# LIPID PROTECTION OF SEVERAL INDONESIAN FEEDS TOWARD PRODUCTION OF HEALTHIER MEAT

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### **ABSTRACT**

An experiment had been conducted in order to evaluate the effectiveness of lipid protection of several Indonesian feeds from rumen microbial attacked (hydrogenation) using formaldehyde. The experiment used the randomized block design in factorial. The first factor was feeds (Kernel Palm Oil Meal/KPOM, Yellow Corn/YC, Rice Bran/RB, and Pasture Grass Brachiaria humidicola/BH); the second factor was level of formaldehyde used (0, 2.5, 5, 7.5, and 10% of Crude Protein of Feed); the third factor was storage time of processed feeds (0,2,4, and 6 days after addition of formaldehyde). The main parameter measured in this experiment is hydrogenation of several unsaturated fatty acids (Oleat/CI8:1; Linoleat/CI8:2; and Linolenat/CI8:3) and degradation of Feed Protein; using Tilley and Terry in vitro technique; digestibilities (Dry Matter and Organic Matter), VFA production and Total Gas Production. The results of the experiments showed that hydrogenation of Oleat was decreased (P<0.05) with 10% formaldehyde for 6 days storage time for RB, BH, KPOM, and YC; hydrogenation of Linoleat and Linolenat was descreased (P<0.05) with 7.5% formaldehyde for 4 days storage time for BH, KPOM, RB, and YC. The NH<sub>3</sub> and VFA production during 4 hours incubation was decreased (P<0.05) at 10% level of formaldehyde, for 6 days storage time. Total Gas Production decreased with 7.5% formaldehyde in 4 days time of storage for KPOM. It was concluded that lipid protection from hydrogenation by rumen microbes in this experiment was possible.

Key words: lipid protection, ruminant, formaldehyde, in vitro.

#### INTRODUCTION

It was started with protection of good quality dietary protein against microbial degradation in the rumen. Formaldehyde treated of those kind protein had been shown to decrease ammonia levels in the rumen (Fergusson et al., 1967), increase the amount of protein reaching the intestines (Faichney and Weston, 1971), reduced urine nitrogen excretion to the greater extent, so that nitrogen retention increased (Faichney, 1974). However, not all of the experiment reported positive results; Wachira et al. (1974) for example did not find the significant performance improvement of the animals given feedstuffs treated with formaldehyde.

Faichney (1972) treated peanut meal (instead of casein) with formaldehyde in order to reduce the breakdown of protein in the rumen. In his experiment the treated peanut meal contained 0.52 g formaldehyde reversibly bound per 100 g crude protein. However he only observed the effect of formaldehyde on protein, not on fatty acid. Fergusson (1967) reported

several feeds treated with formaldehyde related to their effect to wool growth (lupin seed, meat meal, yeast peanut meal, sunflower meal, linseed meal, cottonseed meal, and rapeseed meal); level of formaldehyde used, ranging from 0.80 (for linseed meal) to 1.66% crude protein (for sunflower meal). The whole diet treated with 20 litre volume formalin (18.5% HCHO)/100 kg crude protein. Although a number of factors can be expected to affect the efficiency of treatment, the use of 5% formalin (37% HCHO) from crude protein proved satisfactory for a number of concentrate diets, and twice of this quantity for hay; the optimum level perhaps between 0.5-2% of the crude protein for concentrate diets, and 1.3-2% for hay.

The lipids in the plant material eaten by ruminant animals, generally contain high proportions of Cl8-poly unsaturated fatty acids (e.g. linoleic, and linolenic). Those dietary poly-unsaturated fatty acids in the diet are substantially hydrogenated in the rumen; so when such supplements are given to ruminants those fatty acids do not appear in tissue and milk lipid (Garton, 1967).

Later development, several authors have made possible the production of protected poly-unsaturated fatty acids (e.g. safflower, sunflower, soybeans, etc.) from hydrogenation in the rumen (Cook *et al.*, 1970; Scott *et al.*, 1970) by embedding the (droplets) fatty acids within insoluble formaldehyde treated protein matrix. When ruminant animals are given such supplements, marked changes occur in the fatty acid composition of milk and body fats (Scott *et al.*, 1970). The supplement was prepared by spray-drying an emulsion an equal parts by weight of oil (eg. Safflowers oil) and casein and then treated with formalin (37% formaldehyde), 3.7 g formaldehyde/100 g casein) (Cook *et al.*, 1970).

The objectives of this experiment were: to observe the effect of direct treatment of formaldehyde to several feedstuffs in order to protect unsaturated fatty acids; to observe the concentration of formaldehyde effective in the protection of several feedstuffs, how long the reaction will take to complete; and protection performance itself from microbial process *in vitro*.

### MATERIALS AND METHODS

Several Indonesian feeds i.e forage (Brachiaria humidicola), energy source (yellow corn, Bima variety), protein supplement (Kernel Palm Oil Meal — Elais guinensis Jack, Tenera variety), and supplement between source of energy and source of protein (Rice Bran) were prepared for in vitro study using the following stepwise procedure.

All feedstuffs were dried and ground. One kilogram of each ground sample was sprayed with commercial formaldehyde (37% formaldehyde) in the amount according to the design; volume of formaldehyde solution manually sprayed was: 250 ml to each 1 kg feed sample, mixed thoroughly, before stored in the black plastic bags according to the design.

The experimental design used was Randomized Block, in Factorial 4 x 5 x 4 (Steel and Torrie, 1993) with mathematical model:

$$Yik(1) = \mu + Ai + Bj + Ck + ABCijk + Eijk$$

A = feedstuffs used mentioned above.

B = level of formaldehyde (0, 0.25, 5.0, 7.5 and 10% of the crude protein of the feeds).

C = time of storage (0, 2, 4 and 6 days).

All feedstuffs used in this experiment were analyzed for their dry matter (Harris, 1970), crude protein in order to calculate the formaldehyde needed (Micro-Kjeldahl AOAC, 1980), total lipids (AOAC, 1995) and of course the unsaturated fatty acids before and after fermentation (Gas Chromatograph). In assessing the effectiveness of the protection of unsaturated fatty acids, all samples already prepared (with formaldehyde), fermented *in vitro* (Tilley and Terry, 1963). Other parameters observed, digestibility of DM/OM, N-NH<sub>3</sub> and VFA production during fermentation *in vitro*. Sheep rumen fluid used in this experiment came ftom slaughter house.

### **RESULTS AND DISCUSSIONS**

# Dry Matter, Crude Protein and Fatty Acid Contents of Feedstuffs used in this experiment

Dry matter, crude protein contents of feedstuffs used in this experiment are shown in Table 1, and total lipid and unsaturated fatty acids in Table 2.

Table 1. Dry Matter and Crude Protein (as fed) Contents of Feedstuffs used in this experiment

Feedstuffs	Dry Matter (%)	Crude Protein (%)
Brachiaria humidicola	21.48	7 24
Rice Bran	87.57	13.40
Kernel Palm Oil Meal	86.90	13.21
Yellow Corn	84.95	9.83

It was surprising that kernel palm oil meal contains crude protein only in the same amount of rice bran, and the feedstuffs used in this experiments contain very little (if any) C20:4 fatty acid.

Table 2. Total Lipid and Unsaturated Fatty Acid Content (%) of the Feedstuffs used in the Experiment

Feedstuffs	Total Lipid		Unsaturated	Fatty Acids	
	(%)	C18:1	C18:2	C18:3	C20:4
Brachiaria humidicola	2.69	0.33	0.26	0.22	_
Rice Bran	7.06	1.38	0.75	0.04	_
Kernel Palm Oil Meal	20.37	7.15	0.23	1.23	_
Yellow Com	5.47	1.79	0.93	0.43	_

### Digestibility of Dry Matter (DDM) and Organic Matter (DOM) in vitro.

It is shown from Table 3 that DDM was significantly affected by level of HCHO; the maximum effect at 7.5% HCHO. From protection point of view, 2.5% formaldehyde was the best. It seems that DDM could not clarify the protection effect of HCHO yet, during fermentation. The effect of formaldehyde level to DDM followed quadratic equation (Y=31.514 + 1.1922 X -1.160  $X^2$ ,  $R^2 = 0.06$ ); the maximum DDM was found in the 7.5% level of formaldehyde.

Table 3. The effect of HCHO level on digestibility of dry matter (%) during fermentation of several feedstuffs

Feedstuffs		HCHO (%)					
	0	2.5	5.0	7.5	10	~	
KPO Meal	38.57	31.27	44.22	45.24	37.64	39.39±5.64a	
Yellow Corn	30.44	30.47	33.03	36.25	35.03	33.04±2.63b	
Rice Bran	32.81	28.70	38.84	40.37	33.15	34.77±4.77b	
Grass	30.29	38.69	29.02	38.01	28.63	32.93±4.99b	
Means	33.03±3.87a	32.28±4.40a	36.28±6.65a	40.00±3.90b	33.61±3.80a		

There was no effect of time of storage on DDM *in vitro* (Table 4). Feedstuffs and time of storage had no effect in the beginning, then after 2 days storage difference were appeared in DDM fermentation between feedstuffs, where kernel palm oil meal showed higher DDM-fermentation (48.45%) than yellow corn (37.76%), rice bran (36.97%) and pasture grass (37.37%). At 4 days storage after processing, kernel palm oil meal was still higher, but decreased again after 4 days storage.

Between feedstuffs and level of formaldehyde, DDM of kernel palm oil meal was significantly higher (P<0.05) than the three other feedstuffs. The difference stayed the same with 5, 7.5 and 10% formaldehyde (not with 2.5% of formaldehyde where all of those feedstuffs showed the same DDM during fermentation).

So there was an interaction, although it was not clear or not important or inconsistence conditions. In 0 day storage, feedstuffs sprayed with 5% and 7.5% formaldehyde showed significantly higher (P<0.05) DDM during fermentation than feedstuffs treated with 0, 2.5 and 10%. At the 2 and 4 days storage of time, feedstuffs treated with 7.5% formaldehyde still showed DDM during fermentation significantly high (P<0.05) than other level of formaldehyde; while feedstuffs given 5% formaldehyde showed DDM during fermentation decreased to the same as other DDM fermentation. During storage of feedstuffs there were no significant difference between level of formaldehyde. So, it was suggested to store feedstuffs not more than 4 days after processing with formaldehyde.

The effect of time storage on digestibility of organic matter during fermentation is shown in Table 4.

Table 4. The effect of Time Storage on Digestibility of Organic Matter (%) during fermentation of several feedstuffs used in this experiment.

Feedstuffs		Time of Storage (days)				
	0	2	4	6	-	
KPO Meal	41.33	48.45	45.21	46.21	45.30±2.97a	
Yellow Corn	40.16	37.76	32.37	(t) 39.45	37.43±3.52b	
Rice Bran	39.26	36.97	42.25	38.03	39.13±2.28b	
Grass	36.27	37.37	40.67	39.07	38.34±1.93b	

Different superscript from the same column means differed at (P<0.05)

As found with DDM, DOM during fermentation of Kernel Palm Oil Meal still showed the highest DOM (45.30%) than Rice Bran (39.13%), Yellow Corn (37.43%) and Grass (38.34%). However there were no effect of time storage on DOM to each feedstuffs.

Table 5. The effect of Formaldehyde Level on Digestibility of Organic Matter (%) During Fermentation.

Feedstuffs		НСНО (%)					
	0	2.5	5.0	7.5	10	_	
KPO Meal	44.38	37.33	50.49	50.49	44.15	45.37±5.47a	
Yellow Corn	35.25	33.41	37.38	37.38	39.85	36.65±2.44b	
Rice Bran	36.27	33.93	43.95	43.95	37.02	39.02±4.64b	
Grass	36.36	35.60	40.38	40.38	35.20	37.58±2.59b	
Means	38.06±4.24a	35.07±1.77a	43.05±5.64b	43.05±5.64b	39.05±3.90a		

Different superscript from the same column and rows, means differed at (P<0.05)

The effect of formaldehyde also showed that kernel palm oil meal had higher (P<0.05) DOM (45.37%) than yellow corn (36.65%), rice bran (39.02%) and grass (37.58%); no significant difference (P>0.05) among the other three feedstuffs (Table 5). The highest effect of formaldehyde protection was in 5% concentration (see also DDM).

# Fermentation of Total Lipid and Hydrogenation of Unsaturated Fatty Acids.

The effect of formaldehyde level on fermentation/hydrogenation of total lipid, oleate (18:1), linoleate (18:2) and linolenate (18:3) presented in Table 6, 7, and 9.

Table 6. The effect of level of HCHO on fermentation of total lipid

Level of HCHO (% CP)	Before Fermentation	After Fermentation	Reduction
0	18.59	9.50	9.09a
2.5	11.64	7.99	3.65ab
5	9.99	6.43	3.50ab
7.5	11.25	7.03	4.22ab
10	12.79	10.21	2.58b

Different superscript from the same row means differed at (P<0.05)

Table 6 showed that there was a protection of total lipid against microbial attack with maximum activity at 10% level of formaldehyde. The effect of level HCHO on hydrogenation of oleat, is shown in Table 7.

Table 7. The effect of level of HCHO on hydrogenation of oleate (CI8:1) in several Feedstuffs

Fe	eedstuffs		Le	vel HCHO (9	/ <sub>6</sub> )		Means
		0	2.5	5	7.5	10	_
KPO Meal	BF	2.38	2.64	1.73	2.50	2.54	
	AF	2.34	2.40	1.93	2.04	2.12	
	Reduction	0.04	0.24	-0.20	0.46	0.42	0.19a
Y. Corn	BF	1.44	1.13	0.74	0.67	0.35	0.174
	AF	1.07	0.81	0.37	0.33	0.12	
	Reduction	0.37	0.32	0.37	0.34	0.23	0.33b
R. Bran	BF	1.08	0.78	0.61	0.12	0.07	0.550
	AF	0.94	0.74	0.40	0.13	0.05	
	Reduction	0.14	0.04	0.21	-0.01	0.02	0.08b
Grass	BF	0.50	0.12	0.34	0.10	0.07	0.000
	AF	0.11	0.07	0.06	0.04	0.03	
	Reduction	0.39	0.05	0.28	0.06	0.03	0.16a
Means		0.24a	0.16b	0.16b	0.21a	0.18b	0.104

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

From feedstuffs point of view, C18:1 from rice bran was highly protected with formaldehyde. The least reduction of C18:1 found with 2.5% HCHO (comparable with DDM, DOM). The effect of storage time on protection of C 18;1 after processed with HCHO is shown in Table 8.

Table 8. The effect of storage time on protection of C18:1 after processed with HCHO.

Feedstuffs		······································	G. 5			
1.66	asturis		Storage I	ime (days)		Means
		0	2	4	6	
KPO Meal	BF	2.79	2.75	1.73	1.64	·····
	$\mathbf{A}\mathrm{F}$	1.80	2.00	2.41	2.45	
	Reduction	0.99	0.75	-0.68	-0.81	0.06a
Y. Corn	$\mathbf{BF}$	0.86	0.44	1.27	0.89	0.004
	AF	0.77	0.57	0.53	0.28	
	Reduction	0.09	-0.13	0.74	0.61	0.33b
R. Bran	$\mathbf{BF}$	0.75	0.48	0.45	0.44	0.550
	AF	0.55	0.47	0.65	0.13	
_	Reduction	0.20	0.01	-0.20	0.31	0.08b
Grass	BF	0.15	0.32	0.14	0.30	0.000
	AF	0.06	0.05	0.07	0.05	
	Reduction	0.09	0.27	0.07	0.25	0.17a
M	eans	0.34a	0.22b	-0.02c	0.09d	0.174

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

From Table 8 it can be seen that four days storage was the best from the protected C18:1 point of view. The effect of level HCHO on hydrogenation of linoleate is shown in Table 9.

Table 9. The effect of level of HCHO on hydrogenation of linoleate (CI8:2) in several feedstuffs

Feed	stuffs		Le	vel HCHO	(%)		Means
		0	2.5	5	7.5	10	
KPO Meal	BF	2.53	1.15	1.61	1.36	1.42	~~
	AF	2.39	1.25	1.32	0.88	1.65	
	Reduction	1.14	-0.10	0.29	0.48	-0.23	-0.32a
Y. Corn	$\mathbf{BF}$	0.76	0.36	0.30	0.16	0.07	0.524
	AF	0.68	0.63	0.23	0.13	0.24	
	Reduction	0.08	-0.27	0.07	0.03	-0.17	-0.05b
R. Bran	BF	0.40	0.24	0.15	0.10	0.04	0.000
	AF	0.53	0.32	0.19	0.04	0.01	
	Reduction	-0.13	-0.08	-0.04	0.06	0.03	-0.03b
Grass	BF	0.20	0.10	0.08	0.04	0.05	1,
	AF	0.08	0.02	0.03	0.05	0.02	
	Reduction	0.12	0.08	0.05	-0.01	0.03	0.05c
Me	ans	0.30a	-0.09b	0.09c	0.14d	-0.09b	0.030

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

Table 9 also showed that 2.5% HCHO would be enough to protect CI8:2 from microbial hydrogenation. The effect of storage time on protection of linoleate is presented in Table 10.

Table 10. The effect of storage time on protection of C18:2 after processed with HCHO.

				r	William Caro.
eedstuffs		Storage	e Time	(days)	
	0	2		4	6
BF	1.71	1.79		1.77	1.30
AF	1.25	1.59			1.41
Reduction	0.46	0.02	7, *!		-0.11
BF	0.38	0.44			0.21
' AF	0.53	0.46			0.24
Reduction	-0.15		oh		-0.03
BF	0.25				0.15
AF	0.23		-બોડ્ડ		0.13
Reduction	0.02				-0.08
BF			*.		0.06
AF			115		0.04
Reduction			11:		0.04
Means					-0.05c
	AF Reduction BF AF Reduction BF AF Reduction BF AF Reduction BF AF Reduction	0       BF     1.71       AF     1.25       Reduction     0.46       BF     0.38       AF     0.53       Reduction     -0.15       BF     0.25       AF     0.23       Reduction     0.02       BF     0.11       AF     0.05       Reduction     0.06	BF         1.71         1.79           AF         1.25         1.59           Reduction         0.46         0.02           BF         0.38         0.44           AF         0.53         0.46           Reduction         -0.15         -0.20           BF         0.25         0.10           AF         0.23         0.20           Reduction         0.02         -0.10           BF         0.11         0.11           AF         0.05         0.04           Reduction         0.06         0.07	Storage Time           0         2           BF         1.71         1.79           AF         1.25         1.59           Reduction         0.46         0.02           BF         0.38         0.44           AF         0.53         0.46           Reduction         -0.15         -0.20           BF         0.25         0.10           AF         0.23         0.20           Reduction         0.02         -0.10           BF         0.11         0.11           AF         0.05         0.04           Reduction         0.06         0.07	Storage Time (days)           BF         1.71         1.79         1.77           AF         1.25         1.59         1.50           Reduction         0.46         0.02         0.27           BF         0.38         0.44         0.30           AF         0.53         0.46         0.31           Reduction         -0.15         -0.20         -0.01           BF         0.25         0.10         0.21           AF         0.23         0.20         0.21           Reduction         0.02         -0.10         0.00           BF         0.11         0.11         0.11           AF         0.05         0.04         0.03           Reduction         0.06         0.07         0.08

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

Table 10 showed that the response of feedstuffs were varied; but after 6 days all found the negative results. The effect of HCHO on protection of linolenic (CI8:3) presented in Table 11 while the effect of time storage was shown in Table 12.

Table 11. The effect of level of HCHO on hydrogenation of linolenate (CI8:3) in several feedstuffs.

Fe	edstuffs		Le	vel HCHC	(%)		Means
		0	2.5	5	7.5	10	_
KPO Meal	BF	0.68	0.30	0.61	1.36	1.42	
	AF	1.39	1.25	0.33	0.88	1.65	
	Reduction	-0.71	-0.95	0.28	0.48	<b>-</b> 0.23	-0.23a
Y. Corn	$\operatorname{BF}$	0.76	0.36	0.30	0.16	0.07	
	AF	0.68	0.63	0.23	0.13	0.24	
	Reduction	0.08	-0.27	0.07	0.03	-0.17	-0.05b
R. Bran	$\mathrm{B}\mathbf{F}$	0.40	0.24	0.15	0.10	0.04	
	AF	0.53	0.32	0.19	0.04	0.01	
	Reduction	-0.13	-0.08	-0.04	0.05	0.03	-0.06b
Grass	BF	0.20	0.10	0.08	0.04	0.05	
	AF	0.08	0.03	0.03	0.05	0.02	
	Reduction	0.12	0.07	0.05	-0.01	0.03	0.05c
I	Means	0.16a	-0.31b	0.09c	0.14d	-0.09e	

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

Table 12. The effect of storage time on protection of C18:3 after processed with HCHO.

Fee	dstuffs		Storage	e Time (days)	
		0	2	4	6
KPO Meal	BF	0.60	0.35	1.61	0.76
	AF	0.55	0.37	0.61	0.48
	Reduction	0.05	-0.02	1.00	0.28
Y. Corn	BF	0.31	0.30	0.15	0.12
	AF	0.13	0.12	0.10	0.30
•	Reduction	0.18	0.18	0.05	-0.18
R. Bran	BF	0.43	0.03	0.06	0.03
	AF	0.01	0.01	0.01	0.01
	Reduction	0.42	0.02	0.05	0.02
Grass	BF	0.01	0.01	0.05	0.02
	AF	0.00	0.00	0.04	) 0.10
	Reduction	0.01	0.01	0.01	-0.08
M	leans	0.16"	0.Q5b	0.27e	-O.01b

BF) before fermentation; AF) after fermentation; different superscript under the same column or raws, means significantly different at (P<0.05)

Table 11 and 12 showed that protection of linolenate was similar figure with total lipid, with the maximum protection with 10% formaldehyde. It can be concluded that for protection of dry matter, 2.5% would be enough.

### The effect of formaldehyde on production of NH<sub>3</sub>

Production of N-NH<sub>3</sub> during fermentation in vitro presented in Table 13.

Table 13. The effect of HCHO and time storage on the production of NH<sub>3</sub> (mM).

Feedstuffs	HCHO (%) -		Manna			
		0	2	rage (days) 4	6	- Means
KPO Meal	0	18.00	19.81	12.77	13.03	
	2.5	9.17	5.78	5.41	10.87	
	5.0	8.82	12.60	8.59	8.52	
	7.5	8.86	7.02	7.33	7.26	
	10.0	10.26	6.54	3.85	8.48	
		11.02	10.35	7.59	9.63	9.65±1.48
Y.Corn	, 0	18.52	8.50	10.56 <sub>301</sub> 3	13.80	
	2.5	9.06	9.43	15.22	10.87	
	5.0	6.92	5.40	5.08	6.39	
	7.5	6.35	4.69	6.34	4.40	
	10.0	3.95	5.12	2.88	3.74	
		8.96	6.63	8.02	7.84	7.86±0.96
R. Bran	0	15.72	20.30	15.04	13.03	
	2.5	16.65	17.44	17.78	10.06	
	5.0	8.55	10.40	13.61	9.99	
	7.5	4.99	8.10	14.99	7.16	
	10.0	11.48	10.08	7.22	5.51	
		11/47	13.26	13.73	9.15	11.90±2.08
Grass	0	18.48	13.09	11.00	17.04	
	2.5	12.83	8.10	6.26	15.73	
	5.0	13.78	10.80	11,47	9.88	
	7.5	14.12	3.61	8.18	13.96	
	10.0	16.21	12.12	10.03	9.10	
		15.08	9.54	9.39	13.14	11.79±2.79
Means		11.63±2,54	9.94±2.73	9.68±2.80	9.94±2.26	

There were no effect of storage time on N-NH<sub>3</sub> production. In Table 14, presented the effect of level HCHO on the production of N-NH<sub>3</sub> during fermentation. From the feedstuffs point of view, yellow corn showed significantly lower production N-NH<sub>3</sub> than kernel palm oil meal, pasture grass, and rice bran (P<0.05). Formaldehyde was effective in protecting protein from microbial degradation. The higher level of formaldehyde, the smaller N-NH<sub>3</sub> produced during fermentation. The decrease of production was significantly affected at 5% level of formaldehyde (P<0.05), then no further significant reduction. N-NH<sub>3</sub> production was 15.68  $\pm$  3.64, 11.92  $\pm$ 3.60, 9.52  $\pm$  2.96, 8.58  $\pm$  4.02 and 10.14  $\pm$  5.01 mM at the level 0, 2.5, 5.0, 7.5 and 10% formaldehyde respectively.

14.4

There were no interactions between feedstuffs, formaldehyde level, and time of storage (P<0.05); and so interaction between two factors (between feedstuffs and formaldehyde; feedstuffs and time of storage; level of formaldehyde and time of storage).

Table 14. The effect of HCHO level on the production of N-NH<sub>3</sub> (mM) during fermentation in vitro

Feedstuffs	1.	Means				
	0	2.5	5.0	7.5	10	
KPO Meal	18.00	9.17	8.82	8.86	10.26	11.02±3.94a
Yellow Corn	10.52	9.06	6.92	6.35	3.95	7.36±2.53b
Rice Bran	15.72	16.65	8.55	5.00	10.13	11.21±±4.92a
Grass	18.48	12.83	13.78	14.12	16.21	15.08±2.26
Means	15.68±3.64a	11.92±3.60b	9.52±2.96	8.58±4.02b	10.14±5.01b	-

Different superscript in the same column or rows means significantly different at (P<0.05)

### VFA Production

Production of VFA is shown in Table 15 and 16.

Table 15. The effect of time storage on the production of VFA (mM) during fermentation

Feedstuffs		Means			
	0	2	4 3 NC	6	-
KPO Meal	123.38a	110.38c	128.50a	110.38a	118.16±9.22
Yellow Corn	137.00a	130.88bc	127.00Ь	114.25b	127.28±9.61
Rice Bran	143.12a	162.38a	136.00c	158.00c	149.87±12.39
Grass	129.88a	151.62ab	150.00c	133.75c	141.31±11.10
Means	133.34a±8.57	138.81b±23.03	135.38b±10.51	129.10c±21.82	

Different superscript in the same column or rows means significantly different at (P<0.05)

Feedstuffs were significantly affected VFA production. The lowest VFA production came from grass (141.3±11.10 mM) which was significantly lower than the other feedstuffs (P<0.05). The highest production of VFA came from rice bran (149.87±12.39) and yellow corn

### **CONCLUSION**

From the results of this experiment, it can be concluded that direct employment (sprayed) of formaldehyde to the feedstuffs can protect unsaturated fatty acids from rumen microbial hydrogenation. The results open the path to continue to *in vivo* experiments in order to produce a healthier meats, rich in unsaturated fatty acids, for human.

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