td>0.859</td>
<td>72</td>
</tr>
<tr>
<td>Picolinate</td>
<td>0.183</td>
<td>0.371</td>
<td>0.049</td>
<td>0.960</td>
<td>0.723</td>
<td>0.760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>-0.046</td>
<td>0.240</td>
<td>0.193</td>
<td>0.847</td>
<td>0.526</td>
<td>0.433</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>0.092</td>
<td>0.271</td>
<td>0.341</td>
<td>0.733</td>
<td>0.449</td>
<td>0.635</td>
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</tr>
</tbody>
</table>

$^1$TRAN, transition (three weeks before parturition and four weeks after parturition; N-TRAN, non-transition.

$^2$CI-LB, confidence interval lower bound.

$^3$CI-UB, confidence interval upper bound.

$^4$N, number of comparisons included.
transition and non-transition cows) \((P = 0.234, Q\) statistic = 66.35, \(I^2 = 31.15\)) (Table 2).

**Glucagon**

We identified 6 studies with 20 comparisons that investigated the impact of Cr supplementation in cows on glucagon concentration (Supplementary Table 3; 10.6084/m9.figshare.23804607). The estimated average SMD based on the multilevel random-effects model was \(\mu = 0.303\) for glucagon (95% CI: 0.116 to 0.489) and the average SMD was significantly \((P = 0.003)\) greater for Cr-supplemented cows (Figure 4). The heterogeneity of the SMD for glucagon was low \((I^2 = 0\%), \text{and } Q\) statistic: \(\chi^2 = 12.32\)), so no moderators were included in the model. The symmetrical distribution of all studies around the pooled SMD in the funnel plot (Supplementary Figure 8; 10.6084/m9.figshare.23804607) indicates no publication bias for glucagon data extracted from the studies included in the analysis, which was further supported by the non-significant \((P = 0.437)\) Egger’s test for publication bias.
Nonesterified Fatty Acid. For the NEFA data, 18 studies with 59 comparisons of control vs Cr-supplemented cows met the inclusion criteria for the meta-analysis (Supplementary Table 4; 10.6084/m9.figshare.23804607). The multilevel random-effects model was employed to estimate the average SMD for NEFA, yielding a value of $\mu^* = -0.172$ (95% CI: $-0.391$ to $0.046$). The difference in NEFA levels between control and Cr-supplemented cows was not found to be statistically significant ($P = 0.119$) (Figure 5).

The multilevel variance analysis indicated that 54.61% of the total variance was attributable to within-study sampling variance (Level 1), 0% to variance between effect sizes extracted from the same study (Level 2), and 45.39% to between-study variance (Level 3) (Supplementary Figure 9; 10.6084/m9.figshare.23804607). The Q-test demonstrated moderate heterogeneity in the true outcomes, with an $I^2$ value of 45.39% and a $Q$ statistic of $\chi^2 = 96.29$.

In the meta-analysis of blood NEFA in cows, the funnel plot exhibited a symmetrical distribution of all studies around the pooled effect size (SMD), indicating the absence of publication bias (Supplementary Figure 10; 10.6084/m9.figshare.23804607). Egger’s test for funnel plot asymmetry was also non-significant ($P = 0.092$). However, based on Cook’s distances and the Baujat diagnostic plot (Supplementary Figure 11; 10.6084/m9.figshare.23804607), the study of Kafilzadeh and Targhibi (2012) could be considered overly influential.

The meta-regression analysis indicated that the supplementation of Cr did not have a significant effect on the concentration of blood NEFA in dairy cows (Table 3). This was observed regardless of cow’s body weight ($P = 0.124$, 95% CI: $-0.0008$ to $0.0001$, $Q$ statistic = 85.17, $\hat{F} = 57.4$), the duration of the experiment ($P = 0.141$, 95% CI: $-0.029$ to $0.004$, $Q$ statistic = 97.73, $\hat{F} = 45.32$), or the form of Cr complexes used ($P = 0.128$, $Q$ statistic = 79.54, $\hat{F} = 42.41$). While the dose of Cr supplementation tended to influence plasma NEFA ($P = 0.087$, 95% CI: $-0.044$ to $0.003$, $Q$ statistic = 95.36, $\hat{F} = 46.05$). Similarly, plasma NEFA levels significantly decreased in transition cows as a result of Cr supplementation (SMD = $-0.263$, $P = 0.046$, 95% CI: $-0.522$ to $-0.0039$), but not in non-transition cows (SMD = $-0.053$, $P = 0.708$, 95% CI: $-0.339$ to $0.231$).

Cortisol

For blood cortisol data, a total of 6 studies with 24 comparisons met the inclusion criteria for the meta-analysis (Supplementary Table 5; 10.6084/m9.figshare.23804607). According to the multilevel random-effects model, the estimated average SMD for blood cortisol between control vs Cr-supplemented cows was $\mu^* = -0.1983$ (95% CI: $-0.734$ to $0.337$) and the average SMD was not statistically significantly different from 0 ($P = 0.453$). The multilevel variance analysis indicated that the sampling variance (level 1) of the SMD was 38.17%, the variance between effect sizes extracted from the same study (level 2) was 0%, and the major portion of the variances (61.83%) was associated with among studies variance (level 3) (Supplementary Figure 12; 10.6084/m9.figshare.23804607).

The true outcomes appear to be heterogeneous according to the Q-test ($I^2 = 61.83\%$, and $Q$ statistic: $\chi^2 = 53.14$) (Figure 6). The symmetrical distribution of all studies around the pooled effect size (SMD) for blood cortisol in the funnel plot indicates no publica-
tion bias in the included studies (Supplementary Figure 13; 10.6084/m9.figshare.23804607) and Egger’s test for funnel plot asymmetry was also non-significant ($P = 0.294$). However, the study by Pechova et al. (2002) could be considered overly influential based on the Baujat diagnostic plot (Supplementary Figure 14; 10.6084/m9.figshare.23804607).

The meta-regression model results indicated no statistically significant ($P > 0.05$) impact of Cr supplementation on blood cortisol levels in dairy cows after accounting for various potential moderators such as BW, dose of Cr supplementation, duration of the experiment, blood sampling phase (transition vs. non-transition cows), and the type of Cr complexes used. The summary of the statistical model and moderators for the analysis is presented in Table 4.

**Serum Total Protein**

For serum total protein concentration, a total of 5 studies comprising 18 comparisons met the inclusion criteria for the meta-analysis (Supplementary Table 6; 10.6084/m9.figshare.23804607). According to the multilevel random-effects model, the estimated average SMD for STP between control and Cr-supplemented cows was $\mu = -0.0923$ (95% CI: $-0.316$ to $0.131$) and the average outcome was non-significant ($P = 0.396$).

The true outcome was not heterogeneous ($I^2 = 0\%$, and Q statistic: $\chi^2 = 13.014$) (Figure 7). Due to low heterogeneity, no moderators were included for STP in the model. The symmetrical distribution of all studies around the pooled SMD for STP in the funnel plot no publication bias in the included studies (Supplementary Figure 15; 10.6084/m9.figshare.23804607). Egger’s test for funnel plot asymmetry was also non-significant ($P = 0.600$).

**DISCUSSION**

The main findings of the present meta-analysis were that supplementation of dairy cows with Cr had no effects on blood glucose, insulin, cortisol, and STP, while Cr supplementation increased blood glucagon levels and decreased NEFA concentration in transition cows. The effect of Cr supplementation on these blood parameters in cows was also not affected by the body weight of the cow, Cr dose rate, or supplement duration/experimental duration.

Chromium is an essential element in the body that is crucial for the maintenance of normal carbohydrate and lipid metabolism regulation (Mertz, 1993). There are conflicting reports in the literature regarding the effects of Cr supplementation on glucose and insulin levels in ruminants (Lashkari et al., 2018).
Figure 5. Forest plot of the multilevel random-effects meta-analysis for nonesterified fatty acids (NEFA). The effect size was calculated as the standardized mean difference (SMD). The negative value under the SMD heading indicates a decrease in blood NEFA concentration and vice versa. Ne = number of cows in the experimental group, Me = mean in the experimental group, Se = standard deviation for the experimental group, Nc = number of cows in the control group, Mc = mean in the control group, Sc = standard deviation for the control group.
The mechanisms by which Cr might act on blood glucose and insulin levels are not fully elucidated. It has been postulated that Cr increases the number of insulin receptors and insulin binding at the site of action (Wang and Cefalu, 2010), and Cr might influence glucose supply by modulating the effects of hepatic cellular respiration (García-Roche et al., 2019). The supplementation of Cr-Met may influence hepatic cellular respiration by decreasing pyruvate. Additionally, Cr-Met increased lactic dehydrogenase and nicotinamide adenine dinucleotide activities in dairy cattle, indicating that Cr improved hepatocyte respiration (Wu et al., 2021). Increased hepatic gluconeogenesis in dairy cows would lead to higher blood glucose levels in response to higher hepatic respiratory rates (García-Roche et al., 2019). On the contrary, recent research did not support this hypothesis (Hayirli et al., 2001, Wu et al., 2021). When Cr-Met was fed to lactating cows in these studies, Cr did not influence blood glucose levels. It was also suggested that the increase in milk lactose production induced by Cr-Met supplementation is partly attributable to increased hepatic respiration rate and gluconeogenesis in dairy cows (Wu et al., 2021). Several studies reported that Cr supplementation had no effects on glucose before and after calving (Tomlinson et al., 2008, Khalili et al., 2011, Wu et al., 2021), similar to the findings of the current meta-analysis. These findings strengthen the current meta-analytical results that Cr supplementation had no effects on blood glucose levels in dairy cows.

Some researchers have suggested that Cr plays an important role in the glucose tolerance factor (Bernhard et al., 2012), however, recent research suggests that it might be an artifact of study design or some extraneous factors (Vincent, 2010). Similarly, the current meta-analysis revealed that Cr had no effects on blood insulin levels in dairy cows, with an average SMD of 0.0007 for insulin. Similar findings were also reported by Khalili et al. (2011), who stated that glucose and insulin were not influenced by Cr supplementation, which may be due to the inability of Cr to affect the secretion of insulin from the pancreas. The supplementation of Cr can upregulate insulin receptors and their binding to the cells (Health and Services, 1995). It is believed that Cr acts via the activation of insulin by chromodulin. Chromodulin is an oligopeptide consisting of glycine, cysteine, aspartate, and glutamate (Yamamoto et al., 1987), which is acting as a cofactor for insulin. Insulin activities are mediated by stimulating protein tyrosine kinase, the insulin receptor (Ducros, 1992, Davis et al., 1996, Davis and Vincent, 1997, Spears et al., 2012). The stimulating effect of chromodulin seems to occur without affecting insulin concentration, indicating that this oligopeptide has an intrinsic role in insulin sensitivity (Khan et al., 2014), resulting in increased productivity in farm animals through improved feed intake, growth rate, reproductive parameters and immune functions (Bin-Jumah et al., 2020).

The supplementation of Cr increased blood glucagon, with an SMD of 0.303 (95% CI: 0.116 to 0.489) for control vs Cr-supplemented cows. Therefore, the effect size for glucagon could be considered small to moderate. The hormone glucagon plays an important role in the physiological mechanism regulating blood glucose concentrations. As blood glucose concentration decreases, pancreatic α-cells produce glucagon (Brockman, 1978). However, it remains to be further investigated how Cr influences blood glucagon concentration independent of the mechanisms already described.

**Table 3.** A summary of statistical model and moderators for the blood nonesterified fatty acids meta-analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Estimate</th>
<th>Standard error</th>
<th>z-value</th>
<th>P-value</th>
<th>CI-LB</th>
<th>CI-UB</th>
<th>tau²</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilevel random effect</td>
<td>−0.172</td>
<td>0.109</td>
<td>1.579</td>
<td>0.119</td>
<td>0.391</td>
<td>0.046</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>Moderators</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW ⁵</td>
<td>−0.0003</td>
<td>0.0002</td>
<td>1.565</td>
<td>0.124</td>
<td>0.0008</td>
<td>0.0001</td>
<td>0.212</td>
<td>45</td>
</tr>
<tr>
<td>Dose ⁶</td>
<td>−0.0206</td>
<td>0.011</td>
<td>1.738</td>
<td>0.087</td>
<td>0.044</td>
<td>0.003</td>
<td>0.139</td>
<td>59</td>
</tr>
<tr>
<td>Experimental duration</td>
<td>−0.012</td>
<td>0.008</td>
<td>1.490</td>
<td>0.141</td>
<td>0.029</td>
<td>0.004</td>
<td>0.135</td>
<td>59</td>
</tr>
<tr>
<td>Blood sampling time ²</td>
<td>−0.263</td>
<td>0.129</td>
<td>2.032</td>
<td>0.046</td>
<td>0.522</td>
<td>0.003</td>
<td>0.134</td>
<td>57</td>
</tr>
<tr>
<td>TRAN</td>
<td>−0.053</td>
<td>0.142</td>
<td>0.375</td>
<td>0.708</td>
<td>0.339</td>
<td>0.231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-TRAN</td>
<td>−0.184</td>
<td>0.149</td>
<td>1.239</td>
<td>0.220</td>
<td>0.483</td>
<td>0.114</td>
<td>0.120</td>
<td>59</td>
</tr>
<tr>
<td>Cr-complex Methionine</td>
<td>−0.068</td>
<td>0.226</td>
<td>0.039</td>
<td>0.968</td>
<td>0.462</td>
<td>0.444</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>0.038</td>
<td>0.315</td>
<td>0.123</td>
<td>0.902</td>
<td>0.592</td>
<td>0.670</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1BW, body weight.  
²TRAN, transition (three weeks before parturition and four weeks after parturition), N-TRAN, non-transition.  
³CI-LB, confidence interval lower bound.  
⁴CI-UB, confidence interval upper bound.  
⁵N, number of comparisons included.
Figure 6. Forest plot of the multilevel random-effects meta-analysis for blood cortisol. The effect size was calculated as the standardized mean difference (SMD). The negative value under the SMD heading indicates a decrease in blood cortisol concentration and vice versa. Ne = number of cows in the experimental group, Me = mean in the experimental group, Se = standard deviation for the experimental group, Nc = number of cows in the control group, Mc = mean in the control group, Sc = standard deviation for the control group.

Table 4. A summary of statistical models and moderators for the blood cortisol meta-analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Estimate</th>
<th>Standard error</th>
<th>z-value</th>
<th>P-value</th>
<th>CI-LB ³</th>
<th>CI-UB ⁴</th>
<th>tau²</th>
<th>N ⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilevel random effect</td>
<td>−0.198</td>
<td>0.260</td>
<td>0.761</td>
<td>0.453</td>
<td>0.734</td>
<td>0.337</td>
<td>0.336</td>
<td>26</td>
</tr>
<tr>
<td>Moderators</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW ¹</td>
<td>−0.0007</td>
<td>0.0005</td>
<td>1.221</td>
<td>0.243</td>
<td>0.001</td>
<td>0.0005</td>
<td>0.409</td>
<td>14</td>
</tr>
<tr>
<td>Dose</td>
<td>−0.003</td>
<td>0.031</td>
<td>0.114</td>
<td>0.909</td>
<td>0.067</td>
<td>0.060</td>
<td>0.367</td>
<td>26</td>
</tr>
<tr>
<td>Experimental duration</td>
<td>−0.015</td>
<td>0.023</td>
<td>0.632</td>
<td>0.532</td>
<td>0.063</td>
<td>0.033</td>
<td>0.326</td>
<td>26</td>
</tr>
<tr>
<td>Blood sampling time ²</td>
<td>−0.221</td>
<td>0.281</td>
<td>0.788</td>
<td>0.438</td>
<td>0.801</td>
<td>0.358</td>
<td>0.3599</td>
<td>26</td>
</tr>
<tr>
<td>TRAN</td>
<td>−0.125</td>
<td>0.382</td>
<td>0.327</td>
<td>0.746</td>
<td>0.916</td>
<td>0.665</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-TRAN</td>
<td>0.271</td>
<td>0.459</td>
<td>0.590</td>
<td>0.560</td>
<td>0.679</td>
<td>1.222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>−0.084</td>
<td>0.251</td>
<td>0.355</td>
<td>0.740</td>
<td>0.604</td>
<td>0.435</td>
<td>0.182</td>
<td>26</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.271</td>
<td>0.459</td>
<td>0.590</td>
<td>0.560</td>
<td>0.679</td>
<td>1.222</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹BW, body weight.
²TRAN, transition (three weeks before parturition and four weeks after parturition), N-TRAN, non-transition.
³CI-LB, confidence interval lower bound.
⁴CI-UB, confidence interval upper bound.
⁵N, number of comparisons included.
of glucose concentration. Furthermore, the results should be interpreted and implemented with caution because only 6 studies comprising 20 blood glucagon were available for effect size estimation in the current meta-analysis.

The NEFA concentration decreased significantly in transition cows in response to Cr supplementation, with a small to moderate estimated effect size ($SMD = -0.263$). The decrease in NEFA concentration in Cr-supplemented transition cows was also reported by Kafilzadeh and Targhibi (2012). In contrast, many other studies reported no effect of Cr supplementation on NEFA concentration in transition cows (Smith et al., 2005, Yasui et al., 2014). The increased energy requirements coupled with decreased dry matter intake in transition cows usually result in a negative energy balance (Grummer, 1995, Bradford et al., 2015). The mechanisms underlying the decrease in NEFA due to Cr supplementation are not fully elucidated but might involve reduced mobilization of fat from body stores to support lactation (Stahlhut et al., 2006). Our findings were supported by those of Hayirli et al. (2001) who found that Cr supplementation increased body condition score with increasing supplementation of Cr methionine postpartum, which could be associated with reduced NEFA levels. Another potential mechanism for the reduction in NEFA concentrations with Cr supplementation may involve the upregulation of peroxisome proliferator-activated receptors, which are nuclear receptors that regulate lipid metabolism and inflammation (Amiri Siavashani et al., 2018). Peroxisome proliferator-activated receptors (PPAR) modulate the expression of genes involved in fatty acid oxidation, transport, and storage, and increase the utilization of fatty acids, which might consequently reduce plasma NEFA (Busato and Bionaz, 2020). Chromium may enhance the sensitivity of PPAR to affect NEFA, thereby increasing their clearance and utilization (Amiri Sia-
vashani et al., 2018). The effect size indicated that cortisol was not influenced by Cr supplementation, as observed in many other studies (Soltan, 2010, Kafilzadeh and Targhibi, 2012, Sadri et al., 2012). Similarly, Cr supplementation did not affect STP concentrations in dairy cows, which was observed also in other studies (Mirzai et al., 2011, Wu et al., 2021).

The discrepancies in literature and findings of the current meta-analysis are associated with many factors, e.g., differences in dietary nonfibrous carbohydrates concentrations (Smith et al., 2005), variations in starch fermentability of rations among studies (Rockwell and Allen, 2016), and antagonists to Cr absorption (high concentration of dietary ferrous, zinc and phytate hinder Cr absorption) (Pechova and Pavlata, 2007). Currently, requirements of Cr supplementation in dairy cattle have not been established, due to the availability of several forms of Cr, lack of reliable data on Cr concentrations in feeds (due to low concentrations, μg/kg), contamination of feed samples during processing (using a steel grinder) and variability in absorption mechanisms. In the United States, Cr propionate has been approved for use in animal feed, with the maximum legal rate of 0.5 mg supplemental Cr/kg of the diet dry matter (NASEM, 2021). To evaluate the effects of Cr supplementation dose rates, we used studies that reported DMI in the meta-analysis (Malik et al., 2023).

In these studies, the average dose of Cr supplementation was 7.69 mg/day/cow, and the average DMI was 17.32 kg/day/cow. These data indicate that the average dose of Cr in the literature was 0.444 mg/kg of DM, which approaches the maximum NASEM (2021) recommendation of 0.50 mg/kg DMI.

CONCLUSIONS

In conclusion, dietary supplementation of dairy cows with Cr did not have significant effects on blood glucose, insulin, cortisol, or serum total protein concentration during both transition and non-transition periods in the current meta-analysis. Furthermore, the observed response of blood parameters to Cr supplementation could not be explained by the weight of cows, the dose of Cr supplementation, or the duration of supplementation. However, Cr supplementation was found to increase blood glucagon levels, while decreasing NEFA levels, during the transition period.

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