The Solubility of Cr-Organic Produced by Hydrolysis, Bioprocess and Bioremediation and its Effect on Fermented Rate, Digestibility and Rumen Microbe Population (in vitro)

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Abstract. The research was conducted to study the production of organic chromium from the leather tanning waste and its effect on in vitro rumen fermentation activities. The research was divided into two phases. The first phase was production of organic chromium by alkali hydrolysis, S cereviceae bioprocess, and duckweed bioremediation that perceived solubility in neutral and acid solution. The second phase was the supplementation of organic-Cr in ration seen from in-vitro fermented rate, digestibility and microbe rumen population. Research was conducted experimentally using 4x4 factorial patterns, on the basis of Completely Randomized Design (CRD) with three replications in each experimental unit. The first factor was the type of organic-Cr and the second factor was the supplement in ration at four levels, 1, 2, 3 and 4 ppm. The results of this research indicated that organic chromium can be synthesized by alkali hydrolysis, S cereviseae bioprocess and the activity of duckweed bioremediation. Among the three of processes referred, the highest level of Cr was obtained from S cereviseae bioprocess that was originated from leather-tanning waste. The levels of organic-Cr that was resulted from alkali hydrolysis, bioprocess from $Cl_3Cr.6H_2O$, bioprocess from Cr leather-tanning waste, and from duckweed bioremediation were 354, 1011, 3833 and 310 mg/kg, respectively. Organic-Cr characteristic of each product has relatively similar in ferment ability, dry matter and organic matter digestibility and rumen ecosystem. There is an indication that dry matter and organic matter digestibility and rumen microbe population in ration that was added with organic Cr from alkali hydrolysis was higher than other supplements.

Key Words: organic-Cr, rumen fermentation activities, rumen microbe population

Introduction

Chromium (Cr) is an active component in Glucose Tolerance Factors (GTF) that comprises of Cr^{3+} and two acid molecules of nicotinate and three amino acids (Burton, 1995). Chromium is required in fat metabolism and protein, therefore, the existence of Cr in diet is important. Based on bioavailability, inorganic Cr has lower biological value compared to organic Cr. The organic Cr is non toxic and 25-30% of it is absorbed by the body (Chang and Mowat, 1992). Chromium in the form of hexavalent (Cr^{6+}) is poisonous to livestock, however, in the form of trivalent (Cr^{3+}) is nontoxic, but it only 2% of it is absorbed by the body (Offenbacher et al*.,* 1986).

Chromium exerts its biological function mainly by increasing sensitivity insulin and improving glucose metabolism (Anderson, 1998), though lipids and cholesterols may also be biological targets of Cr (Brautigan et al*.,* 2006; Pattar et al*.,* 2006). Study of Cr on glucose, insulin, and generally metabolism in animal have reported by varying results (Kegley et al*.*, 2000; Sumner et al*.*, 2007).

Organic Chromium naturally bound by protein. The principle of organic-Cr production is the Cr incorporation into protein. In leather-tanning process using chrome, Cr is often used as tanner. The waste of leather tanning in the form of husk-cutting (splitting) or in the form of fiber (shaving) generally contains 3-4% Cr^{3+}, 12-15% inorganic salt, 3-5% fat, and 75-80% protein collagen (Tate and Christopher, 2002). Chromium as a leather tanning waste can be utilized as the source of Cr in organic-Cr

synthesis. In this research, the process of organic-Cr synthesis was conducted in three methods, namely the alkali hydrolysis process, fermentation by *S. cereviseae* bioprocess, and macrophyta (duckweed) bioremediation. The bound Cr by protein in each processes were different in the number and the degree of bounding strength, as well as it solubility in digestive process and its absorption as an active component of glucose (Glucose Tolerance Factor or GTF).

The synthesis of organic Cr with alkali hydrolysis method was conducted by using $Ca(OH)_2$, NaOH or $NaHC0_3$. The optimum pH for the hydrolysis is 8 (Joko, 2003). The chromium in alkali hydrolysis binds more than 25% of the protein (Mua et al., 2003). The final product contains 9.33% glutamic acid, 20.24% glisin and 0.07% sistein (Tate and Christopher, 2002).

Saccharomyces cereviceae is a type of yeast which is often used for organic mineral bioprocess. The advantages of *S. cereviceae* utilization in ruminant nutrition, are 1) As an appetite stimulation. *S. cerevisiae* has a specific flavor of glutamic acid that could improve the palatability; 2) Its high content of vitamin B-complex that represents an essential specific nutrition for gastrointestinal microbes and for metabolism of host animal; 3) It assimilates protein and secretes essential amino acids; 4) Providing minerals as an autolysis product of natural yeast cell minerals that is ready to be absorbed by livestock; 5) The cell wall of active cells has strong absorption capacity and has some roles as nutrient reservoir and pH buffer; 6) Improving homeostasis, as it has the ability to remove oxygen to create anaerobic condition in order to facilitate the growth of anaerobe bacterium (Kelly, 1985; Shin, 1996; Razidlo, 2008).

Duckweed (*Lemna minor*) is usually found on wet lands, it grows quickly, and adapts easily in various aquatic conditions. Some studies indicate that duckweed could accumulate high concentration of various kinds heavy metals and trace minerals such as Ni, Cu, and Mn (Jain et al., 1988). Duckweed is hyper accumulator plant; it can be utilized as some metals accumulators (Kara et al*.,* 2003).

Today, the information of organic Cr production is limited. Therefore, the research was carried on to study the methods of organic Cr synthesis chemically (alkali hydrolysis) and biologically (bioprocess with *S cereviseae* and bio remediation of Duckweed), as well as it's influenced on fermentation activity, dilution of microbe population and *in vitro* ruminal feed digestibility.

Materials and Methods

The research was conducted in two phases experiments, the first phase examined the solubility of supplement in acid and neutral solutions, the second phase examined the effect of the addition of dietary organic Cr on fermented rate, microbe population and *in vitro* feed digestibility. The second phase was conducted using Completely Randomized Design (4x4 Factorial); each experimental unit was repeated 3 times. The first factor was the types of organic Cr, the second factor was the organic Cr level in diet namely 1, 2, 3 and 4 ppm, respectively. The four types of Organic Cr were processed via three different ways, namely alkali hydrolysis, bioconversion, and bioremediation.

The three types of processing of the organic Cr were as follow 1) The synthesis using the method of alkali hydrolysis was conducted by heating leather waste of splitting and shaving process in soluble NaOH to obtain protein hydrolysate that mixed with chromium. From this process, they were three products gained, that were sedimentation, hydrolysate and floating materials. The hydrolysate was taken for next processing. It was mixed with cassava meal then dried. The concentration of Cr based on 100% dry matter was 354 ppm; 2) The

bioconversion of organic Cr synthesis was conducted by cultivating *Saccharomyces cereviceae* on solid substrate of mixture of cassava pomace, cassava meal and heated soy bean (ratio of 48:21:31) diluted by standard soluble mineral and mineral enrichment with a dose according to formulation. Mineral standard condensation consisted of 0.5% NH_4NO_3, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, 0.001% $FeSO_4.7H_2O$, 0.0001% $CuSO_4.5H_2O$ in 1000 ml of aquades. The minerals were in the form of soluble Cr that came from $Cl_3Cr.6H_2O$ (Bioprocess[1]) or from leather tanning liquid waste (Bioprocess[2]). The concentration of Cr at each product based on 100% dry matter was 1011 and 3833 ppm, respectively; 3) The organic Cr bioremediation synthesis was conducted by growing the duckweed on media of 19,200 ml water, 800 ml leather tanning liquid waste and 19,200 ml rice field mud. The growing media were placed on 80 cm x 80 cm basin. The mud volume was determined with a depth of 3 cm and a height of 3 cm. A 500g of duckweed was planted on each basin and a month afterward was yielded. The concentration of Cr based on 100% dry matters was 310 ppm. All obtained Organic Cr was dried and milled thereafter until ready to be used.

There were 16 different rations tested, that were R1 = control ration + hydrolyzed organic Cr, 1 mg/kg of ration; R2 = control ration + hydrolyzed organic Cr, 2 mg/kg of ration; R3 = control ration + hydrolyzed organic Cr, 3 mg/kg of ration; R4 = control ration + hydrolyzed organic Cr, 4 mg/kg of ration; R5 = control ration + bioprocessed[1] organic Cr, 1 mg/kg of ration; R6 = control ration + bioprocessed[1] organic Cr, 2 mg/kg of ration; R7 = control ration + bioprocessed[1] organic Cr, 3 mg/kg of ration; R8 = control Ration + bioprocessed[1] organic Cr, 4 mg/kg of ration; R9 = control ration + bioprocessed[2] organic Cr, 1 mg/kg of ration; R10 = control ration + bioprocessed[2] organic Cr, 2 mg/kg of ration; R11 = control ration + bioprocessed[2] organic Cr, 3 mg/kg of ration; R12 = control ration + bioprocessed[2] organic Cr, 4 mg/kg of ration; R13 = control ration + bioremediated organic Cr, 1 mg/kg of ration; R14 = control ration + bioremediated organic Cr, 2 mg/kg of ration; R15 = control ration + bioremediated organic Cr, 3 mg/kg of ration, and R16 = control ration + bioreme diated organic Cr, 4 mg/kg of ration. The control ration consisted of 30% forages and 70% concentrate on dry matter basis.

Variables measured in the first experiment were product solubility on neutral solution, like as rumen condition), and acid solution (like as abomasum condition) and its solubility ratio. The measurement of organic Cr solubility in neutral solution was conducted by dissolving one gram of organic Cr in a mixture of 10 ml of rumen fluid and 40 ml of McDougall solution, at pH 6.5-6.8 and incubated for 24 hours at 39^oC. Cr content of supernatant was measured by Atomic Absorption Spectrometry (AAS). The measurement of organic Cr solubility in acid solution was almost the same with that of neutral pH. 0.1% HCl was added into the neutral solution until its pH reached 2 -3.

One gram of sampled ration was put into a fermentor tube, before subsequently added by 40 ml of McDougall solution and 10 ml of rumen fluid. CO_2 was exhaled into tube for 30 seconds to make an anaerobic condition, hereinafter covered with ventilated rubber. The tube was incubated in water bath for 3 hours at 39^oC and was shaken every 30 minutes. Afterwards, rubber cover was opened and the fermentation was stopped by dropping 0.2 ml of saturated $HgCl_2$. The supernatant was dissociated by centrifugation at a 3000 rpm for 20 minutes. The concentration of supernatant NH_3 was measured by the method of Conway micro diffusion and the total VFA was measured by gas distillation.

Digestibility was tested by method of Tilley and Terry (1963), which was similar with those

of fermentative incubation. After 24 hours of incubation, the fermentor tube was opened and $HgCl_2$ saturated solution was dropped to kill the microbes. Subsequently, 0.2% pepsin (enzyme activity 1:10.000) in acid condition was added. The mixture of the solutions was re-incubated in waterbath at 39^oC in aerobic condition for 24 hours, before the tube content was filtered with Whatman 41. The residue was analyzed for dry matter and organic matter concentrations.

The active rumen bacteria were counted by colony count method that based on serial dilution calculation. The bacteria were grown in anaerobic condition at a Hungate tube at 39^oC for 7 days and pH 7. The protozoa were counted based on the coloration of Methylgreen Formalin Saline (MFS) solution in physiological NaCl and then was fixated with formalin. The obtained data were analyzed with analysis of variances and was continued with Duncan test.

Results and Discussion

The solubility of organic Cr in neutral and acid solution

The data of organic Cr solubility from hydrolysis process, *S cereviceae* bioprocess, and duckweed bioremediation are presented in Table 2. The lowest solubility in neutral solution was obtained from organic Cr hydrolysis, while the highest one was from organic Cr bioprocess that came from $Cl_3Cr.6H_2O$. The low value of Cr solubility from alkali hydrolysis could be due to the strongly bounded of Cr to protein molecule in the form of organic Cr. This fact indicated that organic Cr from hydrolysis was not available to rumen microbes and could be used as the source of by-pass Cr for digestive system of livestock. The solubility of organic Cr from Bioprocess[1] yielded a high solubility. *S cereviceae* was not able to break down the substrate as it's growth was blocked by Cl_3Cr. As a result, only small amount of Cr was bounded by protein and the huge amount of Cl_3Cr, in the form of mineral salt, was easily dissolved in neutral solution. The lowest solubility Cr in acid solution was came from organic Cr hydrolysis that was 22.27%. It is mean that Cr bounded protein molecules relatively stable at acid condition. Solubility of organic Cr from bioprocess[1] yielded the highest solubility, 68.17%. This indicated that Cr bounded protein molecules at the organic Cr relatively weak at acid condition. The high solubility of Organic Cr in acid solution was assumed to be the best, with a purpose that when Organic Cr resides in abomasum, the Cr fastens protein molecule release, therefore, Cr ions will be bound again by protein from the intestine mucosa in chelated form and is easy to permeated by the intestine wall. Cr ions absorbed by the intestine wall hereinafter comes into blood stream and becomes the precursor to form GTF that act partly in carbohydrate, fat and protein metabolisms.

Solubility ratio in rumen:abomasum expressed the characteristics of diet in digestion process of ruminant. High ratio value expressed that the diet had the character of bypass. Based on Table 2, the higher score was obtained from Organic Cr from Bioprocess[1], which indicated that the supplement was of higher quality. This mean that the lowest amount mineral dissolved in the rumen, the highest amount mineral absorbed by rumen cell wall.

Effect supplementation of Chromium on ration fermented rate in rumen

Diet fermented rate means how easily the diet would degrade by rumen microbes. Fermented rate is expressed by the yields of ammonia (NH_3) and volatile fatty acids (VFA). Rumen microbes always degrade dietary protein in to NH_3, that is required by the microbes for the synthesis of microbial protein.

Rumen microbe activity is depending on N consumption. Low N consumption will inhibit

Table 1. Composition of dietary concentrates

No	Feedstuff	Dry matter (%)	As fed *(%)*
1	Cassava pomace	9.34	11.25
2	Rice bran	28.84	34.75
3	Cassava meal	7.31	8.81
4	Wheat pollard	14.07	16.95
5	Ketchup waste product	18.89	22.76
6	Coconut meal	21.55	25.96
	Total	100.00	120.48

Table 2. Solubility of organic Cr from hydrolysis, *S cereviseae* bioprocess and duckweed bioremediation

Treatment	solubility (%)		
	neutral solution (n)	acid solution (a)	n:a ratio
Organic Cr hydrolysis	7.18	22.27	0.322
Organic Cr bioprocess[1]	86.82	68.17	1.274
Organic Cr bioprocess[2]	33.36	51.16	0.652
Organic Cr bioremediation	7.74	42.33	0.183

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$
[2] = The source of Cr was came from liquid waste of leather tanning

Table 3. Production of NH_3 in rumen fluid at various treatments

Type of supplement	Cr level in diets				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- mM ----------				
Organic Cr hydrolysis	4.24	4.16	3.46	3.54	3.85
Organic Cr bioprocess[1]	4.02	3.86	4.82	4.37	4.27
Organic Cr bioprocess[2]	4.20	4.28	4.23	4.14	4.21
Organic Cr bioremediation	4.50	4.30	3.90	4.10	4.20
Average	4.20	4.10	4.10	4.00	

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$
[2] = The source of Cr was came from liquid waste of leather tanning

rumen microbe activity. Ammonia is a rumen microbe diet and will convert to microbial protein, as it doesn't have transportation system to transport amino acid into cell. Approximately 82% of the microbes can use ammonia.

The high NH_3 production expressed the amount of easily degraded dietary protein by rumen microbes. Based on Table 3, the highest concentration of NH_3 in rumen fluid was obtained in treatment of 1 ppm of organic Cr from duckweed bioremediation, while the lowest concentration of NH_3 in rumen fluid was obtained in treatment that contained 3 ppm of organic Cr from alkali hydrolysis. Statistically, the type of organic Cr and level of Cr in diet were not significantly different. The results showed that the supplementation of organic Cr did not influence degradation system of nutrient especially dietary protein by microbes in the rumen. In general, all diets (sixteen dietary treatments) yielded NH_3 in normal levels that were in the range of 3.46-4.82 mM/L for the growth of rumen microbes. According to

Sutardi (1997), the optimum requirement of NH_3 for rumen microbial activity is 3-12 mM. Therefore, Organic Cr could be used as feed supplement without annoying metabolic activity in the rumen. Each treatment had the same NH_3 production in the rumen fluid. This is supported by previous data that organic Cr yielded at this research had low solubility in the rumen, therefore, did not influence diet degradation in the rumen.

VFA represent the products of dietary organic matter degradation especially structural and non structural carbohydrates. The final product of fermentation in the rumen is VFA, that was represented the fermented rate of structural and non structural carbohydrates. This production related to the ability of microbes, while microbes activity is depended on dietary nutrient composition, rumen condition during fermentation, and time after feeding. Dietary fermented rate as depicted by the production of NH_3 and total production of VFA is presented in Table 4.

Statistically, types of organic Cr used in the treatment had significant effect on total VFA production in the rumen, but level of organic Cr had no significant effect on total VFA. It is indication that solubility of organic Cr in neutral solution correlated with total VFA production. The low production of total VFA expressed that organic matter of organic Cr was difficulty soluble in rumen liquor. Although, Cr was attributed to an increasing G-6-Pase activity in improvement insulin sensitivity and plays an important role in dietary carbohydrate by fermented in rumen, because glucose need of ruminant animal is dependent on gluconeogenesis, as shown previously (Yan et al., 2008; and Yan et al., 2010).

Effect supplementation of Chromium on dry matter digestibility

Based on Table 5, it shows that types of Cr-organic as feed supplement in diets were various effect on dry matter digestibility.

Table 5 shows that dry matter digestibility of diets ranged from 21.59-43.25%. There was no significant influence of organic Cr level on dietary dry matter digestibility and neither interaction between organic Cr levels with the types of supplements in the diet. Similarly, studies on lambs by Kraidess et al. (2009) indicated that dry matter intake during first week of arrival was not affected by supplemental Cr as Cr-yeast compared to control.

However, there was an indication that the average of organic Cr from alkali hydrolysis yielded higher digestibility than other supplements, that was 27.7% higher from organic Cr bioprocess^2, 15.3% from organic Cr bioprocess^1 and 10.8% from organic Cr bioremediation. That data pointed out that tapioca flour (as carbohydrate) was appropriate as a binder, as it was easily degraded and enzymatic digested.

Dry matter digestibility was maximum when the organic Cr level was 3 ppm. In contrary, organic Cr from bioremediation and bioprocess^2 would decreased dry matter digestibility as the level of organic Cr in diet risen.

Table 6 presents that organic matter digestibility of diets in various types and levels of organic Cr ranged from 27.38% - 49.94%. Statistically, organic Cr levels and types did not influence organic matter digestibility of diet. There was no interaction between types of organic Cr and levels in diet. But there was an indication that the averages of organic Cr from alkali hydrolysis yielded a higher organic matter digestibility than treatment of other supplements, that was 29.2% higher from organic Cr bioprocess^2, 20.7% from organic Cr bioproses^1 and 18.8% from organic Cr bioremediation. This finding indicated that tapioca flour as a binder was easily degraded and digested to carbohydrate by the digestive enzymes. The pattern of organic matter digestibility in organic Cr from hydrolysis

Table 4. The production of total VFA in rumen fluid of various diet treatments

Type of supplement	Cr level in diets				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- mM ----------				
Organic Cr hydrolysis	68.5	88.8	63.7	96.3	79.3[a]
Organic Cr bioprocess[1]	107.3	106.8	116.7	146.0	119.2[b]
Organic Cr bioprocess[2]	111.0	92.5	130.0	115.5	112.3[b]
Organic Cr bioremediation	108.5	113.5	110.0	102.7	108.7[b]
Average	98.8	100.4	105.1	115.1	

Values bearing different superscript at the same column differ significantly (P<0.05).

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$

[2] = The source of Cr was came from liquid waste of leather tanning

Table 5. Dry matter digestibility of various dietary treatments

Type of supplement	Cr level in diets				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- % ----------				
Organic Cr hydrolysis	25.29	28.61	43.25	32.17	32.3
Organic Cr bioprocess[1]	28.49	25.32	30.92	27.17	28.0
Organic Cr bioprocess[2]	28.45	21.59	25.06	26.23	25.3
Organic Cr bioremediation	33.40	28.50	27.10	26.30	28.8
Average	28.90	26.01	31.59	27.96	

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$

[2] = The source of Cr was came from liquid waste of leather tanning

Table 6. Organic matter digestibility (%) of diets at various treatments

Type of supplement	Cr levels in diet				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- % ----------				
Organic Cr hydrolysis	32.39	33.82	49.94	37.79	38.5
Organic Cr bioprocess[1]	32.25	31.67	30.82	32.68	31.9
Organic Cr bioprocess[2]	33.32	27.38	29.79	28.68	29.8
Organic Cr bioremediation	38.80	30.50	31.70	28.80	32.4
Average	34.18	30.86	35.56	31.98	

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$

[2] = The source of Cr was came from liquid waste of leather tanning

Table 7. Bacterium population of rumen fluid in various treatments

Type of supplement	Cr levels in diet				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- 10^9 cells/ml ----------				
Organic Cr hydrolysis	43.0	62.3	40.7	50.0	49.0[a]
Organic Cr bioprocess[1]	40.0	39.7	40.0	40.0	39.9[ab]
Organic Cr bioprocess[2]	54.7	45.7	30.3	27.3	39.5[ab]
Organic Cr bioremediation	27.3	26.0	24.0	35.3	28.2[b]
Average	41.3	43.4	33.8	38.2	

Values bearing different superscript at the same column differ significantly (P<0.05).

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$

[2] = The source of Cr was came from liquid waste of leather tanning

Table 8. Population of protozoa in rumen fluid at various treatments

Type of supplement	Cr levels in diet				Average
	1 ppm	2 ppm	3 ppm	4 ppm	
	---------- x10^5 sel/ml ----------				
Organic Cr hydrolysis	15.7	9.7	14.0	17.0	14.1[b]
Organic Cr bioprocess[1]	24.0	23.7	18.0	15.0	20.2[ab]
Organic Cr bioprocess[2]	21.7	34.3	13.0	30.0	24.8[a]
Organic Cr bioremediation	17.3	17.0	23.3	18.0	18.9[ab]
Average	19.7	21.2	17.1	20.0	

Values bearing different superscript at the same column differ significantly (P<0.05).

Note : [1] = The source of Cr was came from $Cl_3Cr.6H_2O$

[2] = The source of Cr was came from liquid waste of leather tanning

reached a maximum rate when Cr in diet was of 3 ppm. Supplementation of organic Cr from bioprocess[1], bioprocess[2], and bioremediation tended to degrade organic matter in line with the increasing levels of organic Cr in diet.

Effect supplementation of Chromium on population of rumen microbes

Bacteria represent the greatest population of microbes that play an important role in crop cell's carbohydrate fermentation. The existence of bacteria in the rumen causes ruminants to have an ability to degrade celluloses, hemicelluloses and other fibers to produce the nergy for the production of meat, milk, or wool.

Statistically, the levels of organic Cr had a significant effect (P<0.05) on the bacterium population of rumen fluid. Supplementary organic Cr from alkali hydrolysis increase rumen bacteria population higher than that of organic Cr from bioremediation. Low bacteria population in organic Cr from bioprocess could be due to antibacterial compound in the duckweed.

The types of supplements of organic Cr in diet had an effect (P<0.05) on protozoa population in rumen fluid. The level of organic-Cr did not have any effect on rumen protozoa population. Supplementary organic Cr from bioprocess[2] affected protozoa population higher than bacteria population of organic Cr from hydrolysis. Protozoa population in rumen fluid was inversely proportional with bacteria population of rumen fluid supplemented by organic Cr from alkali hydrolysis. It is showed that supplementation organic Cr from hydrolysis could depress protozoa population and on the other side, it increases bacteria population.

Conclusions

1. Organic Chromium could be synthesized from alkali hydrolysis, bioprocess activity of *S cereviseae,* and bioremediation activity of Duckweed. Among the three processes, the highest amount Cr is obtained from *S cereviseae* bioprocess, which is comes from leather tanning liquid waste. The concentrations of Cr in organic Cr from alkali hydrolysis, bioprocess of $Cl_3Cr.6H_2O$, bioprocess of leather tanning waste, and duckweed bioremediation are 354, 1011, 3833 and 310 $mg.kg^{-1}$, respectively.
2. The characteristics of organic Cr from each product have similar fermentability, digestibility and rumen ecosystem. However, there is an indication that dietary dry matter, organic matter digestibility and rumen bacteria population of organic Cr from alkali hydrolysis are higher than that of other supplements.

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References

Anderson RA. 1998. Chromium, glucose intolerance, and diabetes. J. Am. Coll. Nutr. 17:548-555.

Brautigan DL, A Kruszewski and H Wang. 2006. Chromium and vanadate combination increases insulin-induced glucose update by 3T3-L1 adipocytes. Biochem and Biophysic. Res. Com. 347:769-773.

Burton JL. 1995. Suplemental Chromium: its benefit to the bovine immune system. Anim. Fed. Sci. Tech. 53:117-133.

Chang X and DN Mowat. 1992. Supplemental Chromium for Stressed and Growing Feeder Calves. J. Anim. Sci. 70:559–565.

Jain SK, GS Gujral, NK Jha and P Vasudevan. 1988. Heavy metal uptake by Pleurotus sajor-caju from metal enriched duckweed substrate. Biol. Wastes. 24:275–82

Joko T. 2003. Penurunan Kromium (Cr) dalam Limbah Cair Proses Penyamakan Kulit Menggunakan Senyawa Alkali Ca(OH)2, NaOH, dan NaHCO3 (Studi Kasus di Pt Trimulyo Kencana Mas Semarang). J. Kes. Lingk. Indon. 2(2):39-45.

Kara Y, D Basaran, I Kara, A Zeytunluoglu and H Gen. 2003. Bioaccumulation of Nickel by Aquatic Macrophyta Lemna minor (Duckweed). Internat. J. Agric. and Biol. 3:281–283.

Kegley EB, DL Galloway and TM Fakler. 2000. Effect of dietary chromium-L-methionine on glucose metabolism of beef steers. J. Anim. Sci. 78:3177-3183.

Kelly WR. 1985. Veterinary Clinical Diagnosis. 3rd. Baylliere Tindall, London. 261-300.

Kraidees MS, IA Al-Haidary, SI Mufarrej, MY Al-Saiady, HM Metwally and MF Hussein. 2009. Effect of supplemental chromium levels on performance, digestibility, carcas characteristic of transport-stressed lamb. Asian-Aust. J. Anim. Sci. 22: 1124-1132.

Mua C, W Lina, M Zhanga and Q Zhub. 2003. Towards Zero Discharge of Chromium-Containing Leather Waste Through Improved Alkali Hydrolysis. Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu-Sichuan, China.

Offenbacher EG, H Spencer, HJ Dowling and FX Pi-Sunier. 1986. Metabolic Chromium balances in Men. Am. J. Cli. Nutr. 44:77-82

Pattar GR, L Tackett, P Liu and JS Elmendorf. 2006. Chromiun picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. Mutat. Res. 610:93-100.

Razidlo DF. 2008. Cell Cycle Checkpoint and Orther Trans-factor Influence Trinucleotide Repeat Instability in *Saccharomyces cerevisiae*. Dissertation. University of Nebrska Medical Centre Omaha. Nebraska.

Shin TH. 1996. Practical Uses of Yeast Cultur CYC-100 in Swine. Poultry and Ruminant Ration. Choong and Chemical Co. Ltd Seoul. South Korea.

Sumner JM, F Valdez and JP McManara. 2007. Effect of chromium propionate on responses to an intravenous glucose tolerance test in growing Holstein heifers. J. Dairy Sci. 90:3467-3477.

Sutardi T. 1997. Peluang dan Tantangan Pengembangan Ilmu-ilmu Nutrisi Ternak. Orasi Ilmiah. Fak. Peternakan Institut Pertanian Bogor.

Tate and R Christopher. 2002. Method of Producing a Metal Nutrient for an Animal Feed. http://www.freepatentsonline.com/6352714.html. (2/3/08).

Yan X, W Zhang, J Cheng, R Wang, DO Kleemann, X Zhu and J Jia. 2008. Effects of chromium yeast on performance, insulin activity, and lipid metabolism in lambs feed different dietary protein levels. Asian-Aust. J. Anim. Sci. 21:853-860.

Yan X, F Zhang, D Li, X Zhu and J Jia. 2010. Effect of chromium on energy metabolism in lamb feed with different dietary protein levels. Asian-Aust. J. Anim. Sci. 23:205-212.