EFFECT OF EXOGENOUSE LOCAL LIGNINOLYTIC CRUDE ENZYMES ON *IN VITRO* DIGESTABILITY AND LIGNIN CONTENT OF ROUGHAGES

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ABSTRACT

In current study use local ligninolytic crude extract enzyme (*HAMU* 8%) to improve IVDMD and reducing lignin content. The result suggest that treated wheat straw (WS), Corncobs (Cc), alfalfa hay (AH), by *HAMU* increased significantly (p <0.05) *in vitro* dry matter digestibility (IVDMD) and reduction in lignin content, However, extra enzyme improvement is shown with Corncobs treated with *HAMU* (average improvement, 19.84%) and lower improvement was shown in alfalfa hay treated with *HAMU* (average improvement, 10.07%). The higher reduction (P<0.01) in lignin content was shown in Cc treated with *HAMU* from 9.8 to 7.901%, with average reduction 1.899%; when the lower reduction was observed significantly (P<0.05) with AH treated from 8.26 to 7.77%, average reduction was 0.49%.It could be recommended, treated low quality roughages with *HAMU* improved the nutritional value of roughages through the digestion of Structural carbohydrates.

Key words: Exogenous local ligninolytic crude enzyme, roughages, IVDMD, lignin
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تاثير استخدام الانزيم المحلي *HAMU* المحلل للكنين على معامل الهضم ومحتوى اللكنين للاعلاف الخشنة

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المستخلص

استخدم الانزيم المحلي(*HAMU*) في الدراسة الحالية بنسبة 8% لتحسين القيمة الغذائية للاعلاف الرديئة النوعية عن طريق تقليل محتواها الاعلاف من اللكنين ، اثبتت النتائج ان معاملة تبن الحنطة Wheat straw(WS)، كوالح الذرة الصفراء Corncobs(Cc) ودريس الجت alfalfa hay (AH). بالانزيم المحلي قبل 24 ساعة من تجربة الهضم المختبري ادت الى زيادة معنوية (P<0.05) في معامل الهضم المختبري للمادة الجافة *in vitro* dry matter digestibility (IVDMD) وقللت من محتوى اللكنين . اعلى تحسين لوحظ عند استخدام الانزيم مع (Cc) بنسبة 19.84% واقل تحسين مع (AH) بنسبة انخفاض10.07%. وادت الى انخفاض عالي المعنوية (P<0.01) في محتوى اللكنين للاعلاف الخشنة ،كان اعلى انخفاض مع (Cc) من (9.8 الى 7.901%) بمعدل 1.899%واقل انخفاض مع (AH) (من 8.26الى 7.77%) بمعدل 0.49%. نستنتج ان معاملة الاعلاف الخشنه الرديئة النوعية بالانزيم المحلي ادى الى تحسين القيمة الغذائية نتيجة تقليل محتواها من اللكنين.

الكلمات المفتاحية :الانزيم المحلي الخام الخارج خلوي *HAMU*، IVDMD، LIGNIN.

INTRODUCTION

The rumen microbial ecosystem is one of the most complicated, diverse, and well-studied microbiological environments. Robert E. Hungate, the father of rumen microbiology, start searching this system in the 1940's and half a decade later, many of his detection still hold true(2).The bacteria in the rumen have co-evolved with the host and produce the enzymes vital to digest plant cell, give the host to get, energy from an another indigestible feed source (Cell wall)(25). The health and productivity of the ruminant is highly dependent on the rumen ecosystem (13,18,23). One of the main issues in the current ruminant animal industry is how the most successful use the employ of feed resources to maximize efficiency of feed digestibility,(27) because feed efficiency ultimately affects profitability and sustainability of the industry. In fact, it has been considered that feed costs account for nearly 75% of the total input costs (17). Moreover, an improvement in feed efficiency has been proposed to bring reductions in nutrient excretion (12) and in methane emissions (11).Forages provide energy and nutrients that are essential for the growth and productivity of ruminant and therefore represent an important feed resource for ruminant production. However, the comparatively high lignin and fiber concentrations in forages limit the extent to which they are digested by ruminant. Therefore, various methods of improving the digestibility of forages have been search. One of the most common and successful forages treatment methods are ammonia or exogenous enzyme.(4)(26)(27). Lignin represents a challenge for enzymatic degradations, for several reasons; first, it has a partially random structure that is racemic and contains several types of ethers and carbon-carbon bonds, which makes more difficult its degradation by specific enzymes. (21).

The aims of this current study are to: Examine the ability of exogenous local crude extract enzyme (*HAMU*) extract from local bacteria *Streptomyces MS* to Degrade various lignin and improve %IVDMD. Of Wheat straw (WS), Corn cobs (Cc), alfalfa hay (AH) as roughages feeds

MATERIALS AND METHODS

Roughages preparation

All roughages was chopped (approximate 1 mm) and divided into two treatments [Untreated roughages, Biological treatments roughages with *HAMU* for (WS, Cc and AH).

Biological treatment

The roughages was placed on a tube (100ml) and spread with 8ml of *HAMU* per 100g of dry matter ,add 0.25ml distilled water Mixed until homogeneity kept in dark at temperature 40^0C for 24 hour. Before us in *in vitro* fermentation. (2).

Rumen collection

Ruminal contents used to prepare the treatment systems were collected from the rumen of slaughtered lamb. Collected ruminal fluids were strained through four layers of cheese cloth into separating tube previously gassed and flush these with CO_2 before use. The strained rumen fluid is kept at 39°C under carbon dioxide and should be prepare adjust before start of the incubation. As the amount of feed taken is 500mg,composition of the medium is according to (14) ; (20).

Preparation artificial saliva

Phosphate-carbonate buffer (artificial salvia) was prepared as described by modified (15) by dissolving 49g Na_2HCO_3, 18.6g Na_2HPO_4, 28.5g KCl, 23.5g NaCl, and 6g $MgSO_4.7H_2O$, 0.5 Resazurine in 800 ml of distilled water. Just before used 2 g $CaCl_2$ was added to the solution and then complete the volume to 1 liter +0.5 L-Cystine, 0.5 Sodium thioglycollate. Kept at 39°C. CO_2 was passed through the solution according to (6;7) The pH of the artificial saliva was adjusted to 7.4 before use (1).

***In vitro* digestibility procedures:**

Approximately 1-2 gm finely ground (particle size < 1 mm) samples of each roughages ,Blank (10 ml ruminal fluids + 40 ml artificial saliva).Control 0.5g roughage (10 ml ruminal fluids + 40 ml artificial saliva).0.5g roughage treated with *HAMU* the enzyme + (10ml ruminal fluids + 40ml artificial saliva).Were weighed in triplicate and placed in 100 ml test tube fitted with gas release valves, CO_2 was being passed through the contents for 10 second then each tube was closed immediately by stopper (Hungate tube).Tubes were incubated at 39°C with periodic shaking for 72 h (6). After 72 h of incubation, fermentation was stopped by attrition of few drops of Hcl to each tube and kept under freezing. The contents of tubes were centrifuged at 2500 rpm for 15 minutes after which the supernatant is poured off. The insoluble residue was filtered off, dried and burned according to standard method of (3). DM residues were recorded after drying and burned to be subtracted from the sample content of DM to provide an estimate of lignin, DM digested, as described by (19).

Chemical analysis:

Proximate chemical analysis of untreated and treated roughages in triplicate per each determination was carried out for dry matter (5).determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) The *In Vitro* digestibility of DM for untreated and treated roughages were determined using the method of (19)(5).

Statistical Analysis

The statistical analysis system-SAS program (21) was used to effect of treatment (*HAMU*) on roughage IVDMD% and lignin content. According to factorial experiment (3x2) (7) multiple range tests was used to significant compare between means in this study. Statistical model: Yijk = μ+ Ai + Bj + AB (ij) + eijk.

RESULTS AND DISCUSSION

Result of local exogenous enzyme (*HAMU*) pre-treatment of roughages on IVDMD% and lignin content .presented in table 1, and table 2.

The effect of treated roughages with exogenous enzyme *HAMU* on IVDMD% is shown in table 1. Treated all roughages (WS, Cc, AH) by *HAMU* increase significantly IVDMD%. However, extra improvement is shown with Corn cobs treated with *HAMU* (average improvement, 19.84) and lower improvement was shown in alfalfa hay treated with *HAMU* (average improvement, 10.07). These differences in IVDMD % across treatments are related to the different source of roughages (different substrate and lignin content).These explanation agree with result reported by (8).when alfalfa hay and wheat straw was treated with high level of exogenous enzyme.

Table 1. Effect of pre-treated roughages with *HAMU* on IVDMD%.

Source of	DM in	IVDMD		IVDMD (%)	Level of
roughages	Fresh%	Untreated	*HAMU*	improvement	sig.
Wheat straw	94.18	20.78	37	16.22	*
Corn cob	94.08	30	49.84	19.84	**
Alfalfa hay	91.72	45	55.07	10.07	**

***(P<0.05).** (P<0.01).**

***HAMU*: local ligninolytic crude extract enzyme (extract from local strain of *Streptomyces* isolated from Iraqi camel degrade feces).**

The effect of pre-treated roughages with exogenous enzyme *HAMU* on lignin content is shown in table 2. All roughages (WS, Cc, AH) pre-treated with *HAMU* indicated significant (P<0.01) reduction in lignin content. The highest reduction (P<0.01)in lignin content was shown in Cc pre- treated with *HAMU* from 9.8 to 7.901%, with average reduction 1.899%; when the lower reduction was observed significantly (P<0.05) with AH pre-treated from 8.26 to 7.77%, average reduction was 0.49%.These reduction in lignin content of roughages after treatment with *HAMU* will improved roughages quality .due to destroy

lignin- carbohydrate linkage and increased soluble carbohydrate released from low quality roughage and improved access to the cell wall matrix by ruminal microorganism which provides additional energy led to enhance and increase microbial colonization of feed particles and increase the rate of digestion (24;22;10;16). (11) found significant reduction in lignin content in WS and AH when pre-treated with exogenous enzyme .

Table 2.Effect of pre-treated roughages with *HAMU* on lignin content (%).

Source of Roughages	lignin content % Untreated	*HAMU*	Average reduction in lignin content %	Level of sig.
Wheat straw	9.4	8.47	0.93	*
Corn cobs	9.8	7.901	1.899	**
Alfalfa hay	8.26	7.77	0.49	**

***(P<0.05). ** (P<0.01).**

***HAMU*: local ligninolytic crude extract enzyme (extract from local strain of *Streptomyces* isolated from Iraqi camel degrade feces)(3).**

CONCLUSION: It could be concluded that ruminant diet pre-treated with 8% or more of *HAMU* improved IVDMD% and reduction lignin content for all roughages, this would be achieved by digestion of structural carbohydrate and destroy lignin–carbohydrate linkage .In addition, biological treatment are preferable than other treatments such as chemical and physical treatments for reduce pollution.

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