Correlation Protein and Amino Acid Content in Feed Ingredients with Zinc Binding Protein

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Abstract. The aim of this study was to assess the correlation between protein content (N) and the amino acid of the feed material to the holding capacity of zinc and to find out the type of amino acids that contribute to bind Zn. Nineteen feedstuffs used in the experiment, namely soybean meal, catton seed meal, coconut meal, palm meal, distillers dried grains with solubles (DDGS), soy sauce waste, tofu waste, blood meal, feathers meal, fish meal, poultry meat meal (PMM) and meat bone meal (MBM), shrimp head meal, cassava leaf flour, sesbania leaf flour, glandiflora leaf flour, leucaenia leaf flour, gliricidia leaf flour, calliandra leaf flour, paraserianthes leaf flour were used. Nitrogen content of all feed ingridient was analyzed using Kejhdahl. The material was immersed in a solution of ZnO with a ratio N: Zn = 10: 1 for 24 hours then dried, subsequently the samples were analyzed to Zn bound in the protein feedstuffs (Zn-proteinate) and amino acid levels. Regression analysis was conducted to determine the correlation between the protein and amino acid content of feed ingredients with Zn-proteinate generated. Results of the study found that the average efficiency of incorporation of Zn-proteinate amounted to 47.87%. Feed ingredients that have the highest level of incorporation of soybean meal (73.71%). There is a positive correlation between crude protein content with the level of incorporation of Zn (Zn-proteinate). The results of non-linear regression analysis of the amino acid to the Zn-proteinate indicate that there were four amino acids that have a positive correlation with Zn-proteinate that aspartic acid, glutamic acid, arginine and tyrosine. The conclusion of this study is to increase the protein content of feed ingredients and amino acids aspartic acid, glutamic acid, arginine and tyrosine lead to increased levels of Zn-proteinate (Zn incorporation).

Key words : Zn proteinatee, amino acid, corelation, feed ingredients.

Abstrak. Penelitian ini bertujuan untuk menemukan korelasi antara protein (N) dan asam amino dari berbagai bahan pakan sumber protein dengan daya ikat (inkorporasi) ion Zn pada pembuatan mineral organik Zn-proteinate. Sembilan belas bahan pakan digunakan dalam percobaan, yaitu bungkil kedelai, bungkil biji kapok, bungkil kelapa, bungkil sawit, *distillers dried grains with solubles* (DDGS), ampas kecap, ampas tahu, tepung darah, tepung bulu, tepung ikan, tepung kepala udang, *poultry meat meal* (PMM), *meat bone meal* (MBM), tepung daun ketela pohon, tepung daun turi, tepung daun lamtoro, tepung daun gamal, tepung daun kaliandra dan tepung daun sengon. Bahan-bahan tersebut direndam dalam larutan ZnO dengan perbandingan N : Zn = 10 : 1 selama 24 jam lalu dikeringkan dan dianalisis kadar Zn-proteinate serta kadar asam aminonya. Uji regresi dilakukan untuk mengetahui korelasi antara kadar protein dan asam amino bahan pakan dengan kadar Zn-proteinate yang dihasilkan. Hasil penelitian mendapatkan bahwa rata-rata efisiensi inkorporasi Zn-proteinate adalah sebesar 47,87%. Bahan pakan yang mempunyai tingkat inkorporasi tertinggi yaitu bungkil kedelai (73,71%). Terdapat korelasi positif antara kandungan protein kasar dengan tingkat inkorporasi Zn (kadar Zn-proteinate). Hasil analisis regresi non linier asam amino terhadap kadar Zn-proteinate menunjukkan bahwa terdapat empat asam amino yang mempunyai korelasi positif dengan kadar Zn-proteinate yaitu asam aspartat, asam glutamat, arginin dan tirosin. Kesimpulan dari hasil penelitian ini adalah peningkatan kadar protein bahan pakan dan kadar asam amino asam aspartat, asam glutamat, arginin dan tirosin menyebabkan peningkatan kadar Zn-proteinate (inkorporasi Zn).

Kata kunci : Zinc*,* protein, asam amino, korelasi*, Zn proteinate.*

Introduction

Mineral Zinc (Zn) has been known as an essential micromineral since 1934 (Fu-Yu et al., 2007). Zn minerals in the body functions are vast including the activities of 200 enzymes, either as a catalyst, stabilize the structure of proteins and serves in nutrient metabolism (McCall et al., 2000). It also serves in cell replication, gene expression, hormone and immune system components (Beerli et al., 2000; Shankar and Prasad, 1998). The predominance of this function is indicated from the concentration of Zn in all parts of the body such as pancreas, liver, kidney, adrenal glands, pituitary gland, intestinal mucosa, bone and teeth (Georgievskii et al., 1982). Therefore, the adequacy of zinc in the diet needs to be considered, especially for cattle in period of lactation or fattening that require high level of Zn.

Zn mineral supplementation would be more efficient when administered in the form of organic minerals, namely in the form of Zn proteinatee (Wright et al., 2008). McCall et al. (2000) found that Zn in the body to function in the form of bonds with the protein and form a most common bond is the tetrahedral bonding where Zn ions bind to three or four side chains of proteins. The majority of Zn ion (82%) and proteins binds to form tetrahidral, and the rest is pentahedral and hexahedral (Wang et al., 2010). Amino acids most commonly found in proteins with Zn bond are Cysteine, histidine, glutamate, tyrosine and arginine. Under these conditions, in the manufacture of organic minerals Zn proteinate need to consider the levels of protein and amino acid feed materials to be used as a binder Zn ions (Zn^{2+}). Tetrahedral Zn bond with nitrogen (N) contained in the protein may be a rationale for the existence of a relationship or correlation between the levels of protein and amino acids with a holding capacity Zn^{2+} ions.

The high protein contained feed (>20%) can basically be used as a binder in the manufacture of Zn-proteinatee minerals. Levels of protein and amino acid content of different possibilities may produce different power of binding. Therefore, this study was aimed at assessing the correlation between protein content (N) and the amino acid of the feed material to the holding capacity of a zinc and finding the type of amino acids that contributed to the binding of Zn.

Materials and Methods

Materials. Nineteen feedstuffs were used as a source of protein for binding Zn^{2+} ions in the manufacture of Zn-proteinatee. Various waste of agriculture industries (soybean meal, kapok seed cake, coconut cake, coconut cake oil, distillers dried grains with soluble (DDGS), soy sauce and tofu dregs), and various kind of waste animal (blood meal, feathers meal, fish meal, poultry meat meal (PMM) and meat bone meal (MBM), shrimp head meal) and various flour derived from leaves (cassava, sesbania glandiflora, leucaenia, gliricidia, calliandra, paraserianthes). Zn^{2+} ion source used is zinc oxide (ZnO).

Zn Proteinate Synthesis Method. All material feed protein sources were subject to proximate analysis to determine levels of protein (N) and Zinc (Zn). N concentration was calculated by multiplying the protein content by 16%. Furthermore, in each of the feed material was added ZnO mineral (inorganic) with a ratio of N: Zn = 10: 1 and water to submerge all ingredients. The solution was left for 24 hours and stirred every 3 hours to bind reaction between the protein and Zn^{2+} ions. Protein at Zn product was further dried at 60°C (Muktiani, 2002).

Chemical Analysis. Product of Zn proteinatee further was analyzed for its Zn content to determine the level of incorporation of Zn in protein feedstuffs. Zn proteinatee in the feedstuff was calculated by dissolving the sample in trichloroacetic acid (TCA) 20%. The precipitate was then analyzed for Zn content by atomic absorption spectrophotometer (AAS) according to AOAC methods (1990). The amount of ten highest Zn proteinate products was selected to analyze the amino acids content using high pressure liquid chromatography (HPLC) according to AOAC methods (1990).

Statiticals Analysis. SPSS 16.0 statistical software package was applied to perform Pearson correlation coefficient (two-tail) and regression analysis. The correlation between Zn-proteinatee and the content of protein and amino acids of feeds ingredient was calculated statistically using this regression analysis method. Levels of Zn-proteinate obtained were then used to calculate the level of Zn incorporation efficiency using the ratio of Zn content in the Zn proteinate mineral products from initial Zn content.

Result and Discussion

Crude Protein and Zink Content. Crude protein (CP) and Zinc (Zn) feed material is presented in Table 1. Some feed ingredients such as coconut meal, palm meal, DDGS, tofu dregs and paraserianthes leaf contained CP below 20%. These results were slightly lower compared to the results of other researchers (Philsan, 2010) and could not be catagorized as protein source since the protein content was less then 20% (Kawas et al., 2012). However, since the protein level in feed ingreden was quite high, those feed ingredients would be potential source of zinc ion binding protein.

Zn content of feed ingredients derived from plants were generally low or less than 30 ppm, except cassava leaves and pulp, while the feed of animals generally had a high Zn levels ranging from 79.70 to 625.25 ppm. This is consistent with the results of Little (1986) that was reported that 60% of the feed material in Indonesia were deficiency of Zn (<30 ppm). Whereas the minimum requirement in beef cattle rations is 30 ppm (NRC, 1996) and the needs of dairy cows at least 40 ppm of ration DM (NRC, 2001). Industrial waste animal feed ingredients on average have higher levels of Zn. There are two feed ingredients (tofu and

Table 1. Dry Matter (DM), Crude Protein(CP), and Zink (Zn) level in feed material research

Feed	DM (%)	CP (%)	Zn Content (ppm)
Soybean meal	88.29	43.76	17.95
Cotton seed meal	88.82	27.12	26.40
Coconut meal	91.59	18.35	23.20
Palm meal	93.62	16.29	24.00
DDGS	90.97	18.04	21.80
Soy sauce waste	90.57	20.81	18.00
Tofu waste	10.17	18.66	146.05
Blood meal	86.69	81.32	17.20
Feather meal	32.60	63.23	652.25
Fish meal	90.43	57.16	161.05
Shrimp head flour	29.9	40.06	155.15
PMM	91.76	56.48	117.40
MBM	94.62	46.93	79.70
Cassava leaf flour	31.48	26.27	67.45
Sesbania leaf flour	20.00	26.64	15.85
Leucaenia leaf flour	24.96	26.34	16.70
Gliricidea leaf flour	23.27	21.10	16.65
Calliandra leaf flour	24.31	23.80	13.15
Paraserianthes leaf flour	35.79	17.97	8.45

[1)] Philsan (2010) ; DDGS = Distillers dried grains with solubles; PMM = Poultry meat meal ; MBM = Meat bone meal

Table 2. Zn content in Zn-proteinate mineral organic product and Zn incorporation efficiency levels

Feed	Zn-proteinate Content (ppm)	Zn incorporation efficiency (%)
Soybean meal	2742.80	73.71
Cotton seed meal	733.31	30.28
Coconut meal	619.65	37.01
Palm meal	646.30	49.89
DDGS	573.78	36.84
Soy sauce waste	824.61	47.99
Tofu waste	955.14	57.00
Blood meal	2169.92	42.23
Feather meal	2276.56	53.46
Fish meal	1826.83	40.93
Shrimp head flour	1700.04	51.30
PMM	2399.25	51.31
MBM	1634.51	41.82
Cassava leaf flour	1165.76	56.07
Sesbania leaf flour	1121.15	49.09
Leucaena leaf flour	1389.07	64.60
Gliricidea leaf flour	941.40	56.99
Calliandra leaf flour	609.32	32.82
Paraserianthes leaf flour	585.28	39.97
Average		47.87

DDGS = Distillers dried grains with solubles; PMM = Poultry meat meal ; MBM = Meat bone meal

Table 3. Zn-proteinate and amino acid content of feed ingredients

Feed	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Met	Val	Phe	Ile	Leu	Lys
Soybean meal	5.33	9.38	2.55	1.08	1.80	1.63	3.11	2.10	1.55	0.27	1.32	2.25	1.31	3.31	2.75
PMM	7.31	11.56	4.33	1.39	5.76	2.83	4.87	5.04	2.59	1.63	2.29	3.07	1.91	5.28	3.93
Feather meal	6.36	11.06	12.57	0.45	6.06	3.98	5.48	4.40	2.78	0.53	4.08	4.48	2.92	6.82	1.53
Blood meal	10.34	8.96	5.27	5.31	3.21	4.52	3.53	7.19	2.45	1.32	4.43	6.12	0.66	10.46	6.05
Fish meal	5.48	9.06	7.67	0.54	5.62	2.81	4.38	4.18	2.22	0.82	2.82	3.30	2.12	5.03	1.83
Shrimp head flour	4.86	7.34	2.71	0.94	2.47	1.98	2.99	3.21	1.92	1.04	1.65	2.31	1.14	2.73	2.34
MBM	4.66	8.00	2.77	1.04	6.05	1.88	3.75	4.51	1.64	0.98	1.63	1.97	1.14	3.66	2.92
Leucaena leaf flour	3.78	3.50	1.42	1.64	1.14	1.07	1.23	1.35	1.00	0.33	1.02	1.27	0.81	1.83	1.35
Cassava leaf flour	2.49	3.10	1.38	0.42	1.13	1.00	1.10	1.39	0.89	0.22	0.75	1.12	0.80	1.79	1.06
Tofu waste	1.94	2.98	1.10	0.36	0.85	0.87	0.87	1.24	0.58	0.24	0.62	0.81	0.59	1.36	1.03

DDGS = Distillers dried grains with solubles; PMM = Poultry meat meal ; MBM = Meat bone meal
Zn-P = Zn Proteinatee, Asp = Aspartic acid, Glu = Glutamic acid, Ser = Serine, His = Histidine, Gly = Glycine, Thr = Threonine, Arg = Arginine, Ala = Alanine, Tyr = Tyrosine, Met = Methionine, Val = Valine, Phe = Phenylalanine, Ile = I-leucine, Leu = Leucine, Lys = Lysine.

feather meal) that has a very high Zn levels, because in the process of drying of the feed material was performed using zinc sheets.

Zn Proteinatee Content in The Product. Zn proteinatee content in feed ingredients and Zn incorporation efficiency levels are presented in Table 2. Four ingredient feed that have highest Zn proteinatee content were soybean meal 3493.93 ppm, feather meal 3228.35 ppm, PMM 3158.49 ppm, blood meal 2982.27 ppm, and fish meal 2465.26 ppm. Zn incorporation efficiency of feed ingredients obtained ranged from 30.28 to 73.71%. The highest efficiency is achieved by the incorporation of soybean meal, while the lowest is cotton seed meal. The average efficiency of incorporation is quite low at 47.87%. When viewed from the average efficiency of the overall result is still lower

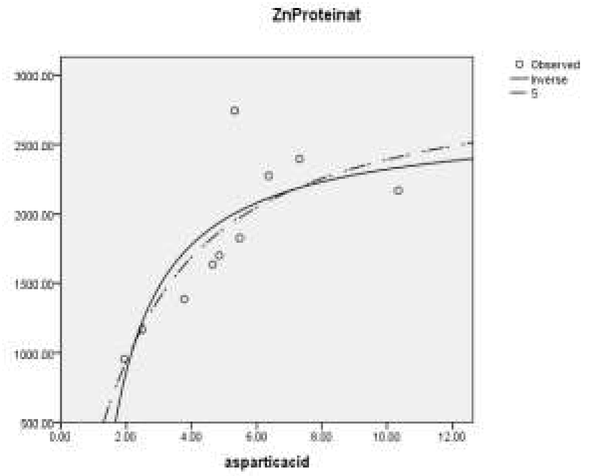

Figure 1. Non-linear regression model between Zn-proteinate and aspartic acid

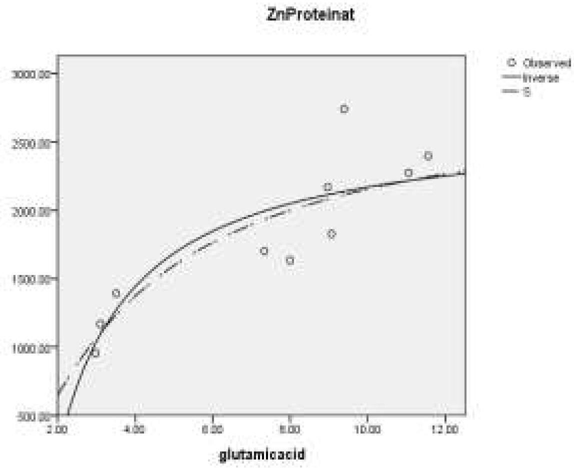

Figure 2. Non-linear regression model between Zn-proteinate and glutamic acid

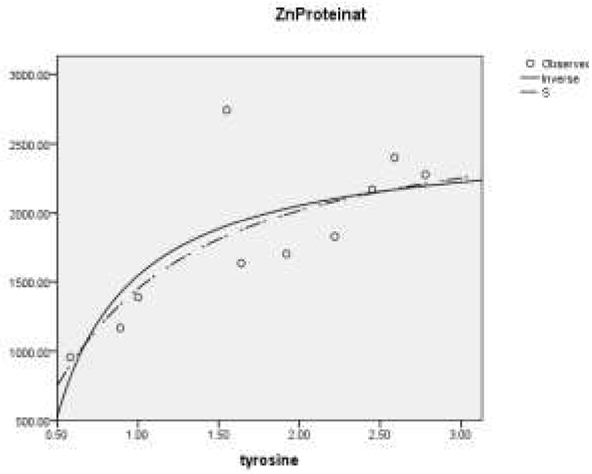

Figure 3. Non-linear regression model between Zn-proteinate and Arginine.

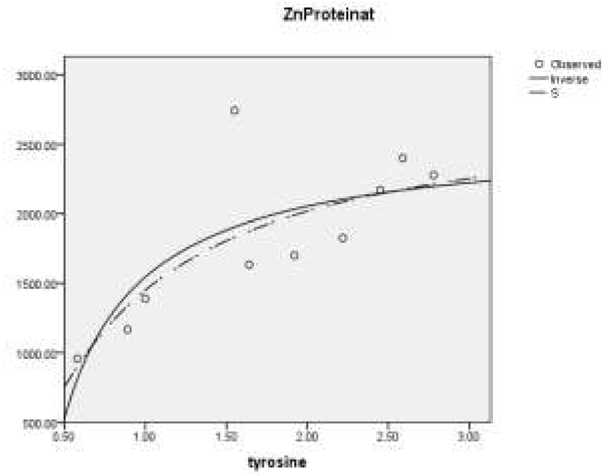

Figure 4. Non-linear regression model between Zn-proteinate and tyrosine

when compared with the results of research Muktiani et al. (2002) which uses basic ingredients cassava and microbes help Sac. serevciae, resulted in the incorporation rate of 73.83%. However, when seen from each feed, soybean meal Zn incorporation results unchanged at 73.71%.

Correlation Between Crude Protein Content and Zn Proteinatee. Significant correlation (P<0.001) was observed between protein content and Zn-proteinatee with a correlation coefficient of 0847 or 84.7%. The correlation coefficient demonstrated a strong positive relationship between the Zn-proteinatee and crude protein content. The coefficient of determination was equal to 0.718 or 71.8%. It showed that the variables would affect to the CP of 71.8% on the overall regression model of the Zn-proteinatee. The regression equation for protein Zn proteinatee was 241.04 + 31.35 CP. Accordingly, variable Zn proteinatee could be predicted by CP variables. This equation can be interpreted that 1% CP increase induced 31.35 ppm average Zn increase. Furthermore, no increase in the CP resulted in 241.04 ppm Zn average. This result clearly strengthened the hypothesis of previous research that the higher the protein content of feed ingredients, the more Zn-proteinaet bond might be synthesized. This result was in line with the results of Wang et al. (2010) that the majority (82%) Zn ions would bind to proteins bond to build tetrahedral form, and the rest was pentahedral and hexahedral. Feed ingredients derived protein sources from waste animal industry in general can form a Zn proteinatee higher than those of agricultural industrial waste, but the several leaf meal turned out to have the ability to bind Zn and efficiency are almost equivalent representing high of crude protein play a role in the proces of Zn binding protein. The differences that emerged allegedly caused by differences in the amino acid content of each ingredient as the main constituent proteins.

Correlation Between Zn Proteinatee and Amino Acid Ingredient Feeds. Analysis of amino acid levels was performed on 10 feed ingredients that produce the highest levels of Zn proteinatee namely soybean meal, feather meal, PMM, blood meal, fish meal, shrimp head meal, MBM, leucaena leaf meal, cassava leaves flour and tofu waste. Amino acid content of ten feed ingredients shown in Table 3. The Kolmogorov-Smirnov test of normality showed that the data of all the variables have a normal distribution, thus the Pearson correlation can be used in the correlation analysis. Bivariate correlation test resulted 0.726; 0.888; 0.749; and 0.746 for aspartic acid, glutamic acid, arginine and tyrosine, respectively, indicating significant correlation to Zn proteinatee.

It was in line with Wang et al. (2010) that the most common amino acids found in protein binding to Zn were Cysteine, histidine, glutamate, tyrosine and arginine. This study reported a new finding of a positive correlation with other amino acids namely aspartic acid which can be used as a basis for predicting the form of bonds or other types of metaloenzym Zn in the body.

Regression analysis showed a correlation coefficient of 96.3% and the coefficient of determination of 92.8%. Analysis of variance also showed that the overall model was significant ($P<0.01$). However, the model did not meet the Goodness of fit model assumptions as a requirement in the multiple linear regression analysis. The results revealed a problem of multicollinearity and auto correlation in the model. High correlation was found among the independent variables namely aspartic acid, glutamic acid, arginine and tyrosine.

Violation against Goodness of fit was usually treated by omitting the variable with high correlation. Since all of the independent variables in this study were highly correlated, the above option was not possible. Therefore, a regression analysis was performed by simple linear regression between Zn-proteinatee with each of the aforementioned amino acid. However, based on the scatter plot between Zn-proteinate with each amino acid there was a possibility of non-linear regression models, then by using the method of curve estimation result the most significant regression model was a model S that produced a coefficient of determination higher than the linear model. Regression analysis non-linear regression model of S was therefore applied to explain the correlation between Zn-proteinate with aspartic acid, glutamic acid, arginine and tyrosine.

Non-linear Regression Between Zn-Proteinate With Aspartate Acid, Glutamic Acid, Arginine And Tyrosine. The non-linear regression model between Zn-proteinate with aspartic acid, glutamate acid, arginine and tyrosine showed that the correlation coefficient for aspartic acid, glutamate acid, arginine and tyrosine were 0.896; 0.907; 0.881 and 0.862 respectively. A strong positive relationship was presumable between the amino acids of the Zn-proteinate. By increasing the amino acids of 1% will increase the amount of Zn-proteinate. The coefficients of determination were 0.803; 0.823; 0.775 and 0774 respectively. The values stated that the amino acids affect of 80.3%; 82.3%; 77.5% and 77.4% in the overall regression model between the amino acid with Zn-proteinate. The rest of the model was influenced by other factors did not examine in this study. Thus in this model, the variable Zn-proteinate was strongly predicted by amino acids variables. Analysis of variance showed that the overall regression model of aspartic acid, glutamate acid, arginine, and tyrosine to Zn-proteinate and outputs (F test) overall regression model generated from those relationships was significant at 1% level. Furthermore, the regression model is described in Figure 1, 2, 3 and 4.

Conclusion

The level 0.02% *Curcuma zedoaria* and 0.03% *Cuminum cyminum* on concentrate improved good condition of rumen ecology by increasing rumen bacteria (50.53 and 118.84%) and total/partial VFA (68.53 - 70.41%) and undetecting Pb in rumen.

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