EVALUATION OF EXOGENOUS FIBROLYTIC ENZYME SUPPLEMENTATION TO IMPROVE FEED UTILIZATION IN RUMINANTS

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ABSTRACT

Exogenous fibrolytic enzyme (EFE) have been shown to increase daily gain and feed efficiency in feedlot animals. So, this study aimed to evaluate effect of using EFE on productive performance of growing lambs. sixteen Barkey lambs (3 months old, 22.31± 1.57 kg) were randomly assigned into two groups, 8 lambs for each according to live body weight. The first group (control) was fed control rations without EFE supplementation, while treated group were fed the control ration plus 2.5 g exogenous fibrolytic enzyme. The groups fed ration supplemented with EFE recorded higher DM, TDN and digestible CP intake. Supplementation of lambs ration with EFE showed no significantly effect on rumen liquor TVFA's and ammonia concentration at 0, 3 and 6 hrs post feeding. While Supplementation of lambs ration with EFE significantly increased rumen liquor pH at 0, 3 and 6 hrs after feeding compared to the control group. Exogenous fibrolytic enzyme significantly improved nutrients digestibility as dry matter, organic matter, crude protein, crude fiber, nitrogen free extract, neutral detergent fiber (NDF) and acid detergent fiber (ADF) as well as feeding values as TDN and digestible crude protein. Exogenous fibrolytic enzyme supplementation to lambs ration showed numerically increased ($P>0.05$) in plasma total protein concentration compared to lambs fed ration not supplemented. While albumin, globulin, triglycerides and creatinine concentration and Alanine Transaminase(ALT), Aspartate Transaminase(AST), alkaline phosphates activity were not significantly ($P>0.05$) affected by Direct-Fed Microbial (DFM) supplementation. Total gain and average daily gain significantly increased

(P≤0.05) for group received rations supplemented with EFE compared to control group. Also supplementation lambs rations with EFE significantly (P≤0.05) improved feed conversion as DM, TDN, CP and DCP compared to the control group. It could be concluded that supplementing lambs ration with EFE resulted in increase feed intake and digestibility, consequently increased average daily gain and feed conversion without any adverse effect on animal health and performance.
Key words: lambs, Exogenous fibrolytic enzyme, feed intake, digestibility, growth performance.

INTRODDUCTION

Improvements of feed utilization and animal productivity are the aims of most of nutrition studies. One of the important of the environment issues is decreasing nitrogen pollution through improving nitrogen utilization in ruminants. These aims could be achieved by producing exogenous fibrolytic enzyme products to use it as feed additives. Many of the feed additives have been used to improve animal performance and feed utilization efficiency.

Recent research has demonstrated that supplementation diets of dairy animal and feedlot with fiber degrading enzymes can improve feed utilization and animal performance by enhancing fiber degradation in vitro (Hristove *et al., 1996;* Gado *et al*., 2007; El-Adawy *et al*., 2008; Rodrigues *et al*., 2008), in situ (Feng *et al*., 1996; Lewis *et al*., 1996; Tricarico *et al*., 2005; Krueger *et al*., 2008) and in vivo (Yang *et al*., 1999; Gado *et al*., 2007; Salem *et al*., 2007; Gado and Salem, 2008). Feeding enzymes is often accompanied by increased feed intake, which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However post-ingestive enzyme effects, such as increased digestion rate and /or extent of digestion (Beauchemin and Rode, 1996; Feng *et al*., 1996; Gado and salem,

2008; Krueger *et al*., 2008) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Adesogan, 2005).

Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle. Dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed and produced 5–25% more milk (Lewis *et al*., 1995; Tricarico *et al*., 2005; Stella *et al*., 2007), improved the energy balance of transition dairy cows (DeFrain *et al*., 2005) and increased milk production in small ruminants (Titi and Lubbadeh, 2004; Stella *et al*., 2007). In feedlot cattle, fibrolytic enzymes have improved live weight (LW) gain by as much as 35% and feed conversion ratio by up to 10% (Beauchemin *et al*., 1995).

So, the objective of this study was to evaluate effect of using exogenous celluletic enzyme on productive performance of growing lambs fed low quality roughage (wheat straw).

MATERIALS AND METHODS

The present study was carried out in Al-Fayroz farms for agriculture and animal production, El-Noubaria, El-Behaira governorate and labs of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University, Egypt.

Exogenous fibrolytic enzyme: A mixture of exogenous fibrolytic enzyme (EFE) were obtained from biofertilizar unit Ain Shams University, faculty of Agriculture. The mixture contain 78 unit cellulose/ gm and 6.23 unit protease.

Animals, diets, feeding and experimental design: Sixteen Barkey lambs (3 months old, 22.31± 1.57 kg body weight) were randomly assigned into two

groups, 8 lambs for each according to live body weight. Each group was assigned randomly to receive one of the two experimental treatments. The experimental rations were formulated to cover lambs allowances according to NRC (1985). The first group (control) was fed control rations without exogenous cellulitic enzyme (ECE), while treated group were fed the control ration plus 2.5 g exogenous cellulitic enzyme (ECE). The chemical composition of the experimental ration ingredients is presented in Table (1). Complete rations were offered twice daily at 8 am and 5 pm in quantities sufficient to allow free choice access to the ration, and animals have free access to clean fresh water. The growth phase lasted 124 days, the animals were weighed monthly to calculate total and daily gain and feed conversion.

Digestibility trials: Through the entire experimental period, the digestibility trial was performed after two months of the experimental beginning, six animals from each treatment were used and fecal bag technique was applied. Fecal collection bags were fixed on the lambs and after a 3 day adaptation period, total feces were collected for 5 consecutive days. Fecal collection bags were emptied twice daily and the contents weighed. Total feces were sub-sampled at a percentage set for individual lambs to obtain approximately 100 g of fecal DM from each animal over the five days collection in order to get representative samples.

Table (1): chemical composition of the experimental rations Ingredients (%).

Item	CFM[1]	Wheat straw
Organic matter (OM)	94.70	90.16
Ash	5.29	9.84
Crude protein (CP)	18.59	4.12
Ether extract(EE)	3.97	1.83
Non fiber carbohydrate (NFC)	39.91	4.73
Cell wall constituents		
Neutral-detergent fiber (NDF)	32.23	83.14
Acid- detergent fiber (ADF)	13.67	54.39
A cid-detergent lignin (ADL)	4.10	7.82
Hemicellulose	18.56	28.74
Cellulose	9.56	46.57
Lignin	3.07	7.59

1 CFM: Concentrate feed mixture

Rumen activity: After 45 days of experimental beginning rumen contents were sampled from 5 animals of each group by stomach tube. Samples were collected before the morning feeding (i.e., t = 0), 3 and 6 h after the morning feeding. The collected rumen fluid was squeezed through 4-layers cheesecloth and immediately after rumen liquor filtration; pH value was measured using pH-meter (Hanna, Italy). Strained rumen liquor was stored in glass bottles (45 ml) with a few drops of toluene and paraffin oil to cover the surface and stored at −18º C.

Chemical analysis: Feeds and feces were analyzed for proximate chemical analyses according to Association of Official Analytical Chemists, (2000). Nitrogen free extract was calculated by difference. The NDF, ADF and ADL were determined according to Van Soest *et al.* (1991). Cellulose and hemi-cellulose were calculated by difference and Non-fiber carbohydrate (NFC) was calculated according to following equations:

cellulose = ADF - ADL

hemi-cellulose = NDF – ADF.

NFC, % = 100− (%ND+%CP + %fat + %ash) (NRC, 2001).

Blood parameters: At the end of growth trial, blood samples were taken from 5 animals for each group. A sample of 10 ml of blood per animal was withdrawn from the jugular vein. The blood sample was directly collected into a clean dried glass culture tubes (after addition of heparin as an anti coagulant) at 3 hrs post feeding. The blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 (rpm) for 15 minutes. Blood plasma was transferred into a clean dried glass vials and then stored in deep freezer at -18º C for subsequent specific chemical analysis. Blood plasma samples were analyzed using commercial kits. Total plasma protein concentrations was determined as described by Cannon *et al*. (1974), albumin concentrations was determined using methods of Doumas *et al.* (1971), blood plasma urea was determined according to Batton and Crouch (1977), alkaline phosphatase was determined according to Belfield and Goldberg (1971), Triglycerides was determined according to Stein (1987), creatinine was determined according to Spencer and Price (1980), Alanin amino transferase (ALT) and aspartate amino transfearse (AST) activities were colorimetrically determined according to AST and ALT kits based on reaction of Young (1990). Globulin and A/G ratio was calculated.

Statistical analysis: The obtained data were statistic analyzed according to statistical analysis system (SAS, 1999). Separation between means was carried out by using Duncun Multiple Range test (Duncan, 1955). Data of

total and daily gain, digestibility, feed conversion and blood parameters were statistically analyzed according the following model: $Y_{ij} = \mu + T_i + e_{ij}$, Where y_{ij} = represents observation, μ=: the overall mean, T_i = effect of treatment (experimental group), e_{ij}: experimental error.

While the data of rumen fermentation parameter was statistically analyzed according the following model: $Y_{ij} = \mu + T_i + S + an(t) + S*T + e_{ij}$

Where: Y_{ij} = The observation on the I^{th} treatment, μ = Overall mean, T_i = Effect of the I^{th} treatment, S = Effect of the period, an (t) = Effect of the animal in the treatment and e_{ij} = Random experimental error

RESULTS AND DISCUSSIONS

Feed intake: The data of table (2) showed effect of lamb rations supplementation with exogenous cellulitic enzyme (ECE). The experimental groups were fed restricted amount of feed (concentrate and wheat straw, according to NRC allowances, 1985) and the orts were recorded. So the data clearly showed that the concentrate feed mixture (CFM) intake was the same for the four experimental groups, while the wheat straw and total DM intake was higher in the group fed ration supplemented with exogenous fibrolytic enzyme (EFE) compared to the group fed un-supplemented ration. Also the data indicated that the groups fed ration supplemented with EFE recorded higher TDN and digestible CP intake. These results could be postulated to feeding exogenous enzymes is often led to increased feed intake, which may partly be due to enzyme activity effects (post-ingestive), such as increase rate of digestion and /or digestibility (Gado and salem, 2008 and Krueger *et al*., 2008) also may be increase hydrolytic enzyme activity in the rumen consequently reduce gut fill and increase feed intake (Adesogan, 2005).

Table (2): Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on feed intake

Item	control	EFE
Concentrate feed mixture, kg d^{-1}	0.784	0.784
Wheat straw, kg d^{-1}	0.202	0.255
Total feed intake, kg d^{-1}	0.986	1.039
Dry matter intake, kg d^{-1}	0.860	0.908
Total digestible nutrient intake, kg d^{-1}	0.708	0.788
Crude protein intake , kg d^{-1}	153.4	155.6
Digestible crude protein intake, kg d^{-1}	110.1	120.0

Rumen fermentation kinetics: Supplementation of lambs ration with EFE showed no significantly effect on rumen liquor TVFA's and ammonia concentration at 0, 3 and 6 hrs post feeding (table 3).

Concerning effect of sampling time, the values of rumen TVFA's and ammonia concentration showed a normal pattern, which the highest values ($p<0.05$) was recorded at 3 hrs post feeding and gradually decreased to reach the lowest values at 0 hrs of feeding (pre- feeding).This may be attributed to that fermentation process of both nonstructural and structural carbohydrates started with a low rate as a result to absence of substrate then increased with the time and reached the maximal level at 3hrs after feeding then deceased up to the next meal, parallel to the gradually disappearance of substrate (Reddy and Reddy, 1989).

Data of Table (3) clearly indicated that Supplementation of lambs ration with EFE significantly increased rumen liquor pH at 0, 3 and 6 hrs after feeding compared to the control group (not supplemented). This result may be due to the higher feed intake from wheat straw (table 2), consequently

increase ruminating process and saliva excretion which enhance the buffering control and resulted in increase the pH value.

Table (3): Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on rumen fermentation kinetics.

	control	EFE	mean	SE
Total volatile fatty acids concentration (TVFA's), m equiv. dL^{-1}				
Before feeding (0 hrs)	7.224	5.32	6.27^{c}	0.29
After 3 hours	8.4	8.5	8.45^{a}	0.29
After 6 hours	6.31	6.90	6.60^{b}	0.29
Mean	7.31^{a}	6.91^{a}		
Ammonia concentration, mg dL^{-1}				
Before feeding (0 hrs)	4.49	4.78	4.64^{b}	0.72
After 3 hours	8.94	9.68	9.31^{a}	0.72
After 6 hours	4.91	7.52	6.21^{b}	0.78
Mean	6.11^{a}	7.33^{a}		
pH value				
Before feeding (0 hrs)	6.51	7.09	6.80^{a}	0.11
After 3 hours	5.51	5.88	5.69^{c}	0.13
After 6 hours	5.83	6.62	6.22^{b}	0.14
Mean	5.95^{c}	6.53^{a}		
Cellulose activity, unit ml^{-1}				
Before feeding (0 hrs)	4.99	3.91	4.873	2.799
After 3 hours	10.238	7.65	11.808	2.799
After 6 hours	17.456	26.96	18.411	2.799
Mean	10.897	12.839		
specific activity of cellulase, unit mg-1 of protein				
Before feeding (0 hrs)	1.097	2.014	1.447	1.28
After 3 hours	5.718	2.709	4.476	1.28
After 6 hours	6.768	9.704	6.1583	1.28
Mean	4.5277	4.809		

a, b and c, Means with different superscripts in the same column are significant ($P< 0.05$) different.

Concerning effect of sampling time, the values of rumen liquor pH showed a normal pattern, the mean value of rumen pH started high at zero time then decreased ($p<0.05$) at 3 hrs, then increased ($p<0.05$) again at 6 hrs after feeding. These results may be related to fermentation processes of both nonstructural and structural carbohydrates and production of volatile fatty acids which increased with proceeding time so that affected the pH values to some limit until they were proportionally and relatively absorbed from the rumen wall resulting in decrease in pH value. These results agree with the conclusion of Roddy and Roddy (1985) who stated that the pH values were inversely related to TVFA's concentration in the rumen.

Numerically increase (not significant) was noticed in cellulase enzyme activity as unit per ml rumen liquor for groups fed ration supplemented with ECE, and no differences was recorded between the two experimental groups in specific activity of cellulose as unit per mg protein (table 3). This may be due to post-ingestive enzyme effects, which may increase hydrolytic activity in the rumen (Adesogan, 2005).

Nutrient digestibility: Exogenous cellulitic enzyme supplementation significantly improved nutrients digestibility as dry matter, organic matter, crude protein, crude fiber and nitrogen free extract (Table 4). Also, EFE supplementation improved, neutral detergent fiber (NDF), acid detergent fiber (ADF), Cellulose and hemicellulose digestibility by about 6.73 and 18.14 % compared to control. Nutritive values in term of total digestible nutrients (TDN), digestible crude protein were significantly improved with rations supplemented by EFE supplementation compared to control ration. The

improvement of nutritive value (TDN and DCP) of rations supplemented with DFM may be due to the improvement of nutrients digestibility as shown in Table 4.

Other reports have also shown increases in digestibility of dry matter particularly fiber with fibrolytic enzyme addition (Gado and Salem, 2008; Hristov *et al.*, 2008). Bowman *et al.* (2002) reported a 25% increase in total tract NDF digestibility with a fibrolytic enzyme product. Exogenous fibrolytic enzymes would be expected to increase fiber digestion by increasing the rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al.*, 1999), alterations in ruminal fermentation (Nsereko *et al.*, 2002) and/or enhanced attachment and colonization to the plant cell wall by ruminal microorganisms (Nsereko *et al.*, 2000; Wang *et al.*, 2001) and/or by synergism with enzymes in rumen fluid (Morgavi *et al.*, 2000).

Table (4): Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on nutrient digestibility coefficients.

Item	control	EFE	P valu
Dry matter, %	68.37±0.64	72.89±0.72	0.0023
Organic matter, %	70.27±0.47	74.67±0.53	0.0005
Crude protein, %	64.65±1.49	69.65±1.67	0.0641
Ether extract, %	79.82±2.12	77.28±2.37	0.4517
Crude fiber, %	55.45±0.78	61.23±0.88	0.0018
Nitrogen free extract, %	77.52^{b} ± 0.64	81.58^{a} ± 0.64	0.003
Cell wall constitutes			
Neutral-detergent fiber (NDF)	57.31±0.67	64.67±0.75	0.0002
Acid- detergent fiber (ADF)	53.18±0.87	62.03±0.97	0.0003
Cellulose	62.88±0.56	74.29±0.63	<0.0001
Hemicellulose	62.42±0.91	67.96±1.01	0.0048
Feeding value			
Total digestible nutrient, %	71.30±0.55	74.50±0.61	0.0062
Digestible crude protein, %	10.98±0.25	11.23±0.28	0.5398

a and b, Means with different superscripts in the same row are significant (P< 0.05) different.

However, increased fiber digestion is unlikely the result of supplemental enzyme activity alone because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin *et al.*, 2001). Wang *et al.* (2001) reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more total polysaccharidase activity to digest feedstuffs. Consistent with this hypothesis,

Blood metabolic parameters: Adding EFE to lambs ration showed numerically increased (P>0.05) in serum total proteins concentration compared to lambs fed ration not supplemented (Table 5). This may be

attributed to that EFE supplementation improve metabolic process as a response to increase nutrients digestibility specially, crude protein and organic matter (table 4) as well as increase flow of microbial protein from the rumen (Yang *et al.,* 1999). Moreover, Kumar *et al.* (1980) and Bush, (1991) postulated that blood total proteins concentration is reflection the nutritional status of the animal and reported a positive correlation between blood total proteins concentration and dietary protein level.

Moreover the group supplemented with EFE recorded significant reduction in urea concentration compared to control group (table 5).

On the other hand, albumin, globulin, tri-glecride and creatinine concentration and ALT, AST, alkaline phosphates activity were not significantly ($P>0.05$) affected by EFE supplementation to lambs ration (table 5). The present values of AST and ALT activity indicated normal activity of the animal liver tissues; consequently, DFM supplementation in the present study had no any adverse effect on the liver function. Which Maxine (1984) reported that the AST and ALT activity are most important indicator for hepatic tissue activity. Furthermore, Kholif *et al.* (2012) and El-Bordeny et al (2015) found that adding exogenous fibrolytic enzyme as a DFM to dairy buffaloes and dairy cows rations had not any significant effect on buffalo's blood metabolites.

Table (5): Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on some blood plasma parameters

	control	EFE	P value
total protien , g dL^{-1}	6.37±0.26	5.88±0.26	0.235
Albumin, g dL^{-1}	2.90±0.15	2.75±0.17	0.524
Globulin, g dL^{-1}	3.46±0.28	3.30±0.31	0.706
A/G ratio	0.86±0.11	0.87±0.12	0.979
Urea, mg dL^{-1}	41.73±1.91	34.99±2.13	0.051
Triglycerides, mg dL^{-1}	98.43±16.24	103.59±16.24	0.827
Creatinine, mg dL^{-1}	0.68±0.15	0.70±0.15	0.930
Alkaline phosphatase, unit L^{-1}	36.10±2.84	31.36±2.54	0.254
AST, unit L^{-1}	10.10±2.38	8.22±1.84	0.556
ALT, unit L^{-1}	56.96±2.06	63.44±2.06	0.06

a and b, Means with different superscripts in the same row are significant ($P< 0.05$) different.

Growth performance: Total gain and average daily gain significantly increased ($P\leq0.05$) for group received rations supplemented with EFE compared to control group. Also supplementation lambs rations with EFE significantly ($P\leq0.05$) improved feed conversion as DM, TDN, CP and DCP compared to the control group. These may be due to 1) the higher intake of CP and TDN for supplemented groups compared to control group (table 2), 2) the recorded higher nutrients digestibility for supplemented groups compared to control group (table 6). Moreover the superiority of feed conversion as DM, TDN, CP and DCP for supplemented group could be attributed to the higher values of average daily gain recorded for supplemented group compared to control.

Table (6): Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on growth performance

	Control	EFE	P value
Initial weight, kg	22.16±1.58	22.42±1.46	0.9055
Final weight, kg	38.33±2.12	44.00±1.97	0.0768
Total gain, kg	16.16±0.79	21.57±0.73	0.0004
Average daily gain. g d^{-1}	0.13±0.00	0.17±0.00	0.0004
Feed conversion kg feed/ kg gain			
Dry matter conversion	6.69±0.27	5.25±0.25	0.0031
TDN conversion	5.50±0.23	4.55±0.21	0.0124
Crude protein conversion	1192.99±49.37	900.01±45.71	0.0011
Digestible protein conversion	856.19±36.02	694.40±33.35	0.0071

a and b, Means with different superscripts in the same row are significant ($P< 0.05$) different.

CONCLUSION

It could be concluded that supplementing lambs ration with EFE resulted in increase feed intake and digestibility, consequently increased average daily gain and feed conversion without any adverse effect on animal health and performance.

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تقييم تأثير إضافة الإنزيمات الخارجية المحللة للألياف لتحسين كفاءة استخدام الغذاء فى المجترات

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المستخلص

استخدام الإنزيمات المحللة للألياف له تأثير على زيادة معدلات النمو وكفاءة التحويل فى الحيوانات، لهذا أُشهد فى هذة الدراسة الى تقييم تأثير استخدام الإنزيمات الخارجية المحللة للألياف على الأداء الأنتاجى للحملان النامية وعلى كفاءة تقليل النيتروجين المفرز فى الروث.

استخدم فى هذة الدراسة ١٦ حمل نامى عمر ثلاثة أشهر بمتوسط وزن ٢٢,٣١±١,٥٧ كجم قسمت عشوائياً على مجموعتين كل مجموعة ٨ حيوانات تبعاً لوزن الجسم، عذيت المجموعة الأولى (الضابطة) على العليقة الضابطة بدون إضافة الإنزيمات المحللة للألياف بينما غذيت المجموعة الثانية (المعاملة) على العليقة الضابطة مع إضافة ٢,٥ جم مستحضر إنزيمات.

أظهرت النتائج ما يلى:

- سجلت المجموعة المعاملة التى غذيت على العلائق مع إضافة الإنزيمات أعلى مأكول من المادة الجافة والمركبات الكلية المهضومة والبروتين الخام .
- لم يلاحظ أى فروق معنوية بين المجموعات التجريبية فى مستوى الأحماض الدهنيه الطيارة والأمونيا فى الأوقات المختلفة (صفر ، ٣ ،٦ ساعات بعد الأكل) .
- لوحظ أن التغذية على علائق تحتوى على الإنزيمات المحللة للألياف أدت إلى زيادة PH الكرش.
- لوحظ أن التغذية على علائق تحتوى على الإنزيمات المحللة للألياف أدت إلى زيادة معدلات هضم المادة الجافة ،المادة العضوية، البروتين الخام والالياف بمكوناتها.

- كما أن إضافة الإنزيمات إلى علائق الحملان أدت إلى إنخفلض يوريا الدم وزيادة غير معنوية فى البروتين الكلى بينما لم يكن هناك أى فروق معنوية فى مكونات الدم الأخرى .
- أدى إضافة الإنزيمات ألى علائق الحملان إلى زيادة معدلات النمو والنمو الكلى وزيادة كفاءة التحويل كمادة جافة أو مركبات كلية مهضومة أو بروتين خام أو بروتين مهضوم بالمقارنة بالمجموعة الضابطه.

والخلاصة أن إستخدام الإنزيمات المحللة للألياف يؤدى إلى تحسين أداء الحيوان وزيادة معدلات الهضم والنمو وتحسين كفاءة التحويل بدون أى تأثير ضار على صحة الحيوان.