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Comment dari Redaksi JAP :

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

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ABSTRACT

The aims 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 a zinc and find out the type of amino acids that contribute to the binding of Zn. Various waste of agriculture industries (soybean meal, catton seed meal, coconut meal, palm meal, distillers dried grains with solubles (DDGS), soy sauce waste and tofu waste), and various kind of waste animal (blood meal, feathers meal, fish meal, poultry meat meal (PMM) and meat bone meal (MBM), head shrimp flour) and various flour derived from leaves (cassava, sesbania glandiflora, leucaenia, gliricidia, calliandra, paraserianthes) have been use in this research. Zinc oxide (ZnO) has been used as Zn^{2+} ion source. Nitrogen content of all feed ingridient was analyzed using Kejhdahl than the obtained data would be used to calculate to addition of Zn oxcide. The final ratio of N:Zn was 10 : 1 was then dipped in water for 24 hours to measure Zn proteinate. The correlation between Zn-proteinate and the content of protein and amino acids of feeds ingredient was calculated statistically using regression analyzed method. The amount of Zn proteinate has been increased along with the increase in crude protein percentage of feed ingredients. Aspartic acid, glutamic acid, arginine and tyrosine showed a strong positive correlation to Zn-proteinate. This result may provide alternative method to produce Zn proteinate from feeds ingrediens.

Keywords : Zn proteinate, amino acid, corelation, feed ingredients.

INTRODUCTION

Mineral Zinc (Zn) has beeb known as an essential micromineral since 1934 (Fu-Yu et al., 2007). Zn minerals in the body functions very broad include the activities of 200 enzymes, either as a catalyst, keeping the stability of the structure of proteins and plays a role in the metabolism of nutrients (McCall et al., 2000). It also plays a role 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 that are in a period of lactation or fattening , because the needs of Zn in this phase is very high.

Zn mineral supplementation would be more efficient when administered in the form of organic minerals, namely in the form of Zn proteinate (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 bond that is most common is the tetrahedral bonding where Zn ions bind to three or four side chains of proteins. The majority of Zn ion (82%) and proteins bind to form tetrahidral, and the rest is shaped pentahidral and heksahidral (Wang et al., 2010). Amino acids are most commonly found in proteins with Zn bond is Cysteine, histidine, glutamate, tyrosine and arginine. Under these conditions, in the manufacture of organic minerals Zn proteinat 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-proteinate 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 find the type of amino acids that contribute to the binding of Zn.

MATERIALS AND METHODS

Materials

Nineteen feedstuffs used as a source of protein for binding Zn^{2+} ions in the manufacture of Zn-proteinate. Various waste of agriculture industries (soybean meal, kapok seed cake, coconut cake, coconut cake oil, distillers dried grains with solubles (DDGS), soy sauce and tofu dregs), and various kind of waste animal (blood meal, flour feathers, fish meal, poultry meat meal (PMM)

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and meat bone meal (MBM), shrimp head flour) and various flour derived from leaves (cassava, sesbania glandiflora, leucaenia, gliricidia, calliandra, paraserianthes). Zn^{2+} ion source used is zinc oxide (ZnO).

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Zn proteinat synthesis method

All material feed protein sources proximate analysis to determine levels of protein (N) and the levels of Zinc (Zn). N concentration is calculated by multiplying the protein content by 16%. Furthermore, in each of the feed material is added ZnO mineral (inorganic) with a ratio of N: Zn = 10: 1 and added water until all ingredients are submerged feed. The solution was left to stand for 24 hours while stirring every 3 hours so that the binding reaction between the protein and Zn^{2+} ions. Furthermore proteinat Zn product was dried at 60°C (Muktiani, 2002).

Chemical analysis

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

Statiticals analysis

We used the SPSS16.0 statistical software package to perform Pearson correlation coefficient (two-tail) and regression analysis. The correlation between Zn-proteinate and the content of protein and amino acids of feeds ingredient was calculated statistically using these regression analyzed method. Levels of Zn-proteinat obtained was then used to calculate the level of Zn incorporation efficiency using the ratio of Zn content in the Zn proteinat 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 less then 20% (Kawas et al., 2012). However, as the protein level in feed ingreden is quitely high, those feed ingredient would be fit for use as a source of zinc ion binding protein.

Table 1. Dry Matter (DM), Crude Protein(CP), and Zink (Zn) Level in Feed Material Research

No.	Feed	DM (%)	CP (%)	Zn Content (ppm)
1	Soybean meal	88.29	43,76	17,95
2	Cotton seed meal	88.82	27,12	26,40
3	Coconut meal	91.59	18,35	23,20
4	Palm meal	93.62	16,29	24,00
5	DDGS	90.97	18,04	21,80
6	Soy sauce waste	90.57	20,81	18,00
7	Tofu waste	10.17	18,66	146,05
8	Blood meal	86.69	81,32	17,20
9	Feather meal	32.60	63,23	652,25
10	Fish meal	90.43	57,16	161,05
11	Head shrimp flour	29.9	40,06	155,15
12	PMM	91.76	56,48	117,40
13	MBM	94.62	46,93	79,70
14	Cassava leaf flour	31.48	26,27	67,45
15	Sesbania leaf flour	20.00	26,64	15,85
16	Leucaenia leaf flour	24.96	26,34	16,70
17	Gliricidea leaf flour	23.27	21,10	16,65
18	Calliandra leaf flour	24.31	23,80	13,15
19	Paraserianthes leaf flour	35.79	17,97	8,45

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

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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 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 proteinate content in the product.

Zn proteinate content in feed ingredients and Zn incorporation efficiency levels are presented in Table 2. Four ingredient feed that have highest Zn proteinate content were soybean meal 3493.93 ppm, feather meal 3,228, 35 ppm, PMM 3.158.49 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 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%.

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

No	Feed	Zn-proteinat Content (ppm)	Zn incorporation efficiency (%)
1	Soybean meal	2742,80	73.71
2	Cotton seed meal	733,31	30.28
3	Coconut meal	619,65	37.01

4	Palm meal	646,30	49.89
5	DDGS	573,78	36.84
6	Soy sauce waste	824,61	47.99
7	Tofu waste	955,14	57.00
8	Blood meal	2169,92	42.23
9	Feather meal	2276,56	53.46
10	Fish meal	1826,83	40.93
11	Head shrimp flour	1700,04	51.30
12	PMM	2399,25	51.31
13	MBM	1634,51	41.82
14	Cassava leaf flour	1165,76	56.07
15	Sesbania leaf flour	1121,15	49.09
16	Leucaena leaf flour	1389,07	64.60
17	Gliricidea leaf flour	941,40	56.99
18	Calliandra leaf flour	609,32	32.82
19	Paraserianthes leaf flour	585,28	39.97
	Average		47.87

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

Correlation between crude protein content and Zn proteinate

We found there was a significant correlation between protein content and Zn-proteinat ($r = 0.847$, $P < 0.001$, $n = 19$). The correlation coefficient stated that there was a strong positive relationship between the Zn-proteinate 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-proteinate. The regression equation for protein Zn proteinate was 241.04 + 31.35 CP. Based on this model, it can be said that the variable Zn proteinate could be predicted by CP variables. This equation can be interpreted as if the CP increased by 1% then the average Zn will increase by 31.35 ppm. Further statement, that if there was no increase in the CP, the average of Zn would be 241.04 ppm. This result clearly strengthen the hypothesis of previous research stated that the higher the protein content of feed ingredients, the more amount of Zn-proteinat bond might be syntezized. This result was in line with the results of Wang et al. (2010) which stated that the majority (82%) Zn ions will bind to proteins bond to build tetrahedral

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form, and the rest is pentahidral and heksahidral. Feed ingredients derived protein sources from waste animal industry in general can form a Zn proteinate 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 proteinate and amino acid ingredient feeds

Analysis of amino acid levels was performed on 10 feed ingredients that produce the highest levels of Zn proteinate 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 proteinate.

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

No	Feed	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Met	Val	Phe	Ile	Leu	Lys
1	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
2	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
3	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
4	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
5	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

6	Head shrimp 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
7	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
8	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
9	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
10	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

Information :

DDGS = *Distillers dried grains with solubles*; PMM = *Poultry meat meal ;* MBM = *Meat bone meal*
Zn-P = Zn Proteinate, 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.

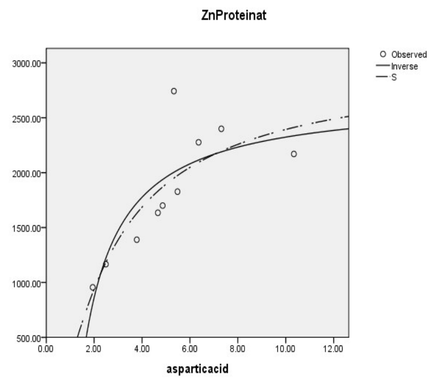
The results was in line with the results of Wang et al. (2010) who found that the most common amino acid found in protein binding to Zn were Cysteine, histidine, glutamate, tyrosine and arginine. This study found a new finding of a positive correlation with other amino acids namely aspartic acid. This new finding 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 fulfill the Goodness of fit model assumptions as a requirement in the multiple linear regression analysis. The results showed that there was a problem of multicollinearity and auto correlation in the model. Among the independent variables namely aspartic acid, glutamic acid, arginine and tyrosine found a high correlation.

Violation against Goodness of fit usually was treated by omitting the variable with high correlation. Due to all of the independent variables in this study were highly correlated then the above option was not possible. Therefore, a regression analysis was performed simple linear regression between Zn-proteinat with each amino acid above. However, based on the scatter plot between Zn-proteinat 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 produce a coefficient of determination higher than the linear model. Based on this finding we used regression analysis non-linear regression model of S to explain the correlation between Zn-proteinat with aspartic acid, glutamic acid, arginine and tyrosine.

Non-linear regression between Zn-Proteinat with aspartate acid, glutamic acid, arginine and tyrosine

The non-linear regression model between Zn-proteinat 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. It can be interpreted that there is a strong positive relationship between the amino acids of the Zn-proteinat. By increasing the amino acids of 1% will increase the amount of Zn-proteinat. 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-proteinat. The rest of the model was influenced by other factors did not examine in this study. Thus in this model, the variable Zn- proteinat 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-proteinat and outputs (F test) overall regression model generated from those relationships was significant at the 1% level. Furthermore, the regression model is described as Figure 1,2,3 and 4.



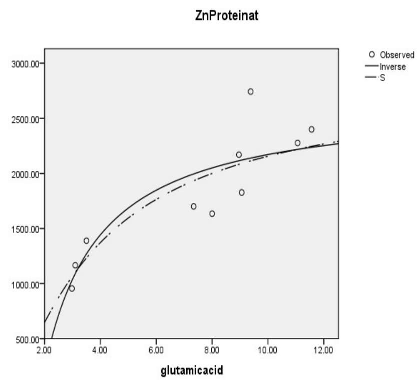
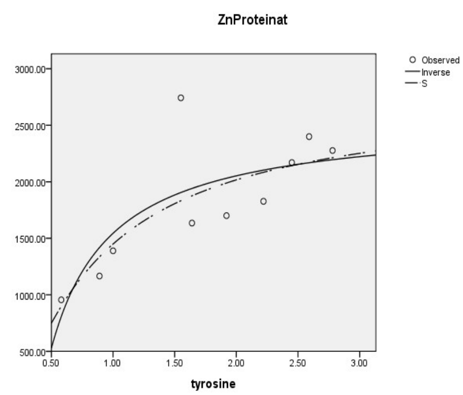


Figure 1. Non-linear regression model between Zn-proteinat and aspartic acid.

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



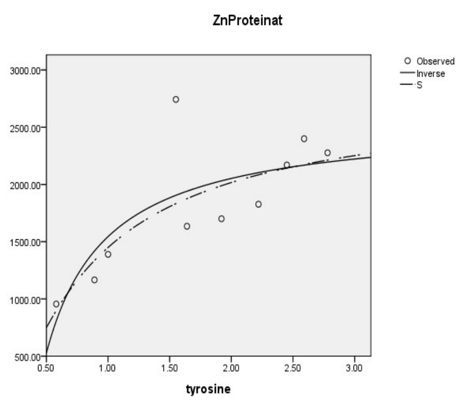


Figure 3. Non-linear regression model between Zn-proteinat and Arginine.

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

CONCLUTION

The result of the present study indicated a positive correlation between crude protein and amino acids with incorporation of Zn. Four amino acids showed a strong positive correlation with Zn-proteinat content namely aspartic acid, glutamic acid, arginine and tyrosine. Feed ingredients such as protein sources from plants, can be used as raw material for the synthesis of organic minerals Zn-proteinat , as well as a protein source from animal.

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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. Various waste of agriculture industries (soybean meal, catton seed meal, coconut meal, palm meal, distillers dried grains with solubles (DDGS), soy sauce waste and tofu waste), and various kind of animal waste (blood meal, feathers meal, fish meal, poultry meat meal (PMM) and meat bone meal (MBM), head shrimp flour) and various flour derived from leaves (cassava, sesbania glandiflora, leucaenia, gliricidia, calliandra, paraserianthes) were used. Zinc oxide (ZnO) served as Zn^{2+} ion source. Nitrogen content of all feed ingridient was analyzed using Kejhdahl than the obtained data would be used to calculate to addition of Zn oxcide. The final ratio of N:Zn was 10 : 1 was then dipped in water for 24 hours to measure Zn proteinate. The correlation between Zn-proteinate and the content of protein and amino acids of feeds ingredient was calculated statistically using regression analyzed method. The amount of Zn proteinate has been increased along with the increase in crude protein percentage of feed ingredients. Aspartic acid, glutamic acid, arginine and tyrosine showed a strong positive correlation to Zn-proteinate. This result may provide alternative method to produce Zn proteinate from feeds ingrediens.

Keywords : Zn proteinate, 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-proteinat. 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 dengan menambahkan setengah bagian onggok sebagai filler. Selanjutnya sampel dianalisis menggunakan *atomic absorbtion spectrophotometer* (AAS) terhadap kadar Zn yang terikat di dalam protein bahan pakan (Zn-proteinat) dengan cara mengendapkan protein terlebih dahulu menggunakan TCA 20%. Pada percobaan Tahap II dipilih 10 bahan pakan yang mempunyai kadar Zn-proteinat tertinggi untuk dianalisis kadar asam aminonya. Uji regresi dilakukan untuk mengetahui korelasi antara kadar protein bahan pakan dengan kadar Zn-proteinat yang dihasilkan. Selanjutnya uji regresi asam amino terhadap kadar Zn-proteinat juga dilakukan untuk menjelaskan fenomena ikatan yang terjadi.

Hasil penelitian mendapatkan bahwa rata-rata efisiensi inkorporasi Zn-proteinat 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-proteinat). Hasil analisis regresi non linier asam amino terhadap kadar Zn-proteinat menunjukkan bahwa terdapat empat asam amino yang mempunyai korelasi positif dengan kadar Zn-proteinat 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-proteinat (inkorporasi Zn).

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

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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, keeping the stability of the structure of proteins and plays a role in the metabolism of nutrients (McCall et al., 2000). It also plays a role 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 that are in a period of lactation or fattening , because the needs of Zn in this phase is very high.

Zn mineral supplementation would be more efficient when administered in the form of organic minerals, namely in the form of Zn proteinate (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 bond that is most common is the tetrahedral bonding where Zn ions bind to three or four side chains of proteins. The majority of Zn ion (82%) and proteins bind to form tetrahidral, and the rest is shaped pentahidral and heksahidral (Wang et al., 2010). Amino acids are most commonly found in proteins with Zn bond is Cysteine, histidine, glutamate, tyrosine and arginine. Under these conditions, in the manufacture of organic minerals Zn proteinat 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-proteinate 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 find the type of amino acids that contribute to the binding of Zn.

MATERIALS AND METHODS

Materials

Nineteen feedstuffs used as a source of protein for binding Zn^{2+} ions in the manufacture of Zn-proteinate. Various waste of agriculture industries (soybean meal, kapok seed cake, coconut cake, coconut cake oil, distillers dried grains with solubles (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 proteinat synthesis method

All material feed protein sources proximate analysis to determine levels of protein (N) and the levels of Zinc (Zn). N concentration is calculated by multiplying the protein content by 16%. Furthermore, in each of the feed material is added ZnO mineral (inorganic) with a ratio of N: Zn = 10: 1 and added water until all ingredients are submerged feed. The solution was left to stand for 24 hours while stirring every 3 hours so that the binding reaction between the protein and Zn^{2+} ions. Furthermore proteinat Zn product was dried at 60°C (Muktiani, 2002).

Chemical analysis

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

Statiticals analysis

We used the SPSS16.0 statistical software package to perform Pearson correlation coefficient (two-tail) and regression analysis. The correlation between Zn-proteinate and the content of protein and amino acids of feeds ingredient was calculated statistically using these regression analyzed method. Levels of Zn-proteinat obtained was then used to calculate the level of Zn incorporation efficiency using the ratio of Zn content in the Zn proteinat 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 less then 20% (Kawas et al., 2012). However, as the protein level in feed ingreden is quitely high, those feed ingredient would be fit for use as a source of zinc ion binding protein.

Table 1. Dry Matter (DM), Crude Protein(CP), and Zink (Zn) Level in Feed Material Research

No.	Feed	DM (%)	CP (%)	Zn Content (ppm)
1	Soybean meal	88.29	43,76	17,95
2	Cotton seed meal	88.82	27,12	26,40
3	Coconut meal	91.59	18,35	23,20
4	Palm meal	93.62	16,29	24,00
5	DDGS	90.97	18,04	21,80
6	Soy sauce waste	90.57	20,81	18,00
7	Tofu waste	10.17	18,66	146,05
8	Blood meal	86.69	81,32	17,20
9	Feather meal	32.60	63,23	652,25
10	Fish meal	90.43	57,16	161,05
11	Head shrimp flour	29.9	40,06	155,15
12	PMM	91.76	56,48	117,40
13	MBM	94.62	46,93	79,70
14	Cassava leaf flour	31.48	26,27	67,45
15	Sesbania leaf flour	20.00	26,64	15,85
16	Leucaenia leaf flour	24.96	26,34	16,70
17	Gliricidea leaf flour	23.27	21,10	16,65
18	Calliandra leaf flour	24.31	23,80	13,15
19	Paraserianthes leaf flour	35.79	17,97	8,45

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

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 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 proteinate content in the product.

Zn proteinate content in feed ingredients and Zn incorporation efficiency levels are presented in Table 2. Four ingredient feed that have highest Zn proteinate content were soybean meal 3493.93 ppm, feather meal 3,228, 35 ppm, PMM 3.158.49 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 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%.

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

No	Feed	Zn-proteinat Content (ppm)	Zn incorporation efficiency (%)
1	Soybean meal	2742,80	73.71
2	Cotton seed meal	733,31	30.28
3	Coconut meal	619,65	37.01
4	Palm meal	646,30	49.89
5	DDGS	573,78	36.84
6	Soy sauce waste	824,61	47.99
7	Tofu waste	955,14	57.00
8	Blood meal	2169,92	42.23
9	Feather meal	2276,56	53.46
10	Fish meal	1826,83	40.93
11	Head shrimp flour	1700,04	51.30
12	PMM	2399,25	51.31
13	MBM	1634,51	41.82
14	Cassava leaf flour	1165,76	56.07
15	Sesbania leaf flour	1121,15	49.09
16	Leucaena leaf flour	1389,07	64.60
17	Gliricidea leaf flour	941,40	56.99
18	Calliandra leaf flour	609,32	32.82
19	Paraserianthes leaf flour	585,28	39.97
	Average		47.87

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

Correlation between crude protein content and Zn proteinate

We found there was a significant correlation (P<0.001) between protein content and Zn-proteinat with a correlation coefficient of 0847 or 84.7%. The correlation coefficient stated that there was a strong positive relationship between the Zn-proteinate 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-proteinate. The regression equation for protein Zn proteinate was 241.04 + 31.35 CP. Based on this model, it can be said that the variable Zn proteinate could be predicted by CP variables. This equation can be interpreted as if the CP increased by 1% then the average Zn will increase by 31.35 ppm. Further statement, that if there was no increase in the CP, the average of Zn would be 241.04 ppm. This result clearly strengthen the hypothesis of previous research stated that the higher the protein content of feed ingredients, the more amount of Zn-proteinat bond might be syntezized. This result was in line with the results of Wang et al. (2010) which stated that the majority (82%) Zn ions will bind to proteins bond to build tetrahedral form, and the rest is pentahidral and heksahidral. Feed ingredients derived protein sources from waste animal industry in general can form a Zn proteinate 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 proteinate and amino acid ingredient feeds

Analysis of amino acid levels was performed on 10 feed ingredients that produce the highest levels of Zn proteinate 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 proteinate.

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

No	Feed	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Met	Val	Phe	Ile	Leu	Lys
1	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
2	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
3	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
4	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
5	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
6	Head shrimp 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
7	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
8	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
9	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
10	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

Information :

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

Zn-P = Zn Proteinate, 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.

The results was in line with the results of Wang et al. (2010) who found that the most common amino acid found in protein binding to Zn were Cysteine, histidine, glutamate, tyrosine and arginine. This study found a new finding of a positive correlation with other amino acids namely aspartic acid. This new finding 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 fulfill the Goodness of fit model assumptions as a requirement in the multiple linear regression analysis. The results showed that there was a problem of multicollinearity and auto correlation in the model. Among the independent variables namely aspartic acid, glutamic acid, arginine and tyrosine found a high correlation.

Violation against Goodness of fit usually was treated by omitting the variable with high correlation. Due to all of the independent variables in this study were highly correlated then the above option was not possible. Therefore, a regression analysis was performed simple linear regression between Zn-proteinat with each amino acid above. However, based on the scatter plot between Zn-proteinat 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 produce a coefficient of determination higher than the linear model. Based on this finding we used regression analysis non-linear regression model of S to explain the correlation between Zn-proteinat with aspartic acid, glutamic acid, arginine and tyrosine.

Non-linear regression between Zn-Proteinat with aspartate acid, glutamic acid, arginine and tyrosine

The non-linear regression model between Zn-proteinat 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. It can be interpreted that there is a strong positive relationship between the amino acids of the Zn-proteinat. By increasing the amino acids of 1% will increase the amount of Zn-proteinat. 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-proteinat. The rest of the model was influenced by other factors did not examine in this study. Thus in this model, the variable Zn- proteinat 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-proteinat and outputs (F test) overall regression model generated from those relationships was significant at the 1% level. Furthermore, the regression model is described as Figure 1,2,3 and 4.

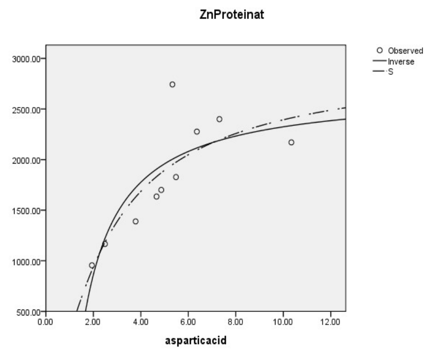

Figure 1. Non-linear regression model between Zn-proteinat and aspartic acid.

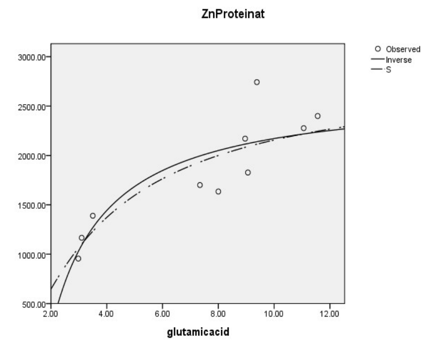

Figure 2. Non-linear regression model between Zn-proteinat and glutamic acid.

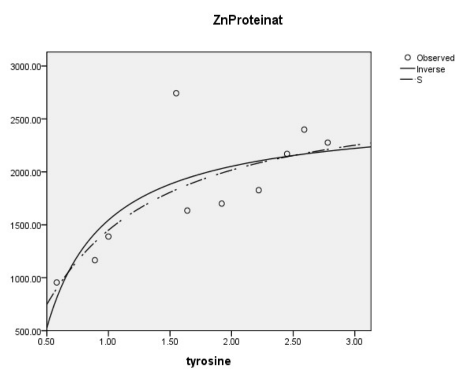

Figure 3. Non-linear regression model between Zn-proteinat and Arginine.

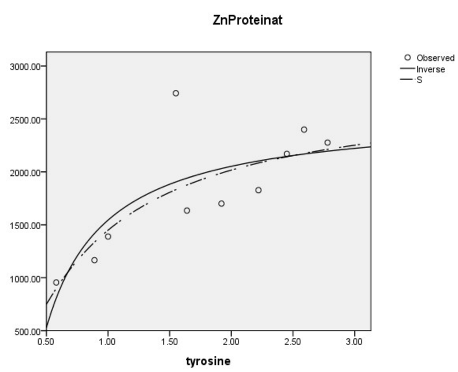

Figure 4. Non-linear regression model between Zn-proteinat and tyrosine

CONCLUSION

The result of the present study indicated a positive correlation between crude protein and amino acids with incorporation of Zn. Four amino acids showed a strong positive correlation with Zn-proteinat content namely aspartic acid, glutamic acid, arginine and tyrosine. Feed ingredients such as protein sources from plants, can be used as raw material for the synthesis of organic minerals Zn-proteinat , as well as a protein source from animal.

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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 as a protein sorrce used in the experiment. 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. 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 was soybean meal (73.71%). There was 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). The feedstuffs such as protein sources from plants, can be used as raw material for the synthesis of organic minerals Zn-proteinat , as well as a protein source from animal.

Keywords : Zn proteinate, nitrogen, amino acid, corelation.

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 sumber protein digunakan dalam percobaan ini. Analisis Kejhdahl dilakukan untuk mengetahui kadar protein dan kadar Nitrogen. Bahan-bahan tersebut kemudian 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). Bahan pakan sumber protein dari tanaman dapat digunakan untuk memsintesis Zn-proteinat sama baiknya dengan bahan pakan sumber protein dari hewan.

Kata kunci : Zinc- proteinat, nitrogen, asam amino, korelasi.

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 proteinate (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-proteinate 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-proteinate. 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 waste, 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, leucaena, gliricidia, calliandra, paraserianthes). Zn^{2+} ion source used is zinc oxide (ZnO).

Zn proteinate synthesis method

All material feed protein sources were 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 proteinate further was analyzed for its Zn content to determine the level of incorporation of Zn in protein feedstuffs. Zn proteinate 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

SPSS16.0 statistical software package was applied to perform Pearson correlation coefficient (two-tail) and regression analysis. The correlation between Zn-proteinate 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 waste 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 ingredient was quite high, those feed ingredients would be potential source of zinc ion binding protein.

Table 1. Dry Matter (DM), Crude Protein(CP), and Zink (Zn) Level in Feed Material Research

No.	Feed	DM (%)	CP (%)	Zn Content (ppm)
1	Soybean meal	88.29	43,76	17,95
2	Cotton seed meal	88.82	27,12	26,40
3	Coconut meal	91.59	18,35	23,20
4	Palm meal	93.62	16,29	24,00
5	DDGS	90.97	18,04	21,80
6	Soy sauce waste	90.57	20,81	18,00
7	Tofu waste	10.17	18,66	146,05
8	Blood meal	86.69	81,32	17,20
9	Feather meal	32.60	63,23	652,25
10	Fish meal	90.43	57,16	161,05
11	Shrimp head flour	29.9	40,06	155,15
12	PMM	91.76	56,48	117,40
13	MBM	94.62	46,93	79,70
14	Cassava leaf flour	31.48	26,27	67,45
15	Sesbania leaf flour	20.00	26,64	15,85
16	Leucaenia leaf flour	24.96	26,34	16,70
17	Gliricidea leaf flour	23.27	21,10	16,65
18	Calliandra leaf flour	24.31	23,80	13,15
19	Paraserianthes leaf flour	35.79	17,97	8,45

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

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

Zn proteinate content in the product.

Zn proteinate content in feed ingredients and Zn incorporation efficiency levels are presented in Table 2. Four ingredient feed that have highest Zn proteinate content were soybean meal 3493.93 ppm, feather meal 3,228, 35 ppm, PMM 3.158.49 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 when compared with the results of research Muktiani et al. (2002) which uses basic ingredients cassava and fermentation mediated by S. cereviceae, resulted in the incorporation rate of 73.83%. However, when seen from each feed, soybean meal Zn incorporation results unchanged at 73.71%.

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

No	Feed	Zn-proteinate Content (ppm)	Zn incorporation efficiency (%)
1	Soybean meal	2742,80	73.71
2	Cotton seed meal	733,31	30.28
3	Coconut meal	619,65	37.01
4	Palm meal	646,30	49.89
5	DDGS	573,78	36.84
6	Soy sauce waste	824,61	47.99
7	Tofu waste	955,14	57.00
8	Blood meal	2169,92	42.23
9	Feather meal	2276,56	53.46
10	Fish meal	1826,83	40.93
11	Shrimp head flour	1700,04	51.30
12	PMM	2399,25	51.31
13	MBM	1634,51	41.82
14	Cassava leaf flour	1165,76	56.07
15	Sesbania leaf flour	1121,15	49.09
16	Leucaena leaf flour	1389,07	64.60
17	Gliricidea leaf flour	941,40	56.99
18	Calliandra leaf flour	609,32	32.82
19	Paraserianthes leaf flour	585,28	39.97

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

Correlation between crude protein content and Zn proteinate

Significant correlation (P<0.001) was observed between protein content and Zn-proteinate with a correlation coefficient of 0.847 or 84.7%. The correlation coefficient demonstrated a strong positive relationship between the Zn-proteinate 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-proteinate. The regression equation for protein Zn proteinate (Y) was 241.04 + 31.35 CP (X). Accordingly, variable Zn proteinate 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-proteinate 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 proteinate 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 process of Zn binding protein. The differences that allegedly caused by differences of the amino acid content of each ingredient as the main constituent proteins.

Correlation between Zn proteinate and amino acid ingredient feeds

Analysis of amino acid levels was performed on 10 feed ingredients that produce the highest levels of Zn proteinate 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 proteinate.

Table 3. Amino acid content of feed ingredients.

No	Feed	Asp	Glu	Ser	His	Gly	Thr	Arg	Ala	Tyr	Met	Val	Phe	Ile	Leu	Lys
1	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
2	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
3	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
4	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
5	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
6	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
7	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
8	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
9	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
10	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

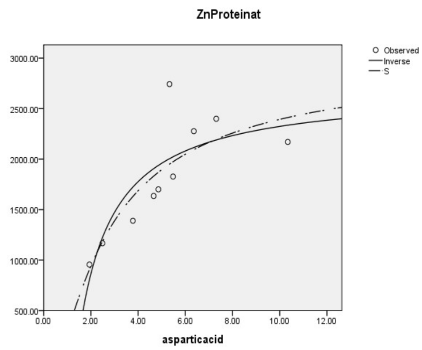
Information :

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

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 of Goodness of fit model 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-proteinate with each of 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.



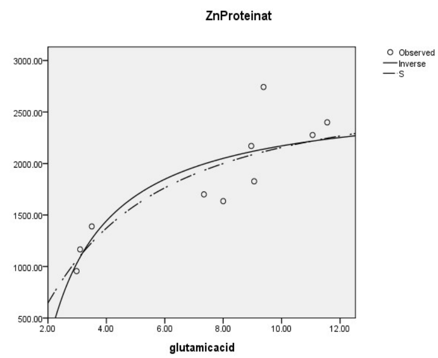
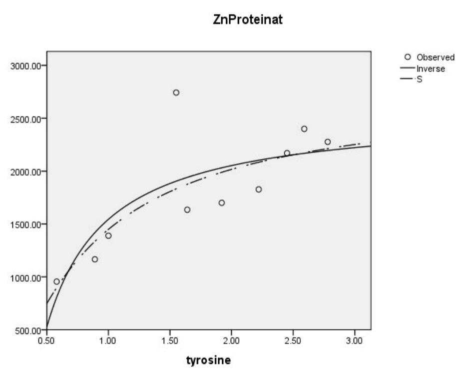


Figure 1. Non-linear regression model between Zn-proteinate and aspartic acid.

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



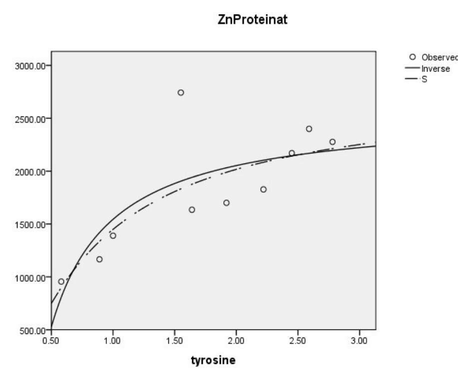


Figure 3. Non-linear regression model between Zn-proteinate and Arginine.

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

CONCLUSION

The result of the present study indicated a positive correlation between crude protein and amino acids with incorporation of Zn. Four amino acids showed a strong positive correlation with Zn-proteinat content namely aspartic acid, glutamic acid, arginine and tyrosine. Feed ingredients such as protein sources from plants, can be used as raw material for the synthesis of organic minerals Zn-proteinat , as well as a protein source from animal.

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Yth Anis Muktiani,

Dengan ini kami sampaikan Letter of Acceptance dari JAP atas artikel anda yang bejudul Correlation Protein and Amino Acid Content in Feed Ingredients with Zinc Binding Protein, untuk terbit di JAP Vol 16 (2) 2014. Terima kasih.

Salam,

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Penulis	Anis Muktiani dan Wahyu Dyah Prastiwi
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Telah diterima dan lolos proses review untuk diterbitkan di jurnal Animal Production. Artikel tersebut akan diterbitkan pada Volume 16 No 2 Tahun 2014.

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