The Use Of Anaerobic Enzymes And Bacteria In Animal Feed Industry

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INTRODUCTION

- Exogenous enzymes have been used extensively :

  - to remove anti-nutritional factors from feeds,

  - to increase the digestibility of existing nutrients,

  - and to supplement the activity of the endogenous enzymes of poultry

- Researchers in the 1960s examined the use of exogenous enzymes but responses were variable and no effort was made to determine the mode of action of these products.

- **Furthermore, production of exogenous enzymes was expensive at the time and it was not economically feasible to apply these preparations at the concentrations necessary to elicit a positive animal response**

- . Recent reductions in fermentation costs, together with more active and better defined enzyme preparations, have prompted researchers to re-examine the role of exogenous enzymes in ruminant production .

- Several studies have attempted to define possible modes of action of these additives. Exogenous enzymes could exert a number of effects, both on the gastrointestinal microflora and on the ruminant animal itself. It is highly probable, therefore, that physiological responses to exogenous enzymes are multi-factorial in origin.

Anaerobic enzymes

- They are enzymes produced by anaerobic bacteria. Their homology are almost similar to those produced inside the reticulo-rumen complex.
- Most of the enzymes are in the markets are produced from fungus. It was proven that are in the back seat if they are compared to those produced from their natural habitat to act in their normal pathways.

Material And Methods

- **the tested roughages :**
- **rice straw, RS;**
- **corn stalks, CS and**
- **sugarcane bagasse,**

- <u>ZAD</u> (patent on: 22155) is biotechnical product made from natural sources to elevated level of cellulase enzyme from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes (cellulase, 8.2 u/gm; hemi-cellulase, 6.2 u/gm; amylase, 64.4 u/g and protease, 12.3 u/gm).

- The experimental treatments were :
- 3 liters of ZAD compound
- added to 1000 litter water
- + 50 kg molasses and
- 20 kg urea for 1 ton of the feedstuff.

- **The two stage technique of Tilley and Terry (1963) as modified by Marten and Barnes (1979) was used to study the effect of the biological treatment of the tested roughages on *in vitro* dry matter (IVDMD)**

- Rumen Liquor was obtained from two fistulated rams kept on high quality hay. The fluid was strained through 4 layers of cheese cloth. The McDougal buffer (shown in the following table) was prepared, then urea was added at the rate of one g/L of buffer and mixed with rumen liquor at the ratio of (2 buffer: 1 rumen liquor).

- This mixture was saturated with CO_2 and warmed at 39°C in a water bath. A volume of 250 ml of the mixture was added to each polyethylene tube containing 0.5g air dried sample (in triplicate), then saturated with CO_2 and tightly closed with rubber stopper fitted with outlet valve and incubated at 39°C in a water bath for 48 hours.

- **Tubes were well shaken twice daily during incubation period. At the end of the incubation period, 6 ml of HCl (20%) was added gradually to each tube followed by 2 ml of 5% pepsin. Tubes were closed and incubated in the water bath at 39°C for another 24 hours.**

- Samples were filtered using a dry weighed Whatman filter paper (No. 54) and the residue was washed with boiled water; the filter paper and residue was dried at 100°C overnight and weighed after cooling in a desiccator

- The IVDMD was calculated using the following equation:
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$$VDMD = \frac{\text{wt of substrate DM} - \text{wt of residual DM}}{\text{wt of substrate DM}} X\, 100$$

- Where wt of substrate DM = dry tube plus sample – dry tube; wt of residual DM = dry tube plus residue – dry tube.

Digestibility trials:

- **Two 3 x 3 Latin square designs were applied in this part of the study in order to determine the *in vivo* digestibility. Three Ossimi rams (with average live body wheat of 45 kg) were used in each design. Sheep were housed in individual metabolic crate.**

- **Feed was offered twice daily at 8:00 and 14.00 hr. Fresh water was available at all times. Samples of feed were taken daily at 8:00 hr in the morning and kept in glass bottle at the laboratory for later analysis. Feces and urine were quantitatively collected daily.**

- Weight of total feces and volume of urine were recorded daily in the morning. The representative sample (10%) were taken from each animal during the collection period; each sample was sprayed with a solution of 10% formaldehyde in addition to 10% H_2SO_4 solution then the sample were dried in the forced air oven at 60-65 °C until constant weight was reached.

- The dried fecal samples for each animal were mixed and kept for labortory analysis. Urine samples were also collected daily for each animal in glass botteles contained fifty ml diluted sulfuric acid (10%) to avoid ammonia losses. Digestibility of all nutrients (CP, CF, EE and NFE were determined for each animal.

- In order to determine the rumen fermentation characters (*p*H, VFA and NH_3-N), samples of rumen fluid were obtained at 0, 2, 4 and 6 hr after feeding. The rumen samples were collected through the cannula.The fluid was strained through 4 layers of cheese cloth and *p*H was immediately determination.

- Then a preservative was added to keep ammonia nitrogen unchanged. The liquor was stored in deep freezer at (-20 C) until chemically analyzed using dried glass bottles with adding 0.5 ml toluene and 1 ml paraffin oil to each sample.

Proximate analysis:

- **Samples of the untreated and treated roughages were ground and subjected in duplicate to proximate analysis. Samples of daily output of feces were taken after drying at 60 °C for 24 hr and then they were also ground. All the ground samples were stored in stoppered bottles.**

- Dry matter (DM), crude protein (CP), and crude fiber (CF) and ash of the feedstuffs and feces samples were determined according to A.O.A.C. (1990). The nitrogen free extract (NFE) was calculated by difference.

- Representative samples of the tested roughages, experimental rations and feces were analyzed for fiber fraction according to Van Soest and Breston (1979) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Cellulose and hemi-cellulose were determined by difference.

ZADO

- It is a powder product (patent) contains group of enzymes from anaerobic sources.
- It could be added during the peltzing operation. It can handle heat and pressure of the manufacturing process.
- It could be added as 5 kg/ton of feed
- It could be offered as 40 gm/head/day for cattle ,w.buffalo and camels.
- It is offered as 10 gm/head/day for sheep

RESULTS AND DISCUSSION

Effect of ZAD on chemical composition of Bagasse, Rice straw and Corn stalks

item	Bagasse		Rice straw		Corn stalks	
	control	with ZAD	control	with ZAD	control	with ZAD
DM	90.1^{b}	83.4^{a}	91.3	90.1	89.3	85.98
OM	95.4^{e}	84.4^{a}	87.70	85.4	92.6^{e}	79.1^{a}
CP	1.9^{a}	11.2^{e}	3.50^{a}	12.50^{d}	4.3^{a}	14.1^{e}
EE	1.40^{a}	4.0^{d}	1.2	2.4	2.4^{a}	3.6^{b}
NFE	46.3^{d}	39.1^{ab}	40.4^{e}	36.5^{b}	44.6^{c}	37.2^{a}
CF	48.3^{f}	30.6^{a}	38.3^{c}	36.7^{a}	36.8^{e}	25.2^{a}

a and b Means with different superscripts on the same column are different at (P<0.01).

Effect of ZAD on fiber fraction of Bagasse, Rice straw and Corn stalks

item	Bagasse		Rice straw		Corn stalks	
	control	with ZAD	control	with ZAD	control	with ZAD
NDF	70.92[b]	59.5[a]	63.67[b]	52.3[a]	61.5[b]	52.4[a]
ADF	50.97[b]	40.4[a]	43.7[b]	32.2[a]	42.1[b]	31.5[a]
ADL	12.01	11.3	11.2	9.86	10.1	9.7

a and b Means with different superscripts on the same column are different at ($P<0.01$).

Table (2): Effect of biological treatment on IVDMD

HOURS	Baggasse		Rice straw		Corn stalks	
	control	with ZAD	control	with ZAD	control	with ZAD
2	7.9[b]	11.5[a]	9.6[b]	14.6[a]	8.3[b]	15.2[a]
4	16.8	17.7	14.3	18.1	18.7[b]	24.1[a]
6	29.6[b]	43.7[a]	31.8	36.8	34.0[b]	40.3[a]
24	37.4[b]	59.8[a]	44.0	44.3	55.3[b]	64.1[a]
48	69.4[b]	83.4[a]	57.3[b]	68.6[a]	69.7[b]	82.5[a]

a and b Means with different superscripts on the same column are different at ($P<0.01$).

Table (3) Effect of biological treatment on digestion coefficient in sheep

Item	Baggasse		Rice straw		Corn stalks	
	control	with ZAD	control	with ZAD	control	with ZAD
DM,%	70.2^{b}	86^{a}	69.4^{b}	82.6^{a}	59.4^{b}	72^{a}
OM,%	79.5^{b}	83.6^{a}	73.8^{b}	87.7^{a}	63.5^{b}	82.6^{a}
CP,%	62.3^{b}	69.6^{a}	35.4^{b}	61.8^{a}	54.3^{b}	77.3^{a}
CF,%	60.4^{b}	76.5^{a}	55.2^{b}	87.8^{a}	60.3^{b}	79.4^{a}
EE,%	82.6^{b}	76.9^{a}	62.4^{b}	88.1^{a}	90	84
NFE,%	83.3	82.2	75.2^{b}	87.6^{a}	70.3	70.8
NDF,%	60.1^{b}	72.8^{a}	69.2^{b}	79.1^{a}	67.3^{b}	86.1^{a}
ADF,%	73.1^{b}	81.6^{a}	71.2^{b}	88.4^{a}	60.3^{b}	88.2^{b}
TDN,%	51.3^{b}	72.2^{a}	54.9^{b}	70^{a}	48.8^{b}	70.2^{a}

a and b Means with different superscripts on the same column are different at ($P<0.01$).

Table (4) : Effect of biological treatment on rumen TVA"s (M.EQU./100 ML) in sheep

Hours	Baggasse		Rice straw		Corn stalks	
	control	with ZAD	Control	with ZAD	control	with ZAD
0	9.1	10.6	9	10	9	10.8
2	10.8[b]	13.9[a]	10.8[b]	12.6[a]	10[b]	12.1[a]
4	13.2	12.9	12[b]	16[a]	11.1[b]	14.2[a]
6	11.1[b]	13.4[a]	10	11.5	9.9[b]	13.4[a]

a and b Means with different superscripts on the same column are different at ($P<0.01$).

Feeding Enzyme Powder to Ruminants

DAIRY

Milk production and composition for cows fed diets containing enzyme treatments applied to different components of a TMR.

	Treatments		
Item	CTRL	with ZADO	SE
Milk production, kg/d			
Actual	14.0[b]	15.6[a]	0.4
4% FCM	12.9[b]	15.5[a]	1.2
3.5% FCM	14.0[b]	16.8[a]	
Energy Corrected Milk,KG	13.9[b]	17.1[a]	
Milk composition, %			
Fat	3.8[a]	3.9	0.1
Protein	3.5	3.6	0.1
Lactose	4.5	4.5	0.05
Milk composition, kg/d			
Fat	0.55	0.62	0.06
Protein	0.49	0.56	0.02
Lactose	0.63	0.71	0.04
Milk/kg DMI	0.85	0.89	0.04

a and b Means with different superscripts on the same column are different at ($P<0.01$).

ZADO and dairy breed

item	Fresian		Brown Swiss	
	con trol	zado	control	zado
No. of animals	147	147	54	54
Milk production/d/head	30.1	32.1	12.2	14.4
Effect of ZADO	6.30%		7%	
fat %	3.5	3.8	3.7%	4.2%
mastitis cases	2	0	0	0
net margin for treatment,egy	0	2.18	0	2.98
ration content				
DMI, KG	19.7	19.7	16.9	16.9
TDN,%	76	76	77	77
CP,%	16.5	16.5	16.8	16.8
ADF,%	20	20	19	19
Nel (Mcal/kg DM)	1.7	1.7	1.68	1.68

a and b Means with different superscripts on the same column are different at (P<0.01).

SHEEP

Effect of ZADO on SHEEP performance

	Control	+ ZADO®	SEM
No. of lambs	50	50	---
Experimental period	90	90	
Initial live-weight, kg	18.3	18.5	0.25
Final live-weight, kg	32.8[c]	38.9[a]	5.05
Live-weight gain, kg/d	0.24[c]	0.34[a]	0.062
DMI,kg/head/day	1.23	1.72	
Net profit, US $/head/day	0.97	1.38	
Cost in US $ of ZADO/head/day	0	0.002	
Feed efficiency, kg DM/kg live weight gain	5.1	5.06	0.07

a and b Means with different superscripts on the same column are different at (P<0.01).

FATTENING CATTLE AND W.BUFFALO

Effect of ZADO on cattle and w.buffalo performance

Item	CATTLE		W.BUFFALO		SEM
	control	plus ZADO	control	plus ZADO	
1st period					
feeding days	91	81	83	78	3.55
no. of animals	54	50	60	62	4.78
initial wt.,kg	152	155	153	149	10.2
final wt.	251	255	248	249	2.5
DMI,kg/d	5	5.1	5	5	0.26
ADG, kg/d	1.1[b]	1.24[a]	1.15[b]	1.28[a]	0.34
Kilograms offeed DM:Kilograms of gain	4.55	4.11	4.35	3.91	1.25

a and b Means with different superscripts on the same column are different at ($P<0.01$).

Item	CATTLE		W.BUFFALO		
	control	plus ZADO	control	plus ZADO	
2nd period					
feeding days	102.4	92.0	109.1	94.2	
no. of animals	54	50	60	62	0.12
initial wt.,kg	250	255	248	250	4.6
final wt.	375	382	379	381	8.4
DMI,kg/d	7.81	7.96	7.84	7.9	6.9
ADG, kg/d	1.22[b]	1.38[a]	1.2[b]	1.39[a]	0.35
Kilograms of feed DM:Kilograms of gain	6.40	5.7	6.5	5.6	8.9

a and b Means with different superscripts on the same column are different at (P<0.01).

Item	Cattle		Water buffalo		±SE
	control	plus ZADO	control	plus ZADO	
3rd period					
Feeding days	56.8	48.6	56.0	52.1	
no. of animals	54	50	60	62	0.12
initial wt.,kg	375	382	379	381	8.4
final wt.	446	452	448	454	9.1
DMI,kg/d	10.2	10.4	10.4	10.4	5.8
ADG, kg/d	1.25^{b}	1.44^{a}	1.23^{b}	1.4^{a}	0.47
Kilograms of feed DM:Kilograms of gain	8.16	7.2	8.4	7.4	9.4

a and b Means with different superscripts on the same column are different at (P<0.01).

Item	CATTLE		W.BUFFALO		±SE
	control	plus ZADO	control	plus ZADO	
The overall calculations					
Feeding days	250.2	221.6	248.2	224.3	12.6
ADG, kg/d	1.19^{b}	1.3^{a}	1.1^{b}	1.3^{a}	0.3
Kilograms of feed DM:Kilograms of gain	6.3	5.7	6.4	5.6	7.5

a and b Means with different superscripts on the same column are different at ($P<0.01$).

SUMMARY

- Using ZAD to treat rice straw , bagasse and corn stalks during the ensiling period of 4 weeks resulted the following:
- 1. decreased the CF for the experimental products in a range from 30.3 to 36.6%
- 2. An improvement in the CP wss recorded more with 80% from microbial protein orgin

- 3.ZAD decreased NDF rice straw , bagasse and corn stalks in range from 30.3 to 36.6%
- 4. The same trend went through for ADF in range of 20.6 to 26.3 %
- 5. INVDMD extent improved in the treat fibrous materials to reach the 80% level in comparison to the control group which didn't exceed the 60's in percentage wise.

- In Vivo results showed an increase in the digestibility for DM, OM, CP,EE, CF, NDF AND ADF which reflected on the TDN value to reach the 70’s in animals fed ZAD treated tested by-products in comparison to animals fed untreated rice straw or bagasse or corn stalks (50’s %)

- Total volatile fatty acids in animals fed ZAD treat rice straw or treated bagasse or treated corn stalks went up with the time progress after feeding. That is showing an increase in the microbial digestion efficiency Also, that will be reflected on available energy through the metabolism system of the animal.

- Feeding ZADO improved milk production by 1.5 / head/day all the milking season
- It increased the meat production by 150 gm /hea/day for cattle and w.buffalo.
- Sheep produced 100 gm head per day as result of feeding zado powder.

Thanks

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