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Effects of Exogenous Enzymes, *Lactobacillus acidophilus* or their Combination on Feed Intake, Digestibility and Performance of Rabbits Fed Sugarcane Bagasse

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ABSTRACT

Abdel-Aziz, N.A., El-Adawy, M.M., Salem, A.Z.M., Cerrillo-Soto, M.A., Camacho, L.M. and Borhami, B.E. 2014. Effects of exogenous enzymes, Lactobacillus acidophilus or their combination on feed intake, digestibility and performance of rabbits fed sugarcane bagasse. Animal Nutrition and Feed Technology, 14: 137-145.

The aim of this study was to evaluate the effect of *Lactobacillus acidophilus* (LAC), exogenous enzymes ZAD® or their combination on feed intake, nutrient digestibility and performance of rabbits fed increasing levels of sugarcane bagasse (SCB). Five rations were allotted randomly to five groups of New Zealand white (NZW) rabbits (838±42.4g average BW at 5 weeks of age. Rabbits were fed: (i) a control diet made of 100% berseem hay and 0% SCB, (ii) 50% berseem hay and 50% untreated SCB (USCB), (iii) 50% berseem hay and 50% SCB treated with *Lactobacillus acidophilus* (LAC), (iv) 50% berseem hay and 50% SCB treated with ZAD® (ZAD), and (v) 50% berseem hay and 50% SCB treated with LAC+ZAD® (LZ). Treatment of SCB with *Lactobacillus acidophilus,* ZAD® and LAC+ ZAD® led to higher CP content and lower contents of ADF, NDF and DM. Total DM intake was not affected by treatments. Digestibility coefficient of CP for LAC and LZ were higher ($P<0.05$) compared to the other groups. The BW for LAC and LZ rabbits was higher ($P<0.05$) than for the other groups, while the BW for LAC rabbit was the highest ($P<0.05$) and for USCB was the lowest. It can be concluded that treating sugarcane bagasse with *Lactobacillus acidophilus*, exogenous enzymes of ZAD® or their combination improved feeding values; however, the treatment with *Lactobacillus acidophilus* was found to be the best.

Key words: Exogenous enzymes, Feed digestibility, *Lactobacillus acidophilus,* Sugarcane bagasse.

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INTRODUCTION

Rabbits have been recognized to have a very important role in the supply of animal protein to humans. Moreover, rabbit occupies a vital midway between and mono-gastric animals and ruminants which can effectively utilize cellulose rich feeds or rations containing less than 20% grain. Rabbits are herbivores which can be successfully raised on diets that are high in roughage such as sugarcane bagasse (Cheeke, 1986). Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below poultry (Hasanat *et al.*, 2006). However, improved feed formulation and strategies for enhancing the production potential of rabbits especially in tropical and subtropical regions of the world have not been fully exploited (Falcão-e-Cunha *et al.,* 2007).

Several studies have been attempted to incorporate exogenous enzymes, *Lactobacillus acidophilus* bacteria and their combination in rabbit diets to improve nutrient availability (Kholif *et al.*, 2005). Some did not observe any significant effect of enzymes on rabbit performances (Falcão-e-Cunha *et al.,* 2004); others showed lower mortality of rabbits (García *et al.,* 2005) besides improved feed conversion ratio (Eiben *et al.,* 2004). Additionally, co-supplementation decreased serum urea but the serum total proteins, AST and ALT were within the normal values (Gado *et al.*, 2006). These have been considered as an advantage, as they improve the impact of the supplemented enzymes on caecal fermentation pattern in the rabbit metabolism (Abdl-Rahman *et al.,* 2010). The objective of this study was to evaluate the effect of *Lactobacillus acidophilus* (LAC), the exogenous enzymes ZAD® or their combination on feed intake, nutrients digestibility and performance of rabbits fed sugarcane bagasse.

MATERIALS AND METHODS

The study was carried out at the Rabbit Research Laboratory of the Animal and Fish Production Department, Faculty of Agriculture (El-Shatby), Alexandria University.

Animals, management and growth trial

A total of 50 male weanlings New Zealand White rabbits of about 5 weeks old and an average initial BW of about 838$\pm$42.4g were allotted at random to five groups ten rabbits each. The animals were housed individually and the experiment was conducted up to 12th weeks of age (marketing weight). Body weight (BW, g) and feed consumption (g/d) were individually recorded each week. Each rabbit was weighed individually before the morning feeding.

Rabbits were individually housed in galvanized metal cages under the same managerial conditions in a well-ventilated building. Fresh water was available all the time through stainless steel nipples available in each cage. The experimental diets were offered pelleted to rabbits *ad libitum*. All rabbits were kept under the same hygienic, environmental and management conditions.

Experimental design and treatments

A control diet was formulated to meet the nutrient requirements for growing rabbits according to NRC, (1984) and De Blas, (1986) in which berseem clover (*Trifolium alexandrinum*) hay was the main source of fibre. The experimental design was a complete randomized design, with five treatments and 10 replications. The treatments comprised: (i) a control diet made of 100% berseem hay and 0% SCB, (ii) 50% berseem hay and 50% untreated SCB (USCB), (iii) 50% berseem hay and 50% SCB treated with *Lactobacillus acidophilus* (LAC), (iv) 50% berseem hay and 50% SCB treated with ZAD® (ZAD), and (v) 50% berseem hay and 50% SCB treated with 25% LAC+ 25% ZAD® (LZ). All diets were iso-nitrogenous (14±0.6% CP) and iso-caloric (2093±50.0 kcal/kg) and contained similar levels of micro-elements (Table 1).

The treatment with *Lactobacillus acidophilus* was carried out according to Verbiscar *et al. (*1981). Briefly, skimmed milk was inoculated with *Lactobacillus acidophilus* at 30°C for 8 days. The SCB was sprayed with 6 L media of plus 50 L water/200 kg SCB and supplemented with 5 kg soya bean plus 10 kg molasses and mixed thoroughly. The treated SCB was then compressed in the form of bales which were covered tightly using polyethylene sheets and kept under room temperature (about 26°C) for 47 days.

The treatment with ZAD (ZAD is biotechnical product made from natural sources and was with the enzyme activity: cellulase, 8.2 U/g; hemi-cellulase, 6.2 U/g; amylase, 64.4 U/g and protease, 12.3 U/g) was carried out by spraying the SCB with packets of ZAD dissolved in 50 L water/200 kg of SCB and supplemented with 5 kg soya beans plus 10 kg molasses and mixed thoroughly. The treated SCB was compressed into bales and incubated as started above. For the treatment with combined *Lactobacillus acidophilus* and ZAD, the SCB was sprayed with 6 L media plus 2 packets of ZAD products dissolved in 50 L water/200 kg SCB and supplemented with 5 kg soya bean plus 10 kg molasses as mentioned earlier.

Digestibility trial

The trial was conducted using 20 male NZW rabbits (1.9±0.30 kg) at the end of the growth performance experiment; four rabbits from each group were randomly chosen to determine the nutrient digestibility coefficients of the experimental diets. Rabbits were kept individually in metabolic cages (60 x 40 x 24 cm) that allowed faecal collection. After seven days of adaptation, the feed actually consumed and total faecal output were measured for six consecutive days according to the European reference method for rabbit digestion trails (Perez *et al.*, 1995). Feed was offered once daily at 10:00 AM and actual feed intake was determined. During the collection period, faeces from each rabbit were collected before offering the daily meal, and the weight determined. Samples of daily faeces (20%) of each rabbit were collected every day, dried at 105°C for 24h, bulked, mixed, grounded to 1 mm and kept for later chemical analysis.

Table 1. Formulation and chemical composition of control and experimental diets

Items	Diets†				
	Control	USCB	LAC	ZAD	LZ
Ingredients (kg)					
Berseem hay	30.0	15.0	15.0	15.0	15.0
Sugarcane bagasse (SCB)	0	15.0	15.0	15.0	15.0
Yellow corn	18.0	18.0	16.0	16.0	15.5
Wheat bran	16.0	16.0	16.0	16.0	16.0
Barley grain	17.0	17.0	17.0	17.0	17.0
Soya bean	15.0	15.0	15.0	15.0	15.0
Wheat straw	0.0	0.0	2.0	2.0	2.5
Molasses	2.0	2.0	2.0	2.0	2.0
Limestone	1.1	1.1	1.1	1.1	1.1
Table salt	0.5	0.5	0.5	0.5	0.5
Vitamin and mineral premix‡	0.2	0.2	0.2	0.2	0.2
DL-Methonine	0.1	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1	0.1
Chemical composition (%, on DM basis)					
Organic matter	87.6	88.3	88.5	88.4	88.6
Crude protein	15.4	13.9	14.4	14.2	14.2
Ether Extract	3.5	3.2	3.4	3.3	3.4
Crude fiber	13.7	14.9	14.3	14.5	14.4
Nitrogen free extract	55.1	56.2	56.5	56.4	56.5
Ash	12.4	11.8	11.5	11.6	11.4
DE (kcal/kg feed DM)**	2016.4	2074.8	2137.8	2102.4	2134.5
Price/100 kg (L.E.)	2200	1960	1975	1975	1990

†Diets: Control is the basal diet; USCB, control diet with 15% untreated SCB; LAC, USCB diet treated with *Lactobacillus acidophilus*; ZAD, USCB treated with ZAD®; LZ, USCB treated with the combination of LAC and ZAD®;

‡Vitamins and mineral premix per kilogram contained: Vitamin A 2,000,000 IU, Vitamin D_3 150,000 IU,Vitamin K 0.33 mg, Vitamin B_1 0.33 mg.,Vitamin B_2 1.0 g., Vitamin B_6 0.33 g., Vitamin B_{12} 1.7 mg., Pantathonic acid 3.33 g., Biotin 33 mg., Folic acid 0.83 g., Choline chloride 200 mg., Zn 11.7 g., Mn 5.0 g., Fe 12.5 g., Mg 66.7 mg., Se 16.6 mg., Co 1.33 mg., Cu 0.5 g., I 16.6 mg., and Antioxidant 10.0 g;

**DE (Kcal/Kg feed DM) was calculated.

At the end of collecting period, all faecal samples for the six days from each rabbit were composited, ground and stored to form one sample for each rabbit. Representative samples of feed offered and faeces of each rabbit were analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) according to AOAC (1995). Acid detergent fibre (ADF), neutral detergent fibre (NDF), cellulose and hemicelluloses were determined following the procedures of Van Soest *et al.* (1991).

Statistical analysis

Data were analysed by General Linear Model (GLM) procedure (SAS, 2000) adapting the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where, Y_{ij}=the observation on the I^{th} treatment; μ=Overall mean; T_i=Effect of the I^{th} treatment; e_{ij}=Random experimental error; Means separation was performed using Duncan Multiple Range test.

RESULTS

The chemical analysis of the diet (Table 1) containing sugarcane bagasse (SCB) showed low content of CP in comparison with the control diet. Contents of CF and NFE in the SCB-diets were higher than that with only barseem hay (control). The CP in LAC, ZAD and LZ treated SCB increased by 1.55, 1.45 and 1.52-fold diets in comparison with the untreated SCB.

The treatment with *Lactobacillus acidophilus* was more effective than the ZAD in reducing the CF content of USCB. The biological treatment of SCB with the combination of LAC and ZAD i.e., LZ resulted in intermediate values for CF and NDF as compared to LAC and ZAD (Table 2).

Replacing 50% of berseem hay with untreated SCB did not affect consumption of feed (DM) during 5-12 weeks of age. Replacing 50% of berseem hay with untreated SCB increased ($P<0.05$) the DMI at 12^{th} week of age and overall average DMI during the period (5-12 weeks of age) with increases ($P<0.05$) of about 11.3 and 5.5%, respectively, when compared to the group fed the control diet. Treating SCB biologically decrease ($P<0.05$) the DMI than those fed USCB diet. The overall DMI in group fed LAC diet was not affected during the whole experimental period (Table 3).

Replacing 50% of berseem hay with untreated SCB in rabbits' rations decreased ($P<0.01$) digestibility of DM, OM, CP and CF. Feeding diets of SCB treated with LAC and LZ increased all nutrients digestibility coefficients compared to USCB and control diets (Table 4).

The replacement of berseem hay with untreated SCB resulted in decreased ($P<0.05$) the BW during most of the experimental weeks. The final BW of rabbits

Table 2. Chemical composition of control and experimental diets (% DM basis)

Chemical composition (%)	Control diet	USCB	Experimental diets		
			LAC	ZAD	LZ
Dry matter	88.7	89.7	42.6	38.9	39.3
Organic matter	86.1	95.9	93.8	93.6	93.6
Crude protein	13.7	2.9	4.5	4.2	4.4
Ether extract	2.4	1.2	2.1	1.9	2.1
Crude fiber	28.4	43.8	28.3	33.2	29.9
Neutral detergent fiber	52.3	77.4	59.6	64.5	62.1
Acid detergent fiber	38.8	96.2	65.3	72.2	68.1
Nitrogen free extract	41.7	48.0	58.9	54.3	57.1
Ash	13.9	4.0	6.2	6.4	6.4

Table 3. Dry matter intake (g/d) of growing NZW rabbits during 5-13 weeks of age

Weeks	Dietary group†					SE	P value
	Control	USCB	LAC	ZAD	LZ		
Week 5-6	492[ab]	543[ab]	546[ab]	579[a]	522[ab]	26.4	*
Week 6-7	526[ab]	530[ab]	517[b]	505[b]	546[a]	15.5	*
Week 7-8	575	590	586	535	574	17.6	NS
Week 8-9	576[ab]	590[a]	586[a]	546[b]	583[ab]	12.1	*
Week 9-10	565[ab]	605[a]	553[b]	558[b]	540[b]	13.4	*
Week 10-11	559	584	541	557	547	15.5	NS
Week 11-12	548[bc]	610[a]	564[ab]	533[bc]	505[c]	13.9	**
Over all	3843[b]	4053[a]	3895[ab]	3813[b]	3825[b]	47.7	*

†Diets: Control is the basal diet; USCB, control diet with 15% untreated SCB; LAC, USCB diet treated with *Lactobacillus acidophilus*; ZAD, USCB treated with ZAD®; LZ, USCB treated with the combination of LAC and ZAD®;

[abcd]Means in the same row bearing different letters, differs significantly; ** ($P<0.001$), * ($P<0.05$) and NS=not significant.

Table 4. Nutrient digestibility of the experimental diets in growing NZW rabbits

Items	Dietary group†					SE	P value
	Control	USCB	LAC	ZAD	LZ		
Dry matter	70.23[c]	65.5[d]	78.9[a]	74.1[b]	77.8[a]	1.7	**
Organic matter	69.5[b]	64.8[c]	78.1[a]	73.1[ab]	77.1[a]	2.2	**
Crude protein	76.6[b]	74.9[c]	83.9[a]	80.6[ab]	81.31[a]	1.9	*
Ether extract	86.9	85.3	89.2	90.5	89.2	2.5	NS
Crude fiber	41.4[b]	32.6[c]	57.0[a]	48.7[ab]	56.9[a]	4.0	*
Nitrogen-free extract	73.8[bc]	69.8[c]	81.4[a]	77.4[ab]	80.5[a]	3.6	**

†Diets: Control is the basal diet; USCB, control diet with 15% untreated SCB; LAC, USCB diet treated with *Lactobacillus acidophilus*; ZAD, USCB treated with ZAD®; LZ, USCB treated with the combination of LAC and ZAD®;

[abcd]Means in the same row bearing different letters, differs significantly; ** ($P<0.01$), * ($P<0.05$) and NS=not significant

Table 5. Body weight of growing NZW rabbits during 5-12 weeks of age

Weeks	Dietary group†					SE	P value
	Control	USCB	LAC	ZAD	LZ		
Initial LBW (week 5)	835.3	849.6	832.0	838.9	832.5	42.4	NS
Week 6-7	948.2[ab]	932.8[b]	966.0[a]	986.8[a]	982.0[a]	24.7	*
Week 7-8	1095.3	1073.3	1103.4	1109.1	1131.1	27.0	NS
Week 8-9	1254.2[a]	1213.0[b]	1264.5[a]	1275.3[a]	1305.2[a]	39.0	*
Week 9-10	1417.9[b]	1368.9[c]	1461.5[a]	1459.5[a]	1477.5[a]	28.3	*
Week 10-11	1579.7[b]	1530.5[c]	1653.5[a]	1611.4[ab]	1630.8[a]	24.1	*
Week 11-12	1707.7[bc]	1655.0[c]	1816.1[a]	1736.5[b]	1745.8[b]	23.4	*
Final BW (week 12)	1874.2[b]	1805.1[c]	1979.2[a]	1883.5[b]	1923.8[ab]	24.7	**

†Diets: Control is the basal diet; USCB, control diet with 15% untreated SCB; LAC, USCB diet treated with *Lactobacillus acidophilus*; ZAD, USCB treated with ZAD®; LZ, USCB treated with the combination of LAC and ZAD®;

[abcd]Means in the same row bearing different letters, differs significantly; ** ($P<0.001$), * ($P<0.05$) NS=not significant.

fed diet containing untreated SCB was lower ($P<0.05$) by 3.7% than those fed the control diet. Feeding SCB treated diets improved ($P<0.05$) the LBW during most of the experimental weeks except for the 7th weeks of age compared to those fed diets which contained untreated SCB. Feeding diets that included treated SCB did result in changes in BW till the 11th week of age. The results showed that feeding rabbits LAC diet reached the highest ($P<0.05$) final BW with a 10% increment than those fed USCB. Rabbits fed on ZAD or LZ the diets registered lower final BW ($P<0.05$) than those fed LAC diet with an increment by 4.3 and 6.6% than those fed on USCB diets, respectively (Table 5).

DISCUSSION

The increase in the CP of the treated diets may be due to the treatment with *Lactobacillus acidophilus,* ZAD® and the is combination. The treatment of SCB decreased the CF and fibre fractions which may be due to the microbial degradability during the incubation period. During the incubation period of the silage, the microorganisms that were found in the treatments produced cellulase enzyme which improved nutrients (Khorshed, 2000; Kholif *et al.,* 2005; El-Banna *et al.*, 2010).

The BW of the rabbits was improved with the treated diets due to the improvement of digestibility and caecal fermentation. El-Banna *et al.* (2010) found that the BW of rabbits fed on potato vines treated biologically with *Lactobacillus acidophilus* was significantly increased compared with the untreated one. El-Adawy and Borhami (2001) and El-Adawy *et al.* (2000) showed that the BW of rabbits fed diets supplemented with Biogen and microbial phytase increased compared with the control. However, El-Banna *et al*. (2010) found that the BW of rabbits fed on SCB treated biologically with brown fungi was inferior to that of rabbits fed the untreated SCB.

The results showed that the replacement of 50% of berseem hay by SCB had a negative effect on the weekly body weight gain and with age this negative impact diminished, this could be due to the difficulty of digesting fibres of SCB in the gastrointestinal tract which may be due to incomplete formation of the cecum and the low microbial activity in the small rabbits (5-7 weeks old). Nonetheless, during 8 to 12 weeks of age, the cecum becomes fully developed and became capable of digesting fibre and take advantage of the digestion products which led to increase in the body weight.

CONCLUSIONS

Data suggested that the replacement of 50% of berseem hay with SCB treated with *Lactobacillus acidophilus* and the combination of *Lactobacillus acidophilus* and ZAD® in growing rabbit diets is recommended due to its positive effects on feed intake and nutrients utilization leading to higher growth performance.

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