Impact of Feeding Exogenous Fibrolytic Enzymes (EFE) on Digestibility, Rumen Fermentation, Haemobiochemical Profile and Productive Performance in Buffalo Calves

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ABSTRACT

This study was fulfilled to assess the impact of supplementing diets with Exogenous Fibrolytic Enzymes (EFE) in buffalo calves on growth performance criteria, rumen fermentation properties and certain biochemical indices. The total number of 12 buffalo calves of (4-6) months age and 123.3 kg average body weight were assigned into two similar groups (6 animals each): Animals of T1 (control) were fed on basal ration and those of T2 (treated) were fed as T1 plus 12 ml Zymogen liquid (ZL)/100 kg of animal weight per head daily. The study was extended for fifteen weeks. Live body weights were individually recorded biweekly in both group Rumen and blood samples were gathered from each animal in both groups at the finishing of study for determination of rumen fermentations properties and certain blood biochemical indices. Results showed that treated group (T2) recorded significant increase (P≤0.05) in dry matter intake (DMI), digestion coefficients, average daily gain, total body weight gain and better feed conversion ratio in comparing with control group. Also, treated group recorded significant increase (P≤0.05) in TVFA’s, ammonia concentration and total protozoa count (TPC) in comparing with control group. Blood biochemical analysis in treated group (T2) showed significant increase (P≤0.05) in total protein, albumin and non-significant decrease in urea, creatinine, triglycerides, AST and ALT levels compared with control group. The results obtained from this study suggested that buffalo calves fed with (EFE) supplemented feed, showed greater daily weight gains, total weight gains and feed conversion rates and rumen fermentation parameters.

Key words: Exogenous Fibrolytic Enzymes (EFE), Buffalo calves, Digestibility, Rumen fermentation, Biochemical profile, Growth performance

INTRODUCTION

In Egypt, agriculturists feed their animals with low quality harvest buildups having high fiber content. Ruminant animals can change over low quality feeds into astounding protein (Satapathy et al., 2018). High fiber substance of feeds keeps the activity of ruminal proteins to the plant cell divider and reduction supplement absorbability. (Elghandour et al., 2015; Abdel-Aziz et al., 2015; Togtokhbayar et al., 2015). Thus to enhance the digestibility, it is important to destruct the linkage between cellulose, hemicellulose and lignin. Buffaloes well known to expand more amount of straw and fodder than that of cattle. However, bad utilization capability of forages by the animal is core restraint in buffalo husbandry. Therefore, there is great want for enhancing the digestibility of the forages in case of buffaloes (Satapathy et al., 2018)

Supplementation of EFE is one of new rumen manipulation biotechnology to improve the quality and digestibility of fibrous forage and enhance production performance of the animals (Patel et al., 2015; Rojo et al., 2015; Salem et al., 2015; Valdes et al., 2015; Raju et al., 2016; Deli Nazmín et al., 2018). The positive effect of inclusion of EFE to ruminant diets can be summarized as pre consumption effect via, partially digest feed or weaken cell wall barriers that limit microbial digestion in rumen and reduce sugars from feedstuffs before consumption (Hristov et al., 1996; Raju et al., 2016) besides enhance rapid microbial attachment in rumen (Forsberg et al., 2000). Ruminal effect includes synergistically work with ruminal microbes to improve

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feed digestion besides increase of attachment and numbers of cellobiose and glucose utilizing bacteria in rumen (Nsereko et al., 2002). Post ruminal benefit of EFE is synergistically work with microbes in small and large intestine (Beauchemin et al., 2004).

In recent years, supplementation of ruminant diet with EFE are increased due to cost active tools of enhancing feed efficiency (Krause et al., 2003), besides its used not corrosive and or / hazardous, unlike chemical treatment for forages (Raju et al., 2016).

Previous studies have reported that use of EFE have a great effect on productivity of cattle (Bhasker et al., 2012), buffaloes (Gaafar et al., 2010), lambs (Salem et al., 2012). The effectiveness of EFE addition depends on many factors such as nature and activity of enzymes, type of ration and level and mode of enzyme supplementation (Shekhar et al., 2010).

So, the current study was fulfilled to assess the effect of inclusion of commercial Exogenous Fibrolytic Enzymes cocktail (EFE) Zymogen® on buffalo calves performance, digestibility, rumen fermentation and certain blood biochemical indices.

**MATERIALS AND METHODS**

This work was allowed in the Experimental Research Station belongs to Faculty of Agriculture, Ain Shams University located in Shalakan village, Qalubia Governorate, Egypt, during the period from February, 2018 to June, 2018.

**The experimental animals and rations**

Twelve growing buffalo calves of (4-6) month's age and 123.3 kg body weight were assigned into two similar groups (6 animals each). Each group was assigned randomly to feed on one of the following two dietary treatments:

- T1: were fed the basal farm ration (NRC, 2001).
- T2: were fed the basal farm ration plus 12 ml Zymogen liquid (ZL) /100 kg of animal weight per head daily.

The basal ration consists of: concentrate feed mixture (CFM), wheat straw and berseem. The CFM composed of 38% ground maize, 15% soybean meal, 34% wheat bran, 5% rice bran, 3% molasses, 1% mineral salts, 2% limestone powder, 1% Sodium Bicarbonate and 1% sodium chloride.

All animals were treated for internal and external parasites and vaccinated for common infectious diseases before the experiment started.

Overall means of the initial body weights of the experimental calves in this experiment were 125.6 and 123.3 kg for T1 and T2, respectively. The experiment was extended for fifteen weeks. Chemical compositions of feed stuffs are illustrated in Table 1.

**Feed additives composition and sources**

**Zymogen liquid (ZL):** This product is a liquid mixture of digestive enzymes, whereas each 1000 ml contains:

- Amylase 1500000 BAU (Bacterial Amylase Unit)
- Protease 2500000 HUT (Hemoglobin Unit Tyrosine)
- Lipase 500000 LU (Lipase Unit)
- Cellulase 1000000 CU (Cellulase Unit)
- Xylanase 1000000 XU (Xylanase Unit)
- Pectinase 2000 Endo-PG (Pectinase Unit) 2000000 PSU
- Propylene Glycol 100 ml
- Water up to 1000 ml (Wisemed Inc, 2017).

**Growth performance parameters**

Live body weights were individually recorded at two weeks intervals. The average daily body weight gain was individually calculated. Daily feed intake was measured for each replicate of a treatment by the difference between the daily offered feed and the daily residual one. Feed conversion ratios were obtained by dividing the amount of feed consumption per calf by the corresponding weight gain in a certain stage (two weeks).

**Digestibility trial**

Through the entire of each study period, digestibility trials were performed for all the experimental buffalo calves using a grab sample method where acid insoluble ash (AIA) was used as an internal marker according to (Schneider and Flatt 1975) for determining the nutrients digestibility. Fecal grab samples were collected handily at 8.0 a.m. and 2.0 p.m. for three successive days from each animal started from the 50th day of the experiment.

**Rumen fluid samples**

Rumen liquor samples were collected from each animal in each group at the finish of experiment using stomach tube 4 hrs post feeding and filtered through four layers of cheese cloth for estimating of rumen parameters. The pH value was immediately documented using digital pH meter, while samples were stored at -20°C until chemical analysis. Ruminal ammonia nitrogen (NH3-N) concentration was measured according to (Conway 1957). Ruminal total volatile fatty acids (TVFA’s) concentration was measured by steam distillation procedure according to (Warner 1964) and total protozoal count was estimating according to (Dehorety, 1986). Blood samples Blood samples were collected from each animal in each group at the finish of experiment. The blood samples were collected at three hours post morning feeding.

**Blood samples**

Blood samples were taken from each animal in each group at the end of experiment. The blood samples were obtained at three hours post morning feeding. A sample of 15 ml of blood per animal was taken from the jugular vein. The blood was taken into a clean dried tube after adding EDTA. The blood plasma was harvested by centrifuging the blood samples soon after collection at 4000 (rpm) for 15 minutes. Blood plasma was transferred into a clean dried tubes vials and then stored in deep freezer at -20°C for subsequent specific chemical analysis.

Blood plasma was determined for total protein (Armstrong and Carr, 1964), albumin (Doumas et al., 1971), urea (March 1965), creatinine (Husdan, 1968), AST, ALT (Reitman and Frankel 1957) and triglyceride (Fassati, 1982), Globulin was calculated by difference.

**Analytical methods**

The chemical composition of the feedstuffs and feaces were analyzed according to the A.O.A.C (1995) methods to determine moisture, DM, OM, CP, CF, EE and ash contents, while NFE content was calculated by difference.
Statistical analysis

The data were measured according to statistical analysis system (SAS) User’s Guide (2001). Separation among means was done by using Duncan multiple tests, (1955).

RESULTS AND DISCUSSION

Dry matter intake

Result were tabulated in Table 2 clearly showed that DMI was increased (P≤0.05) by adding zymogen liquid (ZL) on the basic ration. This result is in good agreement with those obtained by (Thakur et al., 2010), who fed Murrah buffalo calves on a basal diet supplemented by EFE. Also, same findings were recorded by (Arif et al., 2019) on Nilli Ravi early lactating buffaloes, they tested the effect of adding EFE on ration involved of oat silage and concentrates. They recorded an increase in DM intake with treated groups. In addition, (Gomez-Vazquez et al., 2011) on crossbreed steers grazing stargrass plus concentrate and fermented sugar cane supplemented by EFE. They found a positive effects on DM intake. The great impact of enzymes on feed intake may be explained by partly be increase palatability of the diet (Morsy et al., 2016; kholif et al., 2017).

Nutrients digestibility

Data of digestion coefficients were tabulated in Table (3). Animals of T2 (treated group) showed higher (P≤0.05) digestibility of DM, OM, CP, CF, EE and NFE than those of T1 (control group). The same findings were recorded by (Arif et al., 2019) on Nilli Ravi buffaloes. They recorded an improvement (P≤0.05) in all nutrients digestibility coefficients. Also, (Song et al., 2018), they found an improvement of EFE supplementation on OM, CP and fiber fraction digestibility of Chinese domesticated black goats. In addition, (Soliman et al., 2016) fed Barki sheep on a ration supplemented by prebiotic. They recorded a significant enhancing in all digestibility coefficients. Moreover, (He et al., 2015) on Angus cattle recorded an increase of CP and fiber fraction digestibility with diets supplemented by EFE.

Rumen fermentation properties

Rumen fermentation properties (table 4) revealed non-significant increase in pH and significant increase (P≤0.05) in TVFAS, ammonia concentration and total protozoa count (TPC) in EFE supplemented group compared with those in control group.

Rumen pH, NH3-N and VFA concentrations are the important indicators that reflect rumen function and stability of the intra ruminal milieu. In the present study, oral administration of EFE not affect the rumen pH the same finding was recorded by (Yuangklang et al., 2013) in buffaloes, (Poonooru et al., 2015) in buffalo bulls and (Balci et al., 2007) in fattening steers.

Increase ruminal ammonia concentration of the treated group is in agreement with those reported by (Poonooru et al., 2015), Bhasker et al., 2013), Kohilf and Aziz (2014) and Rajamma et al., 2014). In contrast, (Gaafar et al., 2010) reported that EFE decreased (P<0.01) NH3-N concentration. (Pinos-Rodriguez et al., 2008), (Singh and Das 2009) and (Ganai et al., 2011) reported no effect of EFE on NH3-N concentration. This variation might be attributed to difference in environmental factors, feed type, feed allocation method, and type of enzyme blend (Sutton et al., 2003). Similar results of TVFA were observed in buffalo bulls by (Rajamma et al., 2014) and Poonooru et al., 2015).

This may be explained by the great availability of fermentable soluble carbohydrates due to increased fibrolytic activity in rumen. Data of table (4) clearly indicated also significant total protozoa increase in treated group compared with control group.

Blood plasma

Data of Table (5) showed significant increase (P≤0.05) in total protein, albumin, while there was non-significant decrease in triglycerides, BUN, AST, ALT and creatinine levels in experimental group compared with those in control group. The values in both groups were within normal physiological ranges (Kaneko et al., 1997), the same results were recorded by (Beigh et al., 2018) in lambs, (El-Bordeny et al., 2015) and (Peters et al., 2015) in lactating dairy cows fed rations included with exogenous enzymes. These results may be attributed that EFE supplementation enhance metabolic process as a response to increase apparent nutrients digestibility (table 3). In this connection, (Kumar et al., 1980) and (Bush 1991) reported that serum total proteins concentration reflects the nutritional grade of the animal and it has a great link with dietary protein level.

In kidney function attributes, EFE supplemented group had numerically lower levels of urea and creatinine in the blood plasma. The same results were mentioned by (Beigh et al., 2018) in lambs, (El-Bordeny et al., 2015) in lactating dairy cows and (Turkar and Uppal, 2007); (Javid et al., 2008) and (Shekhar et al., 2010) in buffaloes. These results might be attributed to efficient utilization of dietary proteins by addition of the feed additive. Lower N wastage by great utilization of generated ammonia in the rumen was characterized in terms of lower blood urea nitrogen (BUN) levels in EFE supplemented group. So, supplementation had no opposing impacts on glomerular filtration, thus safe for renal functioning. These results of non-significant impacts of exogenous enzymes on plasma BUN and creatinine in the present study are in agreement to those obtained by (Rivero and Salem, 2015) for enzyme feeding in sheep.

Table 1: Chemical composition of the experimental feed stuffs (% on DM basis).

<table>
<thead>
<tr>
<th>Feed stuff</th>
<th>DM</th>
<th>OM</th>
<th>CF</th>
<th>CP</th>
<th>EE</th>
<th>NFE</th>
<th>Ash</th>
<th>AIA**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berseem</td>
<td>90.94</td>
<td>95.38</td>
<td>30.15</td>
<td>19.64</td>
<td>3.80</td>
<td>41.79</td>
<td>4.62</td>
<td>4.42</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>91.73</td>
<td>81.66</td>
<td>51.92</td>
<td>4.15</td>
<td>1.83</td>
<td>23.75</td>
<td>18.34</td>
<td>15.31</td>
</tr>
<tr>
<td>CFM*</td>
<td>91.56</td>
<td>96.24</td>
<td>23.60</td>
<td>16.88</td>
<td>2.99</td>
<td>52.76</td>
<td>3.76</td>
<td>5.62</td>
</tr>
</tbody>
</table>

* CFM: Concentrate feed mixture; **AIA: Acid insoluble ash.
The same results were recorded by (Beigh et al., 2010) on Murrah buffalo calves. They stated that supplementation of EFE improved daily body weight gain. The present results agree also with those obtained by (Thakur et al., 2016) in sheep and goat and (El-Bordony et al., 2015) in lactating dairy cows fed diets included with EFE. These results showed that EFE application in the present study had no an adverse effect on the liver activity. Moreover, (El-Bordony et al., 2010) stated that supplementation of EFE to buffaloe’s rations had not any major adverse influence on blood parameters.

Regarding to the level of triglycerides in experimental group showed non-significant decrease although the values in both the groups were within normal physiological ranges in buffalo calves (Kaneko et al., 1997) The same results were recorded by (Beigh et al., 2018) in lambs and (Morsy et al., 2016) in Egyptian buffaloes.

**Body weight and growth performance criteria**

Data of Table 6 showed higher significant (P≤0.05) total gain and daily gain values in treated group (T2) than control (T1). This may be explained by, 1) Zymogen liquid may be having a stimulating effect on the rumen proper functions and digestion. 2) The higher digestibility that was recorded particularly for group T2 supplemented by (ZL) fermentations parameters

### Table 4: Effect of EFE supplementation on some rumen fermentation parameters

<table>
<thead>
<tr>
<th>Item</th>
<th>G1</th>
<th>G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>6.83</td>
<td>6.93</td>
</tr>
<tr>
<td>Total Volatile Fatty Acids (mmol/l)</td>
<td>80.06B</td>
<td>86.97A</td>
</tr>
<tr>
<td>Ammonia (mmol/l)</td>
<td>100.37B</td>
<td>106.91A</td>
</tr>
<tr>
<td>Total Protozoa Count (x10⁷/ml)</td>
<td>15.32B</td>
<td>18.22A</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Among the indicators of liver functioning activity, AST and ALT were non significantly decreased in experimental group when compared with those in control group although the values in both the groups were within normal physiological ranges (Kaneko et al., 1997). The same data were recorded by (Beigh et al., 2018) in lambs, (Rivero et al., 2016) in sheep and goat and (El-Bordony et al., 2015) in lactating dairy cows fed diets included with exogenous enzymes. The present values of AST and ALT activity indicate normal activity of the animal hepatic tissues, consequently, EFE application in the present study had no an adverse effect on the liver activity. Moreover, (El-Bordony et al., 2010) stated that supplementation of EFE to buffaloe’s rations had not any major adverse influence on blood parameters.

**Table 2: Effect of EFE supplementation on dry matter intake (kg/h/d).**

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 15</td>
<td>4.14±0.65</td>
<td>4.27±1.17</td>
</tr>
<tr>
<td>16 – 30</td>
<td>4.12±0.48</td>
<td>4.28±0.77</td>
</tr>
<tr>
<td>31 – 45</td>
<td>4.22±0.53</td>
<td>4.40±0.56</td>
</tr>
<tr>
<td>46 – 60</td>
<td>4.21±0.32</td>
<td>4.59±1.25</td>
</tr>
<tr>
<td>61 – 75</td>
<td>4.35±0.61</td>
<td>4.60±0.80</td>
</tr>
<tr>
<td>76 – 90</td>
<td>4.37±0.64</td>
<td>4.71±0.56</td>
</tr>
<tr>
<td>91 – 105</td>
<td>4.38±0.52</td>
<td>4.95±1.32</td>
</tr>
<tr>
<td>Average</td>
<td>4.26B</td>
<td>4.54A</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

**Table 3: Effect of EFE supplementation on the nutrients digestibility coefficients of the buffalo calves.**

<table>
<thead>
<tr>
<th>Item</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>61.48±1.03</td>
<td>67.34±0.64</td>
</tr>
<tr>
<td>OM</td>
<td>66.43±1.02</td>
<td>71.08±0.76</td>
</tr>
<tr>
<td>CP</td>
<td>63.33±0.53</td>
<td>68.89±0.49</td>
</tr>
<tr>
<td>CF</td>
<td>58.76±1.82</td>
<td>64.40±0.74</td>
</tr>
<tr>
<td>EE</td>
<td>80.12±1.64</td>
<td>84.39±2.00</td>
</tr>
<tr>
<td>NFE</td>
<td>72.73±0.92</td>
<td>79.25±0.62</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

**Table 5: Effect of EFE supplementation on selected serum biochemical parameters.**

<table>
<thead>
<tr>
<th>Item</th>
<th>G1</th>
<th>G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>5.85B</td>
<td>6.24A</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.90B</td>
<td>2.26A</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.95</td>
<td>3.98</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>28.31</td>
<td>27.88</td>
</tr>
<tr>
<td>Creatinine (g/dl)</td>
<td>1.33</td>
<td>1.31</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>36.92</td>
<td>38.39</td>
</tr>
<tr>
<td>AST (unit/L)</td>
<td>33.62</td>
<td>32.08</td>
</tr>
<tr>
<td>ALT (unit/L)</td>
<td>31.47</td>
<td>30.42</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

**Table 6: Effect of EFE supplementation on changes of body weights and daily gain (kg/h/d).**

<table>
<thead>
<tr>
<th>Item</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protozoa Count (x10⁷/ml)</td>
<td>127.20±2.52</td>
<td>32.08±1.26</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

**Table 7: Effect of EFE supplementation on dry matter conversion (kg DM/kg gain).**

<table>
<thead>
<tr>
<th>Item</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal weight</td>
<td>15.94±0.97</td>
<td>13.78±1.45</td>
</tr>
<tr>
<td>Initial weight</td>
<td>125.60±15.40</td>
<td>117.52±16.70</td>
</tr>
<tr>
<td>Final weight</td>
<td>187.80±18.70</td>
<td>202.33±32.92</td>
</tr>
<tr>
<td>Total gain</td>
<td>62.20±3.52B</td>
<td>81.33±7.54A</td>
</tr>
<tr>
<td>Days Average daily gain (kg/h/day)</td>
<td>0.574±0.09</td>
<td>0.711±0.17</td>
</tr>
<tr>
<td>0 – 15</td>
<td>0.574±0.09</td>
<td>0.711±0.17</td>
</tr>
<tr>
<td>16 – 30</td>
<td>0.571±0.07</td>
<td>0.711±0.09</td>
</tr>
<tr>
<td>31 – 45</td>
<td>0.569±0.10</td>
<td>0.689±0.06</td>
</tr>
<tr>
<td>46 – 60</td>
<td>0.544±0.05</td>
<td>0.800±0.15</td>
</tr>
<tr>
<td>61 – 75</td>
<td>0.572±0.12</td>
<td>0.800±0.10</td>
</tr>
<tr>
<td>76 – 90</td>
<td>0.586±0.11</td>
<td>0.822±0.06</td>
</tr>
<tr>
<td>91 – 105</td>
<td>0.679±0.14</td>
<td>0.889±0.16</td>
</tr>
<tr>
<td>Average</td>
<td>0.586B</td>
<td>0.775A</td>
</tr>
</tbody>
</table>

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).
Feed conversion (kg DM/ kg gain)

Results of Table (7) showed better DM conversion (P≤0.05) for group supplemented by ZL (T2) than the control group (T1). This may be attributed to 1) Zymogen liquid may be having a stimulating effect on the rumen proper functions and digestion. 2) The higher digestibility that was recorded particularly for group supplemented by (ZL) T2 (table 3), which led to increase the absorbed nutrients from small intestine, consequently led to more body weight gain (table 6). 3) Increased protein anabolism due to higher protein digestibility which led to higher blood plasma total protein and albumin concentration (table 5), which resulted an increase in protein biosynthesis in this group.

These results are agree with those reported by (Gomez-Vazquez et al., 2011) on crossbreed (Brahman x Brown Swiss) steers, they fed steers on concentrate, sugarcane fermented and supplemented by EFE. They reported a significant improvement on feed conversion ratio. In addition, (Sudipta Ghosh and Ram Kumar Mehla 2012) on Holstein cross calves recorded an improvement on feed conversion efficiency for calves fed ration supplemented by prebiotic than the control group. Same results are also obtained by (Soliman et al., 2016) on Barki sheep fed ration supplemented by prebiotic.

Conclusions

The obtained results from this study suggested that buffalo calves fed with Exogenous Fibrolytic Enzymes (EFE) supplemented feed, showed better daily weight gains, total weight gains, feed conversion rates and rumen fermentation parameters. Therefore, inclusion complete diets for growing buffalo calves with EFE cocktail provided nutrients more than the recommended diets for growing buffalo calves with fermentation parameters. Therefore, inclusion complete EFE cocktail supplemented feed, showed better daily weight gains, total weight gains, feed conversion rates and rumen fermentation in this group.

These results are also obtained by (Soliman et al., 2011) on crossbreed (Brahman x Brahman) steers. Bulgarian J Vet Med, 61: 29-34.

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