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UTILIZATION OF LIQUID WASTE OF CHITIN EXTRACT FROM SKIN OF SHRIMP PRODUCTS OF CHEMICAL AND BIOLOGICAL PROCESSING AS FEED SUPLEMENT AND ITS IMPLICATION ON GROWTH OF BROILER

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Abstract

The aim of research for getting optimization of condition of process (doze of chemical or microbial and time of processing) on the stage of deproteination and demineralization as chemical and biological on protein and mineral liquefy from chitin extract. The product of chitin extract used as feed supplement for getting optimize level in ration on digestibility value and performance at broiler. The research conducted in three stages using experimental method at Laboratory. The first stage used Nested Design (3x3) consisted three replication. The second and third stage used Completely Randomized Design consisted eight treatments and four. Variables which examined in first stage were the contents of protein, calcium, and phosphor liquefy at liquid product of chitin extract; The second stage were digestibility of dry matter, protein, and organic matter; The third stage: consumption of ration, gain of body weight, and conversion of ration at broiler. The Results were analyzed by variance and deference of chitin extract of waste shrimp as biological through deproteination processed with Bacillus licheniformis at doze 4% time 48 hour, and followed demineralization with Aspergillus niger at doze 2% time 48 hour result the best of protein and mineral liquefy. Liquid product of chitin extract as biological can be feed supplement, and were used about 3% in ration at broiler for result optimized digestibility value and performance.

Key words*: Waste shrimp, chitin extract, digestibility, performance, broiler.*

INTRODUCTION

The ration is a determinant of growth, in addition to seeds and maintenance management. Optimality of broiler performance can be realized if given a quality ration that meets certain requirements in sufficient quantities. Fulfilling the need for food substances in the ration can be done by adding feed additives (feed supplement) in order to improve the quality and efficiency of the ration. One of them is the utilization of liquid waste of chitin extraction from chemical and biologically processed shrimp waste through the deproteination-demineralization stage.

Indonesia is one of the major shrimp producers in Asia. The shrimp production is mostly exported in the form of frozen headless and peeled. Waste from frozen shrimp processing is estimated to be about 60-70% by weight of shrimp (Krissetiana, 2004). Shrimp waste contains high enough protein and minerals, as well as astaxanthin which is a pro-vitamin A for color formation. The high content of protein and minerals to describe the potential of shrimp waste can be used as feed/affixed feed for poultry. The constraint is the presence of chitin, which causes proteins and minerals (in the form of calcium carbonate) bound, making it difficult to digest by poultry digestive enzymes, especially broiler chickens.

The chitin structure of shrimp waste is similar to that of cellulose, with the bonding occurring between the monomers coupled with glucoside at the position of β (1-4). The difference with cellulose is the hydroxyl group attached to the second carbon atom, replaced by the acetamide

group (-$NHCOCH_3$) in chitin so that chitin becomes an N-acetyl glucosamine-linked polymer. Chitin is a macromolecule and can decompose by chemical processes (strong and strong acid) or biologically (bio-degradable) mainly by microbes producing lysozyme and chitinase enzymes (Bising et al., 1995).

The process of shrimp waste treatment (chitin extraction from shrimp waste) can be done chemically through deproteination stages using strong bases and demineralization using strong acids. Chitin extraction from shrimp waste can also be carried out biologically, i.e. through a fermentation process by using microbes producing lysozyme enzyme and chitinase.

Some studies that have been carried out in chemical chitin extraction step from shrimp waste include deproteination by using strong base (Cira et al., 2000), then demineralization using a strong acid (Bisping et al., 2005). The chitinous extraction steps of chitin include deproteination using *Bacillus licheniformis* bacteria (Bisping et al., 2005); then demineralized by lactic acid fermentation by *Lactobacillus* sp. bacteria (Cira et al., 2000). Demineralization can also be done by using *Aspergillus niger* mold which has the ability to create an acidic atmosphere in the process of fermentation. But so far, liquid waste of chitin extraction from shrimp waste has not been studied and used for feed. The experiments designed in this study are intended to utilize liquid waste from the chitin extraction process which is then used as feed additives in broiler ration to improve the quality and benefit value, and the ration efficiency.

Several factors that influence the quality of the liquid product of chitin extraction is the way of the process stages is deproteination then proceed with demineralization and process conditions of each stage. Process conditions include the concentration of chemicals/microbes, processing time, temperature and pH. The chemical used at the deproteination stage is NaOH, and at demineralization stage is H_2SO_4. Microbes used in the deproteination stage are bacteria *B. licheniformis*, and at demineralization, stage using *Aspergillus niger* shell.

The product of chitin salt extract extraction chemically and biologically can be seen the value of its benefits when it is added to feed and biologically tested in broiler chickens because broiler chicken has fast-growing nature and is responsive to ration treatment. Therefore, to see the quality and value of feed additive benefits is measured through the digestibility value, and to see the efficiency added to broiler ration through measurement of performance (consumption of ration, weight gain, and ration conversion).

MATERIALS AND METHODS

First Stage Trial (Chitin Extraction)

The first phase of the experiment was to obtain optimization of chitin extracting process from chemical and biological shrimp waste through deproteination-demineralization process, and determination of process conditions at each stage (chemical or microbial dosage and processing time). Materials used in this study include NaOH, H_2SO_4, Isolate *B. licheniformis* and *Aspegillus nig*er. The main raw material is tiger shrimp waste consisting of skin and head. The tools used are a stenless jar, incubator, autoclave, bunsen burner, Petri dish, centrifuge, funnel, pH meter, spectrophotometer, test tube, and milling machine.

a. Deproteination stages

Chemical Process. Shrimp waste was washed and added 3%, 4%, and 5% potassium hydroxide (NaOH) solution, then boiled for 1 hour, 2 hours and 3 hours at 65^{o}C, then demineralization process.

Biological Process. The optimization experiments were performed with 3%, 4% and 5% doses of inoculum *B. licheniformis*, and the length of the process was 24 hours, 48 hours and 72 hours, which was done at 50^{0}C, then demineralized.

b. Demineralization Stages

Chemical Process. Deproteination product, added 1%, 2%, and 3% sulfate (H_2SO_4) solution, then boiled for 1 hour, 2 hours and 3 hours at 45^{0}C, then the separation between solid and liquid products. Liquid products analyzed the content of soluble proteins and minerals (calcium and phosphorus).

Biological Process. The optimization experiments were conducted at *Aspergillus niger* inoculum dose of 1%, 2%, and 3%, and the length of the process was 24 hours, 48 hours and 72 hours, at temperature 35^{0}C, the

separation of solid and liquid products. Furthermore, the optimum point is determined which produces the optimal dissolved protein and mineral content. The selected product is dried and used as a feed additive.

Experimental design

The first experimental experiments were carried out experimentally in the laboratory using the Authorized Design as much as (3x3) treatment and repeated 3 times. Treatment consists of factor A, an i.e. dose of chemicals or microbes (D1, D2, and D3), and factor B, i.e. chemical or biological process time (W1, W2, and W3). Factor B (time) is nested on factor A (dose). The observed variables are protein and mineral content (calcium and phosphorus) dissolved liquid chitin extraction products from shrimp waste. The data obtained from the observations were analyzed by the variance, and the differences between treatments with Duncan multiple range test (Steel and Torries, 1995).

Second Stage Trial (Determination of Digestibility Value)

Livestock used in this experiment is broiler final chicken strain Cobb stock. The number of chickens used as many as 32 birds aged 7 weeks. The enclosure used is 35 x 20 x 35 cm individual cage as many as 32 units. Treatment rations consist of:

1. R0 = Control rations, rations that do not contain feed suplement with 20% protein content and 3000 kcal/kg of energy.
2. R1 = 99% R0 + 1% of the chemical feed suplement product.
3. R2 = 98% R0 + 2% of the chemical feed suplement product.
4. R3 = 97% R0 + 3% of the chemical feed suplement product.
5. R4 = 99% R0 + 1% of the biological feed suplement product.
6. R5 = 98% R0 + 2% of the biological feed suplement product.
7. R6 = 97% R0 + 3% of the biological feed suplement product.
8. RS = Standard Rations, rations that do not contain feed suplement with 22% protein content and 3000 kcal/kg energy.

The feed ingredients of the ration composition consist of yellow corn, fine bran, soybean meal, coconut meal, fish meal, dicalcium phosphate, $CaCO_3$, coconut oil, premix, chemical feed additive and feed additive biological process.

Trial Procedure

Chickens are placed into individual enclosures, then fasted for 36 hours to remove the previous feed residue from the digestive tract. Feeding force-feeding rations, done in the form of a paste that is inserted into the esophagus as much as 150 grams per bird. Drinking water is given in *ad libitum*. After 14 hours of feeding, the chickens were slaughtered and their colon was expelled to obtain a fecal sample. The formula for obtaining digestibility (Schneider dan Flatt 1973; Ranjhan 1980):

$$\text{Digestibility}(\%) = 100\% - 100\left(\frac{\%\ \text{lignin ration}}{\%\ \text{lignin feces}} + \frac{\%\ \text{protein feces}}{\%\ \text{protein ration}}\right)$$

Experiments were performed experimentally in the laboratory, using Completely Randomized Design with 8 treatments of ration and each repeated 4 times. Differences between treatments performed Duncan Multiple Range Test.

Third Stage Trial (Trial Feeding Trial)

Chicken used is broiler age 1 day (DOC) Cobb strain of 160 birds without sex separator. The used cage measured 0.80 m x 0.70 m x 0.75 m, for every 5 chickens and amounted to 32 units. The ration consisted of control ration (protein 20% and metabolic energy 3000 kcal/kg) and rations with the addition of chemical and biological feed additive products, as well as standard rations (22% protein and 3000 kcal/metabolic energy).

The feed ingredients used and the treatment ration arrangement as in the digestion test. The variables observed were ration consumption, weight gain, and ration conversion.

RESULTS AND DISCUSSIONS

Processing of shrimp waste for feed additives can be done mechanically, chemically, or biologically. The purpose of processing is to extract certain substances, prevent decay, increase palatability and digestibility, which in turn can increase livestock productivity. Therefore, the processing of shrimp waste through chitin extraction, and its products used as feed additives.

Effect of treatment on protein and mineral contents dissolved chemical process products

The rate of protein content, calcium, and phosphorus dissolved in the chemical process product of chitin extraction from shrimp waste through deproteination -demineralization stage in each treatment was statistically analyzed through Random Variables, and the results showed that dose and time in dose were significantly different ($P<0.05$) protein content, calcium and phosphorus dissolved chemical chitin extraction process products from shrimp waste. To know the effect difference between treatments, Duncan Multiple Test Duncan was performed which results can be reviewed in Table 1.

Table 1. Duncan Multiple Range Test - influence of dosage on protein, calcium and phosphorus content of chemical process products dissolved

Treatment	The variables observed		
	Protein dissolved	Calcium dissolved	Phosphorus dissolved
	.....(%).....		
D1 (NaOH 3% + H_2SO_4 1%)	20.09 ^{A}	5.44 ^{A}	0.33 ^{A}
D2 (NaOH 4% + H_2SO_4 2%)	22.67 ^{B}	5.55 ^{B}	0.57 ^{B}
D3 (NaOH 5% + H_2SO_4 3%)	21.91 ^{B}	5.80 ^{C}	0.73 ^{C}

Table 1 shows that the soluble proteins in the treatment of D2 and D3 were not significantly different ($P>0.05$), but both were significantly higher ($P<0.05$) than the treatment D1. The content of calcium and soluble phosphorus in the treatment of D3 was significantly higher ($P<0.05$) than the treatment of D2 and D1, as did the actual treatment D2 ($P<0.05$) higher than the treatment D1. Furthermore, to find out how much influence the time in doses of protein content, calcium and dissolved phosphorus, Duncan test which results are presented in Table 2.

Table 2. Duncan Multiple Range Test - effect of time in dosage on protein, calcium, and phosphorus dissolved chemical process products

Treatment	The variables observed		
	Protein dissolved	Calcium dissolved	Phosphorus dissolved
	.....(%).....		
D3W1 (NaOH 5%, 1 hour + H_2SO_4 3%, 1 hour)	18.01 ^{A}	5.11 ^{A}	0.34 ^{A}
D3W2 (NaOH 5%, 2 hour + H_2SO_4 3%, 2 hour)	24.24 ^{B}	6.11 ^{B}	0.70 ^{B}
D3W3 (NaOH 5%, 3 hour + H_2SO_4 3%, 3 hour)	23.49 ^{B}	6.17 ^{B}	1.14 ^{C}

Table 2 shows that the proteins and calcium dissolved in the treatment of W2 and W3 were not significantly different ($P>0.05$), but both were significantly higher ($P<0.05$) than the W1 treatment. The dissolved phosphorus content in the W3 treatment was significantly higher ($P<0.05$) than the treatment of W2 and W1, as did the actual W2 treatment ($P<0.05$) higher than that of W1 treatment.

Deproteination and demineralization in the chitin extraction process are influenced by the concentration of the solution, temperature and reaction time. The more protein and mineral content released during the chitin extraction process is in line with increasing time, dose and concentration of base and acid used (Bastaman, 1989; Chatelet et al., 1991; Pomeranz, 1991; Benjakul and Sophanodora, 1993). Chemical treatments such as acid or base with higher doses accompanied by longer process/time can release or stretch protein and mineral bonds with chitin and other organic materials on

shrimp shells (Whittenbury et al., 1967; Johnson and Peterson, 1974; Lehninger, 1975; Fennema, 1985). However, warming for a long time can cause protein denaturation so that dissolved protein is reduced (Winarno, 1997).

Effect of treatment on protein and mineral content dissolved biological process products

The rate of protein content, calcium, and phosphorus dissolved in the biological process of chitin extraction from shrimp waste through deproteination-demineralization step in each treatment was statistically analyzed, and the results showed that dose and time in dose, significantly different (P<0.05) to protein content, calcium, and phosphorus dissolved biological products of chitin extraction process from shrimp waste. To know the effect difference between treatments, Duncan Multiple Range Test is used which results can be examined in Table 3.Table 3 shows that the proteins, calcium, and phosphorus dissolved in the treatment of D2 and D3 were not significantly different (P>0.05), but both were significantly higher (P<0.05) than the treatment D1. The table also shows that the higher the dose of inoculum used the greater the content of dissolved proteins and minerals.

Table 3. Duncan Multiple Range Test - influence of dosage on protein, calcium and phosphorus content of dissolved biological process products

Treatment	The variables observed		
	Protein dissolved	Calcium dissolved	Phosphorus dissolved
	.....(%).....		
D1 (*B. licheniformis* 3% + *A. niger* 1%)	20.60 ^{A}	6.27 ^{A}	1.20 ^{A}
D2 (*B. licheniformis* 4% + *A. niger* 2%)	31.73 ^{B}	7.03 ^{B}	1.48 ^{B}
D3 (*B. licheniformis* 5% + *A. niger* 3%)	32.36 ^{B}	7.42 ^{B}	1.51 ^{B}

Furthermore, to determine the effect of time in doses on protein content, calcium and dissolved phosphorus, Duncan test was performed which results are presented in Table 4.

Table 4. Duncan Multiple Duncan Test - effect of time in dosage on protein, calcium, and phosphorus dissolved biological process products

Treatment	The variables observed		
	Protein dissolved	Calcium dissolved	Phosphorus dissolved
	.....(%).....		
D2W1 (*B. licheniformis* 4%, 24 jam + *A. niger* 2%, 24 jam)	22.82 ^{A}	6.36 ^{A}	1.38 ^{A}
D2W2 (*B. licheniformis* 4%, 48 jam + *A. niger* 2%, 48 jam)	36.76 ^{B}	7.54 ^{B}	1.52 ^{B}
D2W3 (*B. licheniformis* 4%, 72 jam + *A. niger* 2%, 72 jam)	35.62 ^{B}	7.20 ^{B}	1.54 ^{B}

Table 4 shows that the proteins, calcium, and phosphorus dissolved in the treatment of W2 and W3 were not significantly different (P>0.05), but both were significantly higher (P<0.05) than the W1 treatment. Treatment of D2W2 (dose of *Bacillus licheniformis* by 4% for 48 hours, and 2% *Aspergillus niger* dose for 48 hours) resulted in optimum protein, calcium and phosphorus content on the chitin extraction of biological shrimp waste through deproteination-demineralization process stages. The content of protein and mineral products of chitin extraction from shrimp waste biologically will increase in line with the length of time of fermentation to a certain time limit then decline again (Sulaiman, 1988). Furthermore, Tanuwidjadja (1975) stated that too much microbial quantity can cause sporulation too fast, so some energy is not used to multiple cells, and vice versa, the number of microbes that are too little growth is not optimal.

Effect of treatment on digestibility

The potential nutritional value of chitin extraction products from shrimp waste can be determined by means of chemical analysis, i.e. proximate analysis. The true value is shown

from the missing part after the ingredients are ingested, absorbed and metabolized (Schneider and Flatt, 1973; Tillman et al., 1991).

The more food substances that can be absorbed by broiler chickens, the digestibility value of chitin extraction products from shrimp waste is higher. Average values of dry matter digestibility, crude protein and organic matter rations contained feed additives, analyzed statistically and the results are shown in Table 5.

Table 5. Meaning of Digestibility value of dry material, crude protein and organic ration material at each treatment

Treatment	The variables observed		
	Dry matter digestibility	Protein digestibility	Organic matter digestibility
	.....(%).....		
R0 (P 20%, ME 3000 kcal/kg; 0% feed suplement)	70.41 ^{E}	70.37 ^{D}	70.91 ^{E}
R1 (99% R0 + 1% chemical feed suplement)	73.29 ^{D}	74.49 ^{C}	72.88 ^{D}
R2(98% R0 + 2% % chemical feed suplement)	74.79 ^{CD}	76.45 ^{BC}	76.07 ^{C}
R3 (97% R0 + 3% % chemical feed suplement)	77.02 ^{BC}	78.09 ^{B}	78.10 ^{B}
R4 (99% R0 + 1% biological feed suplement)	74.04 ^{D}	75.41 ^{C}	75.36 ^{C}
R5 (98% R0 + 2% biological feed suplement)	77.83 ^{B}	78.83 ^{B}	78.48 ^{B}
R6 (97% R0 + 3% biological feed suplement)	80.82 ^{A}	81.52 ^{A}	82.03 ^{A}
RS (P 22%, ME 3000 kcal/kg; 0% feed suplement)	80.80 ^{A}	81.20 ^{A}	81.13 ^{A}

The use of feed ssuplement of biological process products of 3% (R6) in broiler chicken ration is equivalent to standard ration (RS) with a crude protein content of 22%, although at R6 only 20%.

While control rations (R0) containing 20% crude protein, very low digestibility value. Differences in digestibility are due to differrences in the nature of processed foods, including their suitability to be hydrolyzed by broiler digestive enzymes (Kompiang and Ilyas, 1983; Sukarsa et al., 1985; Wahju, 1997). The low digestibility value in R0 treatment is caused by the ration containing only 20% crude protein with the ME of 3000 kcal/kg, not enough support for broiler needs.

The ration with 97% R0 (CP 20%, ME 3000 kcal/kg) plus the added biological feed suplement products as much as 3%, support the nutritional needs for broiler chickens.

Feed ssuplement biological process products have well enough nutritional value such as proteins (amino acids) and minerals that have been dissolved so much easier to be absorbed and digested by broiler chickens.

Feed ssuplement biological process products have a better digestibility value than with the feed addition chemical process.

This is due to the biological process of material changes in the quality of the material caused by the fermentation process by microbes (*Bacillus licheniformis* and *Aspergillus niger*), resulting in chemical changes from one complex compound to a simpler and easier to digest compounds that give a positive effect on digestibility (Schneider and Flatt, 1975; Stanton and Yeoh, 1976; Gumbira, 1989). Other factors affecting digestibility are (1) the level of the proportion of the ingredients in the ration, (2) the chemical composition, (3) the ration protein level and (4) the minerals (Maynard 1979; Bautrif, 1990; Wahju 1997).

Effect of treatment on broiler chicken performances

The treatment of this experiment was the rate of use of feed ssuplement of chemical and biological process products of 1%, 2% and 3% in broiler rations, by measuring the consumption of rations, weight gain, and feed conversion. The data obtained were analyzed statistically and the results are shown in Table 6.

Table 6. Average ration consumption, weight gain and ration conversion on each treatment

Treatment	The variables observed		
	Ration Consumption	Weight Gain	Ration Conversion
	.....(g).....	.....(g).....	...(index)....
R0 (P 20%, ME 3000 kcal/kg; 0% feed suplement)	2353.00 ^{A}	1250.63 ^{E}	1.88 ^{C}
R1 (99% R0 + 1% chemical feed suplement)	2357.00 ^{A}	1276.63 ^{DE}	1.85 ^{C}
R2 (98% R0 + 2% % chemical feed suplement)	2373.00 ^{A}	1302.13 ^{DE}	1.82 ^{BC}
R3 (97% R0 + 3% % chemical feed suplement)	2385.00 ^{A}	1370.56 ^{BC}	1.74 ^{B}
R4 (99% R0 + 1% biological feed suplement)	2358.00 ^{A}	1303.31 ^{DE}	1.81 ^{BC}
R5 (98% R0 + 2% biological feed suplement)	2338.00 ^{A}	1335.25 ^{CD}	1.75 ^{B}
R6 (97% R0 + 3% biological feed suplement)	2339.00 ^{A}	1425.50 ^{AB}	1.64 ^{A}
RS (P 22%, ME 3000 kcal/kg; 0% feed suplement)	2372.00 ^{A}	1446.56 ^{A}	1.64 ^{A}

Table 6 shows that the treatment did not show a significant difference (P>0.01) to ration consumption, but there was a very significant difference (P<0.01) on weight gain and feed conversion. Similar consumption of rations indicates that the addition of feed suplement to broiler rations, both chemical and biological products, does not affect the consumption of rations. Ration consumption is strongly influenced by the palatability of the constituent feed ingredient.

The weight gain in the RS and R6 treatments did not show any significant difference (P>0.01), but both were significantly higher (P<0.01) than those treated with R0, R1, R2, R4 and R5, and between the treatment of R6 with R3 was not significantly different (P>0.01). Similarly, between treatments R5, R4, R3, R2 and R1, and between R2, R1 and R0 show no significant difference (P>0.01). The treatment of R0 was very significant (P<0.01) lower than the treatment of R3, R5, R6 and RS.

The weight gain of broiler chickens increased with the addition of feed additives of chemical process products by 3%, and for biological process products with 2% addition have shown weight gain. The use of feed additives of biological process products as much as 3% in ration (R6) is equivalent to standard ration (RS) containing 22% protein, although R6 ration contains only 20% protein. This is because R6 rations undergo chemical changes from one complex compound to simpler and easily digestible compounds caused by microbial activity so as to have a positive effect on the growth of broiler chickens (Schneider and Flat, 1975; Stanton and Yeoh, 1976).

The conversion value of ration on RS and R6 treatment did not show any significant difference (P>0.01), but both were very significantly (P<0.01) lower than those of R0, R1, R2, R3, R4, and R5. The treatment of R0 was very significant (P<0.01) higher than that of the other treatments. The conversion value of broiler rations decreased with the addition of feed additives of chemical process products by 3%, and for biological process products with 2% addition have shown a decrease in conversion value of rations. The use of feed additives of biological process products of 3% in ration (R6) is equivalent to standard ration (RS) containing 22% protein, although R6 ration contains only 20% protein. Decreasing the conversion value of the ration signifies an increase in the biological value, thus impacting on the growth and efficiency improvement of the ration. It is known that the consumption of rations in each treatment is almost the same, but along with the addition of feed suplement, there is an increase in growth, especially on R6 which is almost the same as standard rations. In line with the opinions of Winarno (1980) and Gumbira (1989), the biological processing can transform an organic material into another useful product and have better-added value. Products that can be produced by a biological process are microbial cells or biomass,

enzymes, primary metabolites and secondary metabolites as well as chemical compounds produced by microbes (Ansori, 1989). Thus, the low conversion value of the ration reflected in increased growth signifies the high quality and efficiency of the ration (Bautrif, 1990), as occurs in R6 rations (addition of 3% of biological process feed additives by *B. licheniformis* and *A. niger*).

CONCLUSIONS

The biological waste chitin salt extraction through the deproteination process by *Bacillus licheniformis* at 4% dose for 48 hours, followed by demineralization by *Aspergillus niger* at 2% dose for 48 hours resulted in the best soluble protein and mineral content. Liquid product of chitin extraction from shrimp waste biologically can be used as a feed suplement in broiler chicken ration. The addition of feed suplements into the basal ration (CP 20%, ME 3000 kcal/kg) of 3%, yielding the optimal digestibility and performance value in broiler chickens.

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