frontiers | Frontiers in Animal Science

SYSTEMATIC REVIEW
published: 17 May 2022
doi: 10.3389/fanim.2022.875944

A Meta-Analysis on the Significance of Dietary Omega-3 Fatty Acids on Bone Development and Quality in Egg- and Meat-Type Chickens

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Poultry egg and meat production continue to be optimized for productivity and efficiency. However, genetic selection focusing on production efficiency has overlooked other aspects critical to bird wellbeing, such as skeletal development. As a result, modern birds are more prone to leg weakness, osteoporosis, and, subsequently, fractures. Dietary omega-3 polyunsaturated fatty acid (n-3 PUFA) enrichment has been proposed to benefit bone development, quality, and strength. However, there is a lack of conclusive and quantitative results across studies. Therefore, a meta-analysis approach was used to evaluate published studies to determine the effects of dietary n-3 PUFA enrichment on bone quality in laying- and meat-type birds. Publications were retrieved from multiple sources (databases and hand searching), and ten studies were selected for inclusion in the final dataset. A model to predict tibial bone ash content (BAC) was developed in Proc MIXED of SAS, treating the study as a random effect. The dietary concentration of n-3 PUFA, n-3 PUFA:n-6 PUFA ratio, calcium (Ca), phosphorus and feeding duration (days) were used as independent variables to predict BAC. The final model included the dietary n-6:n-3 FA ratio and the calcium concentration in the diet. The final model was selected based on the corrected Akaike Information Criteria, the root mean square prediction error (0.999) and its components, and the concordance correlation coefficient (CCC) (0.99). In laying-type birds, BAC was reduced by n-3 PUFA ($p = 0.001$) but was increased by Ca ($p = 0.014$). In contrast, in broiler chickens, BAC was increased by n-3 PUFA ($p = 0.001$) and decreased by Ca ($p = 0.014$). The influence of n-3 PUFA:n-6 PUFA ratio on tibia BAC in laying-type birds was not statistically significant ($p = 0.505$), whereas in meat-type birds, the influence of PUFA ratio was significant ($p < 0.05$). These results may indicate a low biological significance in laying-type birds but not in meat-type birds.

Keywords: layer, broiler chickens, omega-3 fatty acids, bone quality, meta-analysis

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Edited by:
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Specialty section:
This article was submitted to
Animal Nutrition,
a section of the journal
Frontiers in Animal Science

Received: *14 February 2022*
Accepted: *19 April 2022*
Published: *17 May 2022*

Citation:
Thanabalan A, Ellis J and Kiarie EG (2022) A Meta-Analysis on the Significance of Dietary Omega-3 Fatty Acids on Bone Development and Quality in Egg- and Meat-Type Chickens. Front. Anim. Sci. 3:875944. doi: 10.3389/fanim.2022.875944

INTRODUCTION

Due to intensive genetic selection for production parameters, both laying- and meat-type birds experience skeletal metabolic disorders throughout their lifetime. In laying hens, breeding companies have continued to pursue longer production cycles with persistent rates of lay, resulting in osteoporosis and an increased risk of fractures (Bain et al., 2016). Meat-type birds have been continually selected for fast growth rates, better feed efficiencies, and low-cost production cycles, resulting in skeletal frames that are incapable of supporting their heavy bodyweights (Siegel, 2014). Subsequently, nutrition and production strategies for modulating skeletal health have gained interest as a way of attenuating skeletal disorders (Venäläinen and Valaja, 2006; Campbell et al., 2019; Khanal et al., 2021). Based on mammalian research (Watkins et al., 2000), the inclusion of omega-3 polyunsaturated fatty acids (n-3 PUFA) in poultry diets is one route of interest due to its potential to enhance bone quality. Boeyens et al. (2014) reported a strong inhibition of osteoclastogenesis in murine cell lines treated with n-3 PUFA, resulting in increased bone mass retention. However, the effect of dietary n-3 PUFA on bone attributes varies in poultry studies. An increase in the bone ash content of the tibia and the femur was reported in laying hens fed a n-3 PUFA diet when compared with laying hens fed a short-chain fatty acid diet for 58 weeks (Josling et al., 2019). In broiler chickens, the inclusion of docosahexaenoic acid (DHA) at 1 or 2% significantly improved the breaking strength of the humerus, another indicator of bone quality (Ao et al., 2015). Liu et al. (2003) reported similar findings in growing quail fed a n-3 PUFA diet—the tibial bone mineral content was highest in n-3 PUFA supplemented birds. However, Baird et al. (2008) reported no significant effects on the tibial bone mineral content in laying hens fed a high n-3 PUFA diet.

The primary objective of this meta-analysis was to quantify and examine variables that influence bone quality in poultry fed n-3 PUFA enriched diets. For this study, bone quality will be defined by bone ash content (BAC), an indicator of bone mineralization and bone strength.

MATERIALS AND METHODS

Literature Search and Dataset Development

The literature was systematically searched for relevant articles using the Web of Science, Omni, and Google Scholar in March 2021. For the dataset, the following combination of search terms was used: (omega 3 fatty acids OR n-3 OR PUFA OR polyunsaturated fatty acids OR long-chain polyunsaturated fatty acids) AND (skeletal OR bone OR tibia) AND (poultry OR avian OR layer OR broiler) for all fields. No limitations were set on language. However, studies involving broiler chickens were limited to the last 20 years to account for rapid genetic selection and evolution in the chicken industry (Tavárez and Santos, 2016). Highly cited articles and reviews were searched manually for relevant publications, and the reference lists were examined for additional sources.

Study designs with an intervention of n-3 fatty acid deficient diets were excluded in both datasets. Studies that did not report an adequate definition of the fatty acid profile of the diets were also excluded. Furthermore, the pieces of literature were screened for quantifying BAC in the tibia or the femur specifically, such that only studies following one of the two methods described by Hall et al. (2003) for quantifying bone ash were included. Lastly, studies examining keel bone quality in laying hens were excluded from the dataset to create consistency between the types of birds. The literature funnels the dataset is presented in **Figure 1** and adapted from Moher et al. (2009). The final dataset is presented in **Table 1**.

Data Extraction and Model Development

Total dietary n-3 PUFA (g/kg diet), total dietary n-6 PUFA (g/kg diet), dietary Ca concentration (%), available dietary phosphorus concentration (%), treatment duration (days), number of birds, and tibia BAC (%) were extracted into Excel from the included studies (**Figure 1**). Where possible, standard error (SE) or standard deviation (SD) was also extracted and recorded as a metric of mean precision. In studies where data were presented in graphs, means and SE or SD were digitized and extracted using WebPlotDigitizer (Rohatgi, 2021). When dietary Ca and available phosphorus concentrations were not provided, the Poultry NRC (1994) values were used to estimate concentrations based on the ingredient lists provided by the authors ($n = 2$). To ensure consistency, the ratio of n-6 PUFA to n-3 PUFA was calculated manually within each study using the provided fatty acid composition by dividing the total n-6 PUFA by the total n-3 PUFA as a means of standardizing varying enrichment concentrations. In addition, data were identified by study type of the bird (laying type or meat birds) to account for genetic differences. For model development, a total of 40 treatment means from 10 studies were used.

All statistical analyses were performed using SAS Studio (SAS Inst. Inc., Cary, NC). Continuous independent X variables included in this meta-analysis were the ratio of dietary n-6 PUFA to n-3 PUFA and dietary Ca concentration, while the type of bird (laying or meat type) was a categorical independent X variable. Initial data assessment included examination of the means and SE (PROC MEANS) for continuous variables by the categorical variables (**Table 2**). The normality of data was also visually examined (PROC UNIVARIATE). PROC CORR was used to identify potential independent variables highly correlated according to Spearman's rank correlation (data not shown). After identifying variables that could be used in the model together (low collinearity), the PROC REG backward selection procedure was used for an initial assessment of potential continuous variable models. Based on these preliminary explorations of the data, potential continuous variables of interest ($p < 0.05$) included duration of treatment, dietary available phosphorus concentration, the ratio of n-6 PUFA to n-3 PUFA, and dietary Ca concentration.

In this meta-analysis, the study was treated as a random effect, accounting for sources of between-study variance (St-Pierre, 2001; Sauvant et al., 2008). The PROC MIXED procedure in SAS was used for model development while considering the random study effect, such that:

$$Yij = b0 + b1^{*}b3^{*}X1ij + b2^{*}b3^{*}X2ij + si + eij, \tag{1}$$

where i is the number of studies, j is the number of observations, Y_{ij} is the value of the response variable (BAC, %), b_0 is the overall intercept, b_1 are the coefficients of independent predictive variable 1 ($X1ij$, N6:N3 ratio), b_3 is the species level (meat-type or layer-type), $b2$ are the coefficients of independent predictive variable 2 ($X2ij$, Dietary Ca, %), s_i is the intercept of the random

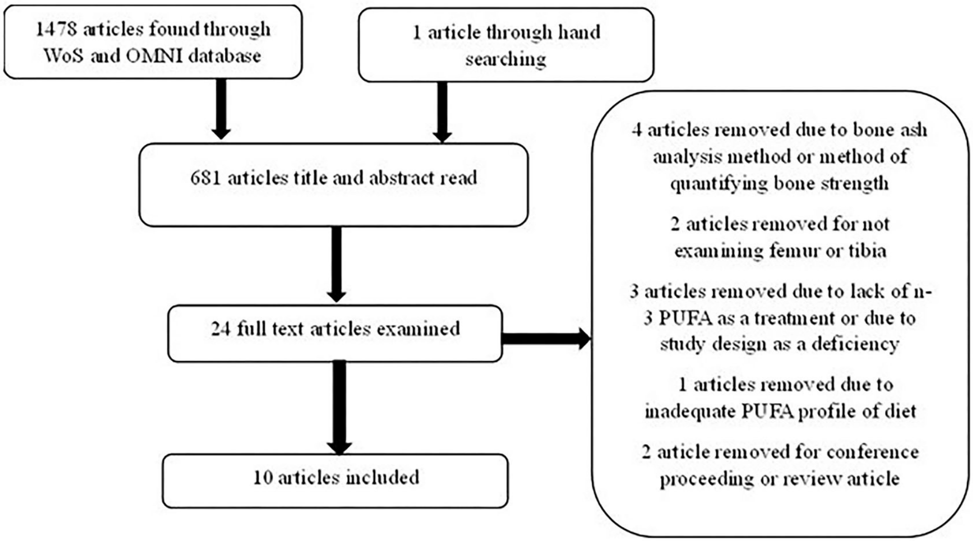

FIGURE 1 | Literature funnel for dataset development examining direct omega-3 polyunsaturated fatty acid (n-3 PUFA) feeding effects on bone ash content (BAC).

TABLE 1 | Summary of studies included in the direct feeding influence dataset.

Study	Species	Type	Experimental design	Treatment	N[1]
Abdulla et al. (2016)	Broiler	Meat	2 × 2 factorial design	Three concentrations of calcium Three sources of oil (soybean, palm and linseed) of varying FA concentrations	378
Ebeid (2011)	Layer	Laying	Completely randomized design with four treatments	Control Three low n-6:n-3 ratio (fish oil, linseed oil and linseed + fish oil)	100
Ebeid et al. (2011)	Quail	Meat	Completely randomized design with five treatments	Control Three low n-6:n-3 ratio (fish oil, linseed oil and linseed + fish oil) One high n-6:n-3 ratio (vegetable oil)	300
Ehr (2017)	Layer	Laying	Completely randomized design with three treatments	Two low n-6:n-3 ratio (fish oil and flaxseed oil) One high n-6:n-3 ratio (soy oil)	234
Josling et al. (2019)	Layer	Laying	Completely randomized design with five treatments	Control One low high n-6:n-3 ratio (fish oil) Three high n-6:n-3 ratio (sunflower oil, high oleic acid sunflower oil and tallow)	200
Leskovec et al. (2018)	Broiler	Meat	3 × 2 factorial design	Three low n-6:n-3 ratio (linseed oil) Three high n-6:n-3 ratio (walnut oil)	36
Liu et al. (2003)	Quail	Meat	Completely randomized design with four treatments	One low n-6:n-3 ratio (fish oil) Three high n-6:n-3 ratio (soybean oil, hydrogenated soybean oil and chicken fat)	120
Liu et al. (2004)	Quail	Meat	Completely randomized design with four treatments	One low n-6:n-3 ratio (fish oil) Three high n-6:n-3 ratio (soybean oil, hydrogenated soybean oil and chicken fat)	240
Puzio et al. (2012)	Broiler	Meat	Completely randomized design with four treatments	Control (sunflower oil) One low n-6:n-3 ratio (*camelina sativa* oil) Two high n-6:n-3 ratio (conjugated linoleic acid and sunflower + conjugated linoleic acid)	96
Skrivan et al. (2020)	Cockerel	Meat	2 × 3 factorial design	Four low n-6:n-3 ratio (combination of hemp seed and extruded flaxseed) Two high n-6:n-3 ratio (just hemp seed and basal wheat/corn/soybean)	540

[1] *Number of animals used in total during the experiment.*

TABLE 2 | Descriptive statistics of bone ash content (BAC, %) and independent variables (treatment duration (days), bird age (days), age at sampling (days), dietary calcium to phosphorus ratio, dietary n-6:n-3 ratio, and dietary calcium concentration (%) in the dataset.

Bird type	Variable	N[1]	Mean (SD)	SE	Min	Max	Median
	Dependent variable						
Laying	Bone ash content (BAC, %)	12	55.8 (6.99)	2.02	46.15	65.30	57.88
Meat	Bone ash content (BAC, %)	28	42.3 (3.91)	0.74	35.67	48.64	41.20
Laying	*Independent variables*						
	Age at sampling (days)	38	497.2 (151.49)	24.57	210.0	938.0	490.0
	Bird age (days)	38	342.5 (203.9)	33.07	112.0	854.0	392.0
	Dietary Ca:P ratio	38	9.7 (2.26)	0.37	8.0	14.5	8.4
	Dietary calcium (%)	38	3.6 (0.28)	0.05	3.2	4.2	3.5
	n-6:n-3 FA	38	7.8 (10.11)	1.64	0.4	48.7	7.5
	Treatment duration	38	188.5 (120.28)	19.51	63.0	378.0	98.0
Meat	*Independent variables*						
	Age at sampling (days)	33	124.5 (153.70)	26.76	35.0	392.0	42.0
	Bird age (days)	33	10.8 (11.98)	2.09	1.0	28.0	1.0
	Dietary Ca:P ratio	33	3.12 (2.00)	0.35	1.2	12.3	2.6
	Dietary calcium (%)	33	1.5 (0.81)	0.14	0.78	4.2	3.5
	n-6:n-3 FA	33	6.9 (6.85)	1.92	0.4	22.10	4.49
	Treatment duration	33	114.2 (143.75)	25.02	17.0	364.0	42.0

[1] *Number of observations in dataset.*

effect portion of the model, and e_{ij} is the unexplained residual error (St-Pierre, 2001). Variables in the model were considered statistically significant at $p < 0.05$ to be included in the model.

The Cook's Distance test was used in PROC MIXED to identify influential outliers, which were subsequently examined and considered for exclusion. Conditional studentized residual plots were visually assessed to ensure homogeneous and normally distributed residual errors. The homogeneity and normality of the random effect of the study (on model intercept) were also visually assessed, using a Q–Q plot (PROC SGPLOT). The optimal variance-covariance structure was determined by comparing the corrected Akaike's Information Criterion (AICc) of various random-effects statement structures.

Model Evaluation

The developed model was assessed for accuracy and precision of predictions using the original dataset, using methods described by Tedeschi (2006). The mean square prediction error (MSPE) assesses the mean bias, the error due to regression slope deviation from unity and random errors, and can be calculated as:

$$MSPE = \left(\bar{f}\,(X_1, \ldots, X_P) - Y\right)^2 + (s_{f(X_1, \ldots X_P)} - r \times s_y)^2 + \left(1 - r^2\right) \times s_y^2, \quad (2)$$

where $\bar{f}\,(X_1, \ldots, X_P)$ is the mean of the model predicted values, Y is the mean of the observed values, $s_{f(X_1, \ldots X_P)}$ is the SD of the model predicted values, r is the Pearson correlation coefficient, s_y is the SD of the observed values, and r^2 is the coefficient of determination. The root MSPE (RMSPE) was also calculated, expressed as a percentage of the observed mean, estimating the overall prediction error relative to the data scale.

The model was also evaluated *via* the concordance correlation coefficient (CCC) to account for both accuracy and precision simultaneously (Lin, 1989) and calculated as:

$$CCC = r \times C_b, \quad (3)$$

where r is the Pearson correlation coefficient, a measure of precision and C_b is a measure of accuracy. The C_b formula contains multiple useful metrics, such as measures of scale shift (V) and location shift (μ) about the data scale. The scale shift represents the change in SD between predicted and observed data; therefore, a large shift indicates a large difference in variance captured in predictions compared with the observations. Location shift indicates underprediction or overprediction by the model as indicated by a negative or a positive value, respectively.

Finally, the models developed were visually assessed using (conditional) predicted vs. observed plots and (conditional) residuals vs. predicted plots.

RESULTS AND DISCUSSION

Influence of Dietary n-3 PUFA on Bone Development in Laying- and Meat-Type Birds

To the best of our knowledge, this is the first meta-analysis conducted regarding the dietary enrichment of n-3 PUFA and its effects on bone strength in poultry. Bone strength is a concern in poultry production as genetic selection for production goals, such as high rates of lay in hens and fast growth in broilers, has neglected bone quality and can lead to skeletal disorders (Julian, 2005). In broiler- or meat-type birds, rapid muscle tissue accretion supersedes the rate of bone development, resulting in a wide and thick tibial growth plate unable to support the bird's heavy body weight (Butterworth, 1999; Julian, 2005). In laying hens, high egg production rates and lay persistency in conjunction with inadequate dietary Ca supply can lead to osteoporosis (Bain et al., 2016; Rufener et al., 2019). Bone strength is affected by bone mineralization, where poor mineralization has been associated with increased fracture risks (Onyango et al., 2003). Bone mineralization can be evaluated by bone ash, the mineral portion of the bone remaining after incineration, and is the dependent variable in the models developed here (Rao et al., 1993).

Models Predicting the Influence of Dietary n-3 PUFA on Bone Development in Laying- and Meat-Type Birds

The final model for predicting bone strength, as represented by BAC in avian species fed diets enriched with n-3 PUFA, is as follows:

Laying-Type Birds

$$\begin{aligned} BAC\,(\%) = {} & 44.01\ (\pm 5.229,\ P = <0.001) \\ & + [0.03\ (\pm 0.49,\ P = 0.505) \times n-6:n-3] \\ & + [3.26\ (\pm\ 1.558,\ P = 0.047)\ \times\ Ca\,(\%)] \end{aligned} \qquad (4)$$

Meat-Type Birds

$$\begin{aligned} BAC\,(\%) = {} & 44.01(\pm 5.229,\ P =< 0.001) - \\ & [0.25\ (\pm 0.060,\ P =< 0.001)\ \times n-6:n-3] - \\ & [0.07\ (\pm\ 2.780,\ P = 0.979)\ \times Ca\,(\%)] \end{aligned} \qquad (5)$$

While the n-6:n-3 × bird type interaction was significant, the coefficient for the n-6:n-3 ratio in laying-type birds (0.03 ± 0.049, parameter estimate) was not significantly different from zero ($p = 0.505$), indicating a low biological significance. Similarly, the coefficient for dietary Ca in meat-type birds (−0.07 ± 2.781, parameter estimate) was not significantly different from zero ($p = 0.979$), indicating a small influence of Ca on BAC in these birds. Interestingly, the model did not consider phosphorus or the ratio of calcium to phosphorus to be significant.

Model evaluation was conducted using conditional predictions (such as, the fixed and random components of the model developed) and is presented in **Table 3**. The majority of errors (MSPE) were random errors, indicating a high precision and accuracy of the model (**Table 3**). Furthermore, the CCC of the model was 0.99, indicating a high agreement between predictions and observations. Predicted vs. observed and residual vs. predicted plots are also presented in **Figure 2**, with both "raw" (considering just the fixed effect portion of the model) and conditional adjusted predictions. The difference between the two sets of plots highlights the variation accounted for in the "study effect."

TABLE 3 | Evaluation of model equation for BAC (%) in avian species.

Fit statistic	Model
N[1]	37
AICc[2]	156.9
Observed mean	46.32(0.985)
Predicted mean (SE)	46.32 (1.328)
SD[3]	8.077
MSPE[4]	0.998
RMSE[5], % mean	0.999
Slope bias, % MSPE[6]	5.06^{-27}
Regression slope deviation, % MSPE	0.079
Random error, % MSPE	99.92
CCC[7]	0.99
R[8]	0.999
C_b [9]	0.999
V[10]	1.011^{-16}
μ[11]	8.701

[1]N, number of observations. [2]Corrected Akaike Information Criterion as a measure of goodness-of-fit where lower is better. [3]Standard deviation (SD) of the predicted values. [4]MSPE, mean square prediction error. [5]RMSE, root mean square prediction error. [6]Presented as a percentage of the MSPE. [7]CCC, concordance correlation coefficient. [8]Pearson's correlation coefficient. [9]Bias correction factor. [10]Scale shift. [11]Location shift.

Influence of Dietary n-3 PUFA on Bone Development in Laying Birds

In the database used for model development, the reduction in the n-6:n-3 ratio is primarily a result of increased n-3 content in the diet. Therefore, the coefficient estimate in the model ($b_1 = 0.03$) for laying hens indicates that increasing the inclusion of dietary n-3 reduces BAC. While the size of the database is limited ($n = 12$ treatment means for layers and $n = 25$ for meat birds), within each study, BAC did decrease numerically with n-3 PUFA enrichment. The meta-analysis approach has been shown to increase statistical power (Cohn, 2003), and thus a (perhaps) smaller, previously non-significant effect was picked up as significant herein.

Toscano et al. (2015) previously reported that changes in bone properties due to n-3 PUFA are dependent on the type of n-3 PUFA provided. Furthermore, while only studied in mammals, dietary n-3 PUFA enrichment is thought to attenuate osteoporosis through the receptor activator of the nuclear factor-kappa-B ligand (RANKL)/receptor activator of nuclear factor-kappa-B (RANK)/Osteoprotegerin (OPG) pathway, which is responsible for osteoclastogenesis, the process wherein bone-resorbing cells known as osteoclasts are formed (Kajarabille et al., 2013). The main regulatory cytokine involved in this process is arachidonic acid (AA) (which is derived from n-6 PUFA, LA—it is present in quantities in soy oil) derived lipid mediator prostaglandin E2 (PGE_2) and acts to stimulate RANKL and RANK, leading to maturation of osteoclast precursors into activated osteoclasts, causing bones to enter a resorptive state (Boyce and Xing, 2008; Shen et al., 2008). Manipulation of dietary fatty acids results in higher levels of n-3 PUFA in cellular membranes, increasing production of EPA and DHA-derived

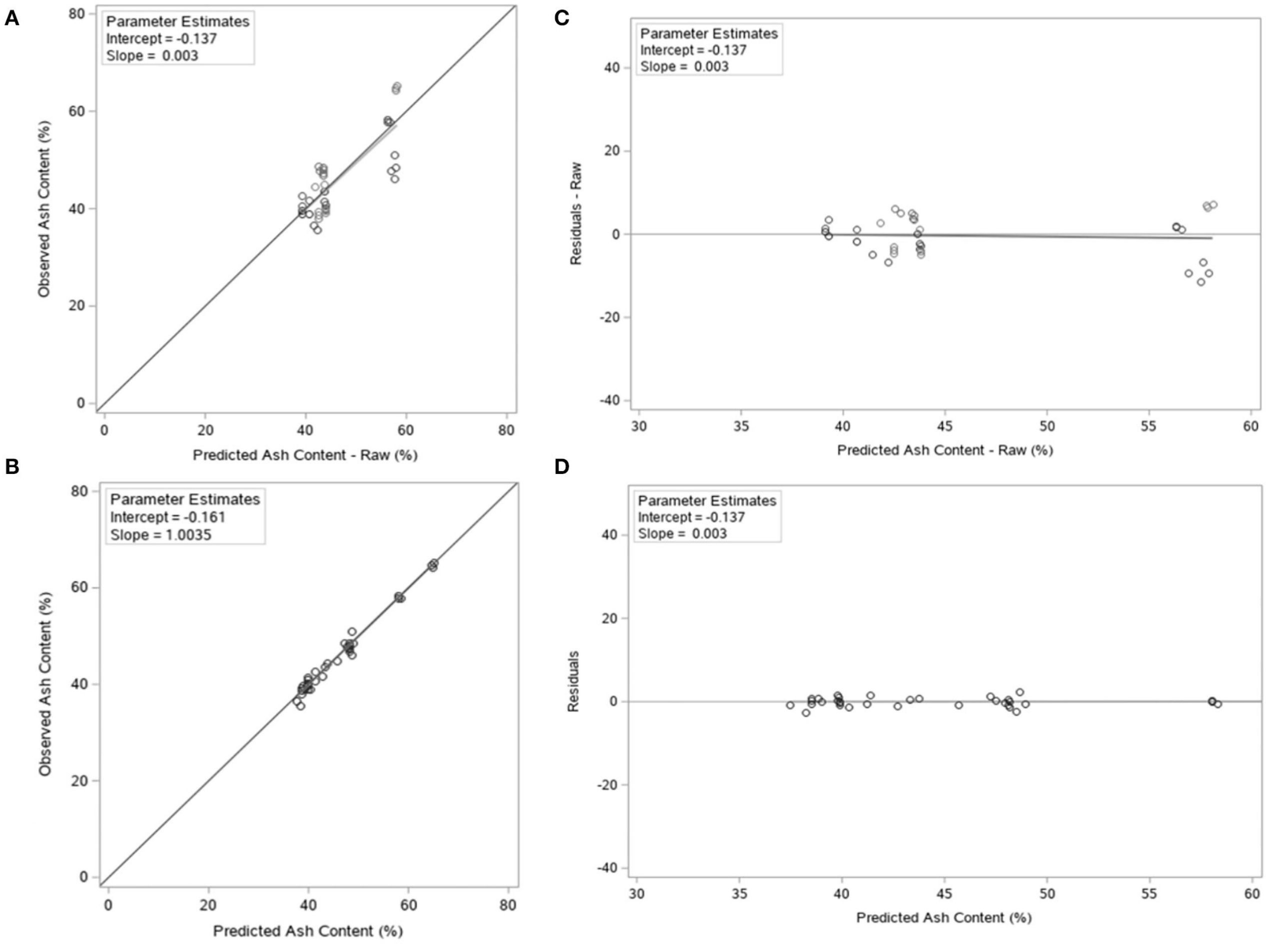

FIGURE 2 | (A) Observed vs. raw predicted values plot for the model equation. **(B)** Observed vs. conditional predicted values plot for the model equation. **(C)** Raw residuals vs. predicted plot for the model equation. **(D)** Conditional residuals (predicted – observed) vs. predicted plot for the model equation.

eicosanoids and decreasing AA-derived eicosanoid production—suppressing osteoclastogenesis (Kajarabille et al., 2013). The conversion of alpha-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and DHA, the active metabolites, is relatively poor, and therefore, the authors hypothesize that the source of dietary n-3 PUFA in the studies analyzed may have influenced the results. However, due to little variance in the source of PUFA (studies provided flaxseed oil, a source of ALA or soybean oil, a source of LA), the source or type (e.g., ALA, e.g., ALA vs. DHA) of PUFA could not be considered in the model(s) developed.

Unfortunately, this database (and thus the model) could not consider the time of intervention (growing, pre-lay, or laying phase) or the treatment duration as most studies in the dataset were considered long-term feeding and after the onset of production. At the onset of lay, there is a shift from structural cortical bone formation to the medullary bone, a reservoir of Ca for eggshell production (Whitehead, 2004). In cases where dietary Ca is insufficient, the medullary bone is replenished by resorption of structural bones, which may lead to osteoporosis (Khanal et al., 2021). Radiographic density reports indicated a higher bone mineral density and a higher amount of medullary bone in the keel bone of egg-laying birds (Eusemann et al., 2020). While the medullary bone has a high level of mineralization, it does not contribute to bone strength due to its structure (Whitehead, 2004). The keel bone, a structural bone, is widely studied in laying-type birds as a measure of skeletal health. Ultimately, studies examining keel bone quality in laying hens were excluded from the dataset to create consistency between the types of birds in this meta-analysis. However, it would be beneficial to examine the effects of feeding omega-3 FA perhaps separately on keel bone BAC in laying hens, specifically due to the availability of literature (Tarlton et al., 2011; Toscano et al., 2012).

To put the effect of n-3 on egg-laying birds into perspective, compared with the relatively larger impact of dietary Ca concentration, the influence of n-3 PUFA enrichment is minimal and may not cause detrimental effects on bone ash (**Table 4**). **Table 4** demonstrates the model predictions using the egg-type model—the change in dietary Ca concentration influenced the

TABLE 4 | Predicted BAC (%) for layers using the developed model, where BAC (%) = 44.01 + 0.03 (dietary n-6:n-3) + 3.26 (Ca concentration diet, %).

Ca (%)[1]	n-6:n-3[2]	Predicted bone ash content in layer (%)
2.10	1	50.89
	3	50.95
	5	51.01
	9	51.13
	12	51.22
	15	51.31
	18	51.40
	22	51.52
	25	51.61
3.50	1	55.45
	3	55.51
	5	55.57
	9	55.69
	12	55.78
	15	55.87
	18	55.96
	22	56.08
	25	56.17
4.06	1	57.28
	3	57.34
	5	57.40
	9	57.52
	12	57.61
	15	57.70
	18	57.79
	22	57.91
	25	58.00

[1] Calcium concentrations were chosen based on nutrient guidelines from Hendrix Genetics (2020) for prelay (2.1%), layer 1 (3.5%), and layer 2 (4.1%) phases. [2] n-6:n-3 ratios were chosen arbitrarily for demonstration purposes.

TABLE 5 | Predicted BAC (%) for meat birds using the developed model, where BAC (%) = 44.01 – 0.25 (dietary n-6:n-3) – 0.07 (Ca concentration diet, %).

Ca (%)[1]	n-6:n-3[2]	Predicted bone ash content in meat type birds (%)
0.96	1	43.69
	3	43.19
	5	42.69
	9	41.69
	12	40.94
	15	40.19
	18	39.44
	22	38.44
	25	37.69
0.87	1	43.70
	3	43.20
	5	42.70
	9	41.70
	12	40.95
	15	40.20
	18	39.45
	22	38.45
	25	37.70
0.81	1	43.70
	3	43.20
	5	42.70
	9	41.70
	12	40.95
	15	40.20
	18	39.45
	22	38.45
	25	37.70

[1] Calcium Concentrations were chosen based off nutrient guidelines from (Ross, 2019) 708 Broilers for starter (0.96%), grower (0.87%), and finisher (0.81%) phases (2019). [2] n-6:n-3 ratios were chosen arbitrarily for demonstration purposes.

BAC much greater than the dietary n-6 PUFA:n-3 PUFA within each Ca concentration.

Influence of Dietary n-3 PUFA on Bone Development in Meat-Type Birds

In contrast, the meat bird model indicated increased BAC as diets were increasingly enriched with n-3 PUFA despite the negative Ca coefficient (**Table 5**). The negative calcium coefficient may be because of a decreased concentration of calcium in the diet as meat birds age, indicating decreasing importance metabolically. In the meat-type bird studies, the broilers were fed starting from their hatching, and therefore dietary influence on developing birds could be more significant. For example, growing rats fed a n-3 PUFA diet had an increased Ca balance and small intestine absorption, translating to increased intestinal availability of Ca, potentially increasing incorporation into the bone matrix (Lau et al., 2013). Another proposed mechanism of action involving n-3 PUFA and bone development involves bone marrow containing mesenchymal stem cells that can differentiate into osteoblasts (bone-forming cells) and adipocytes, suggesting that the higher the number of bone marrow cells available, the higher the potential for osteoblastogenesis (Kruger et al., 2010). However, as previously mentioned, the effect of treatment duration was not significant (perhaps due to limited data representation) and therefore not included in the model.

Furthermore, in a typical broiler ration, LA constitutes over 50% of total PUFA compared with the ∼3–3.5% ALA, due to the predominance of corn, soybean, and other sources of dietary fat high in n-6 PUFA, generally leading to a pro-inflammatory state in broilers (Cherian, 2011). Enrichment with n-3 PUFA while decreasing the n-6 PUFA to n−3 PUFA ratio results in the decrease of proinflammatory n-6 PUFA-derived cytokines, such as IL-6 (Tarlton et al., 2013). Cytokines, such as interleukin-6 (IL-6), and PGE_2 induce each other's production and prompt osteoclastogenesis by inhibiting OPG production and upregulating RANK production, indicating that an inflamed state could perpetuate bone loss and weakening (Liu et al., 2003).

The relationship between immune response, dietary n-3 PUFA, and bone quality is another mechanism that could explain our model. Ultimately, this model suggests that enriching meat-type birds' diets with n-3 PUFA could attenuate skeletal disorders.

CONCLUSION AND FUTURE DIRECTIONS

The foregoing meta-analysis developed models to predict the bone quality of both egg- and meat-type birds when fed a n-3 PUFA diet with consideration of dietary Ca concentration as an important covariate. In laying-type birds, the enrichment of dietary n-3 PUFA resulted in decreased BAC whereas, in meat-type birds, the enrichment increased BAC. It is important to note that this exercise was not able to account for treatment duration, source of FA, or genetic breed differences (which may all influence BAC) due to a lack of available data. Future studies should consider examining and reporting the age of dietary intervention. Furthermore, BAC incorporates both Ca and P; however, our models did not have dietary phosphorus as a significant fixed effect. The developed models suggest the relationship between n-3 PUFA inclusion and BAC in broiler and layer diets; however, more data is needed to account for all possible influencing variables.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AT is a graduate student of EK and conducted the meta-analysis under the supervision of JE. All authors reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank the contributions of current and former graduate students and research associates of EK Monogastric Nutrition Research Laboratory at the University of Guelph.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.