Fayoum University
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OPTIMAL PRODUCTION OF PECTINASE AND ITS USE FOR IMPROVING PRODUCTIVE PERFORMANCE OF LACTATING BUFFALOES

By

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5. SUMMARY AND CONCLUSION

The present study was carried out at farm and laboratory of Animal Production Department, Faculty of Agriculture, Fayoum University. While, pectinase production experiments and microbiological analyses were performed at the laboratory of Dairy Sciences Department, National Research Centre، Dokki, Giza, Egypt.

The objective of this study was to produce pectinase under the optimum conditions and evaluated its potential activity compared to commercial pectinase enzyme source. Also to investigate the impact of adding pectinases to lactating buffaloes ration on nutrients digestibility, milk yield and composition, feed conversion and some blood parameters.Also, simple economical evaluation of the tested rations was considered.

This study was performed at two stages:

1. The first stage [Laboratory experiments]

a. Culture conditions for pectinase production

Pectinase was produced by *Fusarium avenaceum*, *Asperigillus fugimatus*, *Cephalosporium acremonium*, *Trichoderma viride* , *Asperigillus flavus NRRL 5522, Aspergillus niger, Fusarium oxysporum, Aspergillus terreus,and Penicillium chrysogenum.* The grown culture was employed as inocula for experimental 1000 ml conical flasks containing 100 ml (BPPM) to test its ability to utilize beet pulp powder as a major carbon source to produce pectinase.

b. *In vitro* experiment of the tested rations:

The *In vitro* DM, OM, NDF, ADF, pH value, TGP, NH3-N, SCFA, ME and NEL for the tested rations were determined. The tested rations consisted of 50% concentrates feed mixture, 20% Egyptian clover, 20 % sugar beet pulp and 10% orange by-products. Locally produced pectinase or commercial one (SMIZYME®) were added to the tested rations at levels of 0, 1, 2, 3, 4 and 5 g / kg DM).

2. The second stage [Farm experiment]:

Twelve lactating Egyptian buffaloes (in their 3rd to 5th lactation seasons and weighed 480±8 kg in average) were used in this study. Twenty days after parturition, buffaloes were randomly assigned to three groups, four animals per each group by using complete randomized design. The experimental period was 70 days.

The first animal group was fed on ration of 50% concentrates feed mixture, 20% Egyptian clover, 20 % sugar beet pulp and 10% orange by-products (control ration).The two pectinase enzymes were supplemented to the rations at the optimum rate which recommended from the *in vitro* experiment. The second group (R1) was fed control ration supplemented with the produced enzyme at level of 3g /kg DM, while the third group (R2) was fed control ration supplemented with SMIZYME® (commercial enzyme) at level of 3 g/kg DM . The effects of adding pectinases on lactating buffalo's performance were studied .

The obtained results revealed that:-

A- Culture conditions for pectinase production revealed the:-

1- *Penicillium chrysogenum* exhibited the highest pectinase activity reached 10.87 µmole/ml/min; therefore it was selected for production of laboratory pectinase enzyme (the produced enzyme) under the optimum fermentation conditions.

2-The maximum production of pectinase enzyme by *Penicillium chrysogenum* was achieved at incubation period of 3 day was 31.55 µmole/ml/min, initial pH 4 was 28.44 µmole/ml/min, yeast extract as a nitrogen sources at a concentration of 0.33 g N/l achieved 33.97 µmole/ml/min and pomegranate peel as a carbon source at a concentration of 15 % (W/V) to give 29.53 µmole/ml/min.

B- The *in vitro* experiment:-

1. All levels of the produced enzyme and SMIZYME® supplementation increased (P≤ 0.05) DM,OM, NDF, ADF, TGP , SCFA, ME and NEL compared to control ration.

2. All levels of the produced enzyme and SMIZYME® supplementation decreased (P≤ 0.05) pH value compared to control ration.

3. No significantly difference was found among control and fibrolytic enzymes ration of *in vitro* NH3-N.

4-Increasing the produced enzyme and SMIZYME® supplementation levels up to 3g enzyme /kg DM gave the highest values of both IVDMD of ration.

C-The *in vivo* experiments:-

1- Apparent nutrient digestibility coeffients:-

Rations supplemented with the produced enzyme (R_1) and SMIZYME® (R_2) significantly (P≤0.05) increased DM, OM, CP and CF digestibility compared to the control ration. No significant differences were found between the produced enzyme and SMIZYME® rations concerning DM, OM and CP digestibilities. Moreover there were insignificant increase among all the tested rations in regarding NFE and EE digestibilities.

2- Nutritive values of the tested rations:-

Rations supplemented with pectinases significantly (P≤ 0.05) increased TDN, SV % and DCP% compared to control ration. On the other hand, there were insignificant increases in TDN, SV and DCP between rations supplemented with pectinases (R1 and R2).

3- Milk yield and its composition:-

Rations supplemented with pectinases ($R_1$and R_2) increased (P≤0.05) actual milk yield and average 4% fat corrected milk yield compared to control ration. Control ration recorded the lowest milk yield, being 5.89 Kg/h/d followed by R_1, being 6.61 Kg/h/d. The highest value was detected for R_2, being 6.78 Kg/h/d. There were insignificant increases in actual milk yield and average fat corrected milk between rations supplemented with pectinases ($R_1$and R_2). There were insignificant increase among all the tested rations in milk compositions percentage, while there were significant (P≤0.05) increase by rations supplemented with pectinases in milk component's yields of total solids,

SNF, fat and total protein compared to control ration. On the other hand, there was insignificant increase among all the tested rations in milk lactose and ash yields**.**

4- Feed conversion:-

No significant differences were found between the tested rations regarding feed intake of DM, TDN, SV and DCP. On the other hand, feed conversion of DM and DCP of control ration was significantly decreased compared to ($R1_{)}$ and ($R2_{)}$ rations. On the other hand there were insignificant differences between the tested rations in feed conversion of SV and TDN. Also, there were insignificant differences between R1 and R2 regarding feed conversion.

5-Some blood serum parameters:-

Total protein:-

The values of serum total protein content were 7.13, 8 and 7.8 g/dl, for control, $R_1$and R_2, respectively. Rations supplemented with pectinases ($R_1$and R_2) increased ($P\leq0.05$) serum total protein compared to the control ration.

Albumin:-

The overall means of serum albumin content were 3.63, 3.87 and 3.70 g/dl, for control, $R_1$and R_2, respectively. No significant differences were found between the tested rations for serum albumin.

Urea:-

The values of serum urea were 33.67, 31.7 and 32 (mg/dl) for control, R1 and R_2, respectively. No significant differences were found between the tested rations for serum urea.

Creatinine:-

Averages of serum creatinine were 1.2, 0.95 and 1.1 (mg/dl) for control, R_1 and R_2, respectively. No significant differences were found between the tested rations for serum creatinine.

Aspartate aminotransferase (AST):-

The overall means of serum AST were 114, 117.3 and 111.7 (IU/ L) for control, R1 and R_2, respectively. The differences between rations were not significant.

Alanine aminotransferase (ALT):-

The values of serum ALT were 39, 36 and 34.8 (IU/L) for control, R_1 and R_2, respectively. The differences between rations were not significant.

Glucose:-

Rations supplemented with pectinases ($R_1$and R_2) increased ($P \leq 0.05$) serum glucose compared to the control ration. The values of serum glucose were 78.33, 85.5 and 88.1 (mg/dl) for control, R1 and R2, respectively.

Total cholesterol:-

The values of serum cholesterol were 84, 83.6 and 87 (mg/dl) for control, R1 and R2, respectively. The differences between rations were not significant.

6- Simple economical evaluation of experimental rations:-

The economical values expressed as net revenue (L.E./h/70d) were enhanced for lactating buffaloes fed the produced enzyme and SMIZYME® rations compared to control one.

Conclusion:-

Fungal pectinase enzyme was locally produced (the produced enzyme) under the optimum conditions. This would contribute for reducing the cost of importation. The evaluated pectinases (the produced enzyme and SMIZYME®) were increased DM, OM, NDF and ADF degradation, also increased production of TGP, SCFA, ME and NEL. Also, additions of the produced enzyme and SMIZYME® to the rations of lactating buffaloes lead to marked increasing most of nutrient digestibilities and improving ($P \leq 0.05$)milk yield and 4% fat corrected milk yield. From economical point of view, the produced enzyme ration was the best one.Moreover; these studies recommend producing pectinase enzyme and adding it to the ration of lactating buffaloes.